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Plasma lipidome patterns associated with cardiovascular risk in the PREDIMED trial: a case-cohort study

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Conflicts of interest

ER and RE received grants from the California Walnut Commission and personal fees from pharmaceutical and beverages companies. The remaining authors reported no conflicts of interest related to the study.

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Abstract

Background—The study of the plasma lipidome may help to better characterize molecular mechanisms underlying cardiovascular disease. The identification of new lipid biomarkers could provide future targets for prevention and innovative therapeutic approaches. In the frame of the PREDIMED trial, our aim was to examine the associations of baseline lipidome patterns or their changes with the risk of clinical CVD events.

Methods—We included 983 participants in our case-cohort study. The end-point was the incidence of major CVD during 4.8 years of median follow-up. We repeatedly measured 202 plasma known lipid metabolites at baseline and after 1-year of intervention. Principal component analysis was used to identify lipidome factors. Among the 15 identified factors, 7 were significantly associated with CVD. Considering common patterns among factors, lipids were grouped (summed) into scores.

Results—After adjustment for traditional CVD risk factors, scores of baseline polyunsaturated phosphatidylcholines (PC)/lysoPC/PC-plasmalogens and polyunsaturated cholesterol esters (CE) showed inverse associations with CVD ($p = 0.036$ and 0.012 , respectively); whereas scores of monoacylglycerols (MAGs)/diacylglycerols (DAGs) and short triacylglycerols (TAGs) showed a direct association with CVD ($p = 0.026$ and 0.037 , respectively). Baseline phosphatidylethanolamines (PEs) and their 1-y changes tended to be associated with higher CVD risk ($p = 0.066$ and 0.081, respectively). We did not find a significant effect of the intervention with the Mediterranean Diet on these scores.

Conclusions—Our study suggests that polyunsaturated PCs and CEs may confer protection against CVD. In contrast, MAGs, DAGs, TAGs and PEs appeared to be associated with higher CVD risk.

Keywords

Cardiovascular disease; lipidomics; Mediterranean diet; case-cohort; primary prevention

1. INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of global mortality accounting for 17.9 million of deaths in 2015. In this scenario, prevention strategies to reduce the huge burden of CVD remain a high priority for public health. Dietary interventions are very likely to represent one of the most promising approaches for preventing CVD. The PREDIMED study, a randomized trial for primary prevention of CVD with a nutritional intervention, revealed that the Mediterranean diet (MedDiet), supplemented with either extra virgin olive oil (EVOO) or nuts, reduced the risk of cardiovascular events by 30% after a median follow-up of 4.8 y [1].

Low levels of high-density lipoprotein cholesterol (HDL-C), high concentrations of low-density lipoprotein cholesterol and triacylglycerols (TAGs) are well established as independent predictors of CVD risk. However, some pharmacologic approaches aimed to improve lipid profiles have failed to reduce CVD risk [2]. Thus, there is a need to find further lipid biomarkers which may contribute to a better explanation and classification of CVD risk. The identification of these biomarkers may provide future targets for prevention and innovative therapeutic approaches.

Associations between some lipid metabolites and body mass index (BMI) [3], diabetes [4] and CVD [5] have been reported in studies of the lipidome. Hence, analyzing the lipidome in a randomized trial using a dietary intervention to modify the overall food pattern, would provide further insights in the search for new lipid biomarkers to better understand molecular mechanisms that may account for the benefits of a nutritional intervention. Moreover, the definition of the MedDiet *per se* and the results of PREDIMED have indicated that a relatively fat-rich dietary pattern may contribute to effective prevention of CVD. Thus, one of the potential mechanisms underlying this beneficial effect of MedDiet may be explained by changes in the circulating lipidome. Beyond individual lipid species, it is interesting to identify combinations of molecules (i.e. lipid patterns) that may present a joint effect on the risk of CVD and may be changed by a dietary intervention with beneficial effects on hard CVD events. Therefore, we examined the associations of baseline lipidomic patterns or their 1-year changes with the risk of CVD in the PREDIMED trial.

2. MATERIALS AND METHODS

2.1. Study population and design

The PREDIMED study (www.predimed.es) is a randomized, primary cardiovascular prevention trial conducted in Spain in 7,447 participants at high vascular risk. The methods and design have been reported elsewhere [1,6]. Briefly, participants were randomly assigned to one of three nutritional interventions: MedDiet supplemented with 1) extra-virgin olive oil (MedDiet+EVOO), 2) or mixed nuts (MedDiet+Nuts), and 3) a control diet consisting of advice to reduce intake of fat.

For the present study, an unstratified case-cohort study nested in the PREDIMED trial was designed. We included a random sample of ~10% of PREDIMED participants at baseline and also 233 incident cases of CVD (55 of the 288 incident cases of the PREDIMED trial

had no available plasma samples). We excluded 5 participants because of unavailable lipid metabolites data and 2 participants in the initial quality check. Finally, 983 participants were included in our analysis: 230 incident cases and 790 participants in the subcohort (including 37 overlapping cases). In addition, 907 participants (777 participants in the subcohort and 160 cases, including 30 overlapping cases) had available plasma samples after 1-year of follow-up and were included in the lipidome change analyses.

The Institutional Review Boards of all the recruitment centers approved the overall PREDIMED trial design according to the ethical guidelines of the Declaration of Helsinki. The Institutional Review Boards of the University of Navarra, the Broad Institute of MIT and Harvard, and the Harvard TH Chan School of Public Health, approved the case-cohort subproject. All participants gave written informed consent. Clinical trial registry number: Controlled-Trials.com number, ISRCTN35739639.

2.2. Lipidomics profiling

Fasting blood samples were collected at baseline and yearly thereafter during follow-up. Plasma EDTA tubes were aliquoted, coded and stored at 80 °C.

Pairs of samples (baseline and first-year) of the selected participants were randomly ordered and shipped on dry ice to the Broad Institute of Harvard&MIT for the metabolomics analyses. Specifically, plasma polar and nonpolar lipids were profiled. Detailed description about the method can be found in the supplemental material. A total of 202 known lipid metabolites were analyzed for the present study.

2.3. Outcome ascertainment

The primary endpoint of the PREDIMED trial was a composite outcome of non-fatal acute myocardial infarction, non-fatal stroke and cardiovascular death. Further details can be found in the supplemental material.

2.4. Covariate assessment

At baseline and on yearly follow-up visits, a questionnaire was administered about lifestyle variables, educational achievement, history of illnesses, medication use, and family history of disease. Physical activity was assessed with the use of the validated Spanish version of the Minnesota Leisure-Time Physical Activity questionnaire [7]. A 137-item validated semi-quantitative food-frequency questionnaire was used by trained dietitians to ascertain dietary habits [8]. We used Spanish food-composition tables to estimate energy and nutrient intakes [9]. Anthropometric and blood pressure measurements were directly measured and registered by trained nurses.

2.5. Statistical analysis

Baseline characteristics of the participants, according to their primary outcome status, were described as means and standard deviations (SD) for quantitative traits and as percentages for qualitative traits.

Missing values for 30 lipid species (four of them with >5% of missing values and 26 with <1% of missing data) were replaced by the half of the minimum detected value, assuming that the missingness was a result of lower concentrations than the detectable threshold.

Baseline individual lipid values were normalized and scaled in multiples of 1 SD with Blom's inverse normal transformation [10]. Changes in the lipid values (1-y value minus the baseline value) were calculated and the resulting difference was also normalized and scaled.

The statistical assessment of the association between lipid patterns and CVD was done following two sequential steps: 1) We conducted an exploratory analysis using principal component analysis (PCA) to identify lipidome factors. PCA was performed considering the 202 lipid metabolites as candidates to be included in the obtained factors, and those factors with an eigenvalue higher than 2 were retained. Fifteen factors (not correlated) were extracted explaining 83% of the total variance. An orthogonal rotation (varimax) was used to better interpret the results. Individual metabolites with absolute loadings >0.40 were considered relevant components of the identified factors (table1S), as previously done based on convention [11]. Subsequently, Cox regression models weighted with Barlow weights [12] were used to calculate the hazard ratios (HR) and their 95% confidence interval (95% CI) for the risk of CVD for each factor categorized into quartiles. Quartile cut-off points were generated considering only the subcohort and thereafter cases were categorized according to the same cut-off points. Each factor was introduced, as a continuous variable and categorized in quartiles, in an individual model adjusted for age, sex, BMI, smoking, family history of early coronary heart disease, leisure-time physical activity and intervention group and additionally adjusted for the rest of the PCA-identified factors. Similar models were used to evaluate the linear trend among factors considering the median value of each quartile as a quantitative variable. 2) After identifying the patterns of lipids commonly represented in those factors associated with CVD, we evaluated these lipid patterns grouping the metabolites based on their direct or inverse association with CVD and based on their lipid family trying to clarify biological mechanisms. Therefore, one score (lipid group A) resulted from the sum of all the metabolites represented among factors inversely associated with CVD and another one from the pattern of lipids identified within factors directly associated with CVD (lipid group B). Moreover, one individual score *per* each implicated lipid family was built by summing up the values of metabolites identified in the PCA, which belonged to the same lipid class (according to their chemical structure). Each lipid group (A and B) and each lipid family score were introduced as quartiles and as continuous in a weighted Cox regression model adjusted for age, sex, BMI, smoking habit, family history of early coronary heart disease, leisure time physical activity and stratified for intervention group to analyze their associations with incident CVD. Quartile cut-off points of each group or score were generated considering only the subcohort and thereafter cases were categorized according to the same cut-off points. Areas under the Receiver Operating Curves (AUC) were estimated to assess the predictive ability of each group or score beyond the already known predicting factors: age, sex, BMI, smoking habit, family history of early coronary heart disease, leisure time physical activity and intervention group.

Our next step was to study the effects of changes in these lipid groups after 1-y intervention. Thus, following the same approach that at baseline, changes for each lipid group A and B,

and one score for each implicated lipid family was calculated. After excluding those events occurred during the first year of intervention, each group or score of change was introduced (as continuous and quartiles) in a Cox regression model adjusted for its respective baseline score, and the same baseline confounding factors to analyze their effects on CVD risk.

All the statistical procedures were carried out with STATA 12.0 software. Statistical significance was set a priori at <0.05.

3. RESULTS

Baseline characteristics of the population according to their outcome status are shown in table 1. As expected, the subcohort presented similar baseline characteristics to those observed in the whole PREDIMED study [1]. We observed that cases presented higher levels of the classical cardiovascular risk factors than the subcohort members: older age and a higher proportion of men, smokers, diabetics and less active participants.

3.1. Factor analysis

Fifteen factors with eigenvalues ≥2 were extracted from the PCA analysis conducted on 202 candidate lipid species measured at baseline. Seven extracted factors were significantly associated with CVD, 3 of them showed an inverse association with CVD and the other 4 were directly associated with CVD (Table 2). Supplementary table 1S shows the factor loadings for each factor. The association with a higher risk was especially strong for factor 11, mainly represented by phosphatidylethanolamines (PE). Table 2S describe the lipid species included in each of these factors, which were found to be significantly associated with the risk of CVD.

3.2. Baseline lipid groups and CVD

Based on our first exploratory PCA results (table 3), in a second step we decided to group lipids according to whether the identified patterns were directly or inversely associated with CVD and also depending on their lipid families (chemical structures).

We labeled lipid group A to the sum of that lipids, identified among factors, which were inversely associated with CVD and lipid group B to the sum of metabolites directly associated with CVD. Lipid group A was composed of three families of metabolites: 1) phosphatidylcholines (PC) family grouping PCs, LysoPCs and PC-plasmalogens presenting ≥5 double bonds (PC score); 2) cholesterol esters (CE) with >3 double bounds (CE score); and 3) long-chained triacylglycerols (TAG) with ≥52 carbon atoms containing ≥6 double bonds (long TAG score). For the lipid group B, four families of metabolites were identified: 1) all the monoacylglycerols (MAG) and diacylglycerols (DAG) (MAG & DAG score); 2) short-chained TAGs containing ≤4 double bonds (short TAG score); 3) PEs excluding those with saturated fatty acids (PE score) and 4) all the hydroxyPC (hPC) (hPC score). We also analyzed the association of each of the seven individual scores with CVD.

Baseline characteristics of the population and drug intake distribution according to the extreme quartiles of lipid groups A and B are presented in tables 3 and 4. The results of the associations of both lipid groups A and B and each of the seven scores built according to

lipid families with CVD are shown in table 5. For the lipid group A we found a statistically significant association with lower risk of CVD (p for linear trend=0.015). Within this lipid group, PC and CE scores were significantly associated with lower risk of CVD (p for linear trend 0.036 and 0.012, respectively). In the case of the long TAGs score, we found a significant inverse association with CVD when considering it as a continuous variable (for each SD, HR: 0.84; 95% CI: 0.71–1.00). Lipid group B was significantly associated with a higher risk of CVD (p=0.006). When analyzing separately each family of lipids implicated in the group B, we observed that MAGs&DAGs, short TAGs and PEs scores were directly associated with a higher risk of CVD across their successive quartiles; however, the linear trend was only statistically significant for MAGs&DAGs and short TAGs scores (p=0.026 and 0.037, respectively).

Nevertheless, we found a significant association for the 4 individual scores (MAG&DAG, short TAG, PE and hPC) when analyzing their effect as continuous variables (per each SD) (table 5).

When we additionally adjusted for the use of medication (statins, other lipid lowering drugs, insulin, other oral antidiabetics, antiplatelet agents, ACE inhibitors/AIIRA, diuretics, other antihypertensives and hormone replacement therapy), we found an attenuation of the effect (HR per each SD: 0.85; CI95%:0.69–1.04) in the association between baseline lipid group A and CVD. For lipid group B, we found no substantial differences in their association (HR per each SD: 1.29; CI95%:1.07–1.58) at baseline with CVD after adjustment for the use of medication. When the association between the 1-year changes in the lipids and CVD was considered, results hardly changed after adjustment for drugs (data not shown).

The predictive ability of both groups A and B was evaluated using ROC curve analyses, considering age, sex, BMI, smoking habit, family history of CVD, leisure time physical activity and intervention group as the basic model to assess the improvement in prediction by adding these groups of lipids. We observed that the lipid group B (Fig.1S) was able to significantly improve the prediction of CVD beyond that of risk factors (AUC excluding lipid patterns= 0.692 (CI 95%:0.65–0.73) and AUC including lipid group B = 0.709 (CI 95%:0.67–0.75); p=0.0492 for the comparison).

3.3. Lipids groups changes and CVD

In order to analyze the potential effects of the intervention on lipidome changes, we considered the same baseline metabolite groups and, after calculating 1y-baseline changes, we calculated the scores of lipid changes.

We found that neither changes in the lipid group A nor changes in the group B were associated with CVD events occurring after 1 year (table 3S). When analyzing changes in each lipid family, we only found that the score reflecting 1-y changes in PEs presented a trend to be associated with an increased risk of CVD (p for linear trend=0.081) in consistency with our findings for baseline levels of PEs (tables 5 and 3S).

3.4. Effect of the predimed intervention on changes in lipidome factors

Finally, we analyzed the effects of the PREDIMED intervention on lipid changes by assessing the observed 1-y changes for each lipid group in each of the three arms of the trial. We did not observe any significant changes in these lipid groups for any of the 2 MedDiet groups in comparison with the control group (Fig.1). However, subjects allocated to MedDiet+EVOO presented a marginally significant trend to reductions in the lipid group B (associated with a higher risk of CVD at baseline) (Fig. 3b). This reduction was based on lower levels of short TAGs, MAGs, DAGs and PEs compared to the control group (Fig. 3b).

4. DISCUSSION

The present work was aimed to identify patterns of the lipidome that could account for reduced or increased CVD risk after a dietary intervention in the PREDIMED trial. Our main findings were: 1) at baseline, two different lipid patterns associated with CVD were identified: a) one lipid pattern, composed by 3 families of metabolites, showed inverse association with CVD events (lipid group A): polyunsaturated PCs, lysoPCs, PC-plasmalogens, CEs, and long TAGs; b) the other pattern (lipid group B) was composed by 4 families of metabolites and showed a direct association with CVD: short TAGs (saturated/monounsaturated), hPCs and, especially, MAGs&DAGs and PEs. 2) Changes after 1-y of intervention pointed at the role of PEs on higher subsequent CVD risk. And 3) a 1-y intervention with MedDiet showed no sound effects on lipidome changes, with only trends to lower short TAGs, PEs, MAGs and DAGs in the group receiving intervention with MedDiet+EVOO.

A beneficial effect of dietary polyunsaturated fatty acids on CVD risk has been consistently reported in the literature [13,14]. However, studies related to plasma polyunsaturated status have yielded inconsistent results [15–17]. In our study, we observed that especially the PC lipid family and some CEs likely to be carrying eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) or long TAGs likely to be carrying EPA and arachidonic acid (AA) were associated with a lower CVD risk. This finding is in concordance with some results from other studies [18,19].

A higher risk of CVD in our population was mainly associated with high circulating levels of MAGs, DAGs, and PEs. Increased circulating levels of DAGs have been associated with visceral fat deposition [20] and in liver biopsies, short chain DAGs containing a small number of double bonds (≤3) have been associated with liver steatosis [21]. Moreover, in the Framingham study, a higher content of short TAGs with low number of double bonds was associated with insulin resistance [4]. In addition, in the Bruneck study, these lipids were associated with higher CVD risk [5]. These findings are highly consistent with our results, as we found an association of saturated/monounsaturated short TAGs and their precursors with higher CVD risk, especially when taking into account that 64% of the CVD cases were diabetics and 45% were obese. Moreover, high levels of saturated and short DAGs in steatotic liver appeared to lead to altered levels of TAGs and PEs [21]. Other authors have found an imbalance of PCs/ PEs in the membrane of hepatocytes of fatty liver presenting an abnormally high content of PEs [22,23]. In fact, we found that PEs were the group of metabolites showing the strongest direct association with CVD risk. These findings may

suggest that the risk of CVD explained by MAGs, DAGs, short saturated TAGs and PEs in our population may be linked to an excess of visceral fat, which disrupts the normal metabolism of abdominal tissues.

When we assessed changes in lipids after 1-y of intervention, we found that the association between increased levels of PEs and higher CVD risk after 1-y was persistent, supporting the hypothesis that PEs may play an important role in the development of CVD. Thus, it appears important to further study the mechanism of PEs in other tissues, beyond the liver, by which elevated levels of PEs may be one of the mechanisms underlying the development of future CVD events.

Finally, we observed no significant changes in metabolites due to the intervention with MedDiet. However, we observed a trend among subjects allocated to MedDiet+EVOO had reduced PEs, short TAGs, MAGs and DAGs after 1-year compared to the control group. This finding was consistent with the reduced CVD risk found for the MedDiet+EVOO group in PREDIMED study after a median follow-up period of 4.8 y [1]. Lack of statistical significance may be explained by the fact that 1 year represents a short induction period, and to the potential initial adaptation of several tissues responding to a progressive higher adherence to the MedDiet. Perhaps, our observation period for changes in the lipidome on 1y might be not long enough to observe the reflection in plasma of the stable cell metabolism of an established MedDiet pattern as intended by our intervention. In addition, we discarded 126 lipid metabolites because they were not represented in the identified baseline factors (PCA), addressed to identify patterns associated with CVD, and we followed the same approach when looking at 1-y changes. Though, it is possible that MedDiet may be exerting its main effects trough other lipids, we did not find any robust evidence to support that substantial lipidome changes may account for the benefits observed in the PREDIMED trial. Other factors, such as changes in the antioxidant capacity [24] of the diet or enrichment of the anti-infammatory potential [25] of the diet might have been more important. In fact, some of the benefits of the MedDiet can be mediated by non-lipid mechanisms, such as the high intake of bioactive polyphenols with beneficial anti-oxidant and anti-inflammatory properties that are characteristic of the overall Mediterranean food pattern [26,27].

4.1. Study limitations

Some limitations of this study deserve to be acknowledged. It is possible, that with the initial PCA approach we have missed interconnections or interactions between lipid species/groups after 1-y of intervention that may better explain the potential mechanisms underlying changes caused by MedDiet. Second, our results may not be fully generalized to other populations because of the high baseline vascular risk presented in the sample of the PREDIMED trial. Third, the number of incident cases was relatively low and we may have suboptimal statistical power to detect some associations. And fourth, it would have been very interesting to have inflammation biomarkers data to test the hypothesis of the effects of both lipids and inflammation on CVD risk. Unfortunately, the data about inflammation biomarkers are limited to a very few participants among the case-cohort randomly selected

for this substudy. These biomarkers were measured only to conduct the pilot study [28] in only a small subset (roughly 10%) of the PREDIMED participants.

4.2. Study strengths

Our manuscript presents important strengths. First, the design of the study enables us to both describe a baseline lipidome pattern labelling the initial risk of participants and 1-y changes driven by the intervention in a randomized controlled trial. Second, considering the whole lipidome we are capturing potential relationships between different lipid species and not only isolated effects of each lipid species.

5. CONCLUSIONS

In conclusion, our study suggests that specific patterns of lipid species carrying polyunsaturated fatty acids may confer protection against CVD, including PC, long-chain TAGs and CE. In contrast, PEs may play an important role in increasing CVD risk. In the same direction mono-, di- and short tri-acylglycerides, saturated or monounsaturated, appeared to contribute to CVD risk. Further assessments should explore, in further detail, the effect of interactions within lipids in networks or combined metabolic pathways to better understand the role of the dietary intervention in lipid-mediated changes in CVD risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, Gomez-Gracia E, Ruiz-Gutierrez V, Fiol M, Lapetra J, Lamuela-Raventos RM, Serra-Majem L, Pinto X, Basora J, Munoz MA, Sorli JV, Martinez JA, Martinez-Gonzalez MA. Investigators PS. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med. 2013; 368:1279–1290. DOI: 10.1056/NEJMoa1200303 [PubMed: 23432189]
2. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, Cooney MT, Corrà U, Cosyns B, Deaton C, Graham I, Hall MS, Hobbs FDR, Løchen ML, Löllgen H, Marques-Vidal P, Perk J, Prescott E, Redon J, Richter DJ, Sattar N, Smulders Y, Tiberi M, van der Worp HB, van Dis I, Verschuren WMM. 2016 European Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2016; 37:2315–2381. DOI: 10.1093/eurheartj/ehw106 [PubMed: 27222591]
3. Ho JE, Larson MG, Ghorbani A, Cheng S, Chen MH, Keyes M, Rhee EP, Clish CB, Vasan RS, Gerszten RE, Wang TJ. Metabolomic profiles of body mass index in the framingham heart study reveal distinct cardiometabolic phenotypes. PLoS One. 2016; 11:e0148361.doi: 10.1371/journal.pone.0148361 [PubMed: 26863521]
4. Rhee EP, Cheng S, Larson MG, Walford GA, Lewis GD, McCabe E, Yang E, Farrell L, Fox CS, O'Donnell CJ, Carr SA, Vasan RS, Florez JC, Clish CB, Wang TJ, Gerszten RE. Lipid profiling identifies a triacylglycerol signature of insulin resistance and improves diabetes prediction in humans. J Clin Invest. 2011; 121:1402–1411. DOI: 10.1172/JCI44442 [PubMed: 21403394]

5. Stegemann C, Pechlaner R, Willeit P, Langley SR, Mangino M, Mayr U, Menni C, Moayyeri A, Santer P, Rungger G, Spector TD, Willeit J, Kiechl S, Mayr M. Lipidomics profiling and risk of cardiovascular disease in the prospective population-based Bruneck study. Circulation. 2014; 129:1821–31. DOI: 10.1161/CIRCULATIONAHA.113.002500 [PubMed: 24622385]
6. Martinez-Gonzalez MA, Corella D, Salas-Salvado J, Ros E, Covas MI, Fiol M, Warnberg J, Aros F, Ruiz-Gutierrez V, Lamuela-Raventos RM, Lapetra J, Munoz MA, Martinez JA, Saez G, Serra-Majem L, Pinto X, Mitjavila MT, Tur JA, Portillo MP, Estruch R. Investigators PS. Cohort profile: design and methods of the PREDIMED study. Int J Epidemiol. 2012; 41:377–385. DOI: 10.1093/ije/dyq250 [PubMed: 21172932]
7. Elousa R, Garcia M, Aguilar A, Molina L, Covas I, Marrugat J. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish Women. Med Sci Sport Exerc. 2000; 32:1431–1437.
8. Fernandez-Ballart JD, Pinol JL, Zazpe I, Corella D, Carrasco P, Toledo E, Perez-Bauer M, Martinez-Gonzalez MA, Salas-Salvado J, Martin-Moreno JM. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br J Nutr. 2010; 103:1808–1816. DOI: 10.1017/S0007114509993837 [PubMed: 20102675]
9. Mataix, FJ. Tablas de composición de alimentos. [Food composition tables.]. Instituto de Nutrición y Tecnología de Alimentos, Universidad de Granada; Granada: 2003.
10. Blom, G. Statistical Estimates and Transformed Beta-Variables. John Wiley & Sons A/S; New York: 1958.
11. Hunter WG, Kelly JP, McGarrah RW, Khouri MG, Craig D, Haynes C, Ilkayeva O, Stevens RD, Bain JR, Muehlbauer MJ. Metabolomic profiling identifies novel circulating biomarkers of mitochondrial dysfunction differentially elevated in heart failure with preserved versus reduced ejection fraction: evidence for shared metabolic impairments in clinical heart failure. J Am Heart Assoc. 2016; 5:e003190.doi: 10.1007/s11897-016-0289-5 [PubMed: 27473038]
12. Barlow WE, Ichikawa L, Rosner D, Izumi S. Analysis of case-cohort designs. J Clin Epidemiol. 1999; 52:1165–1172. DOI: 10.1016/S0895-4356(99)00102-X [PubMed: 10580779]
13. Guasch-Ferre M, Babio N, Martinez-Gonzalez MA, Corella D, Ros E, Martin-Pelaez S, Estruch R, Aros F, Gomez-Gracia E, Fiol M, Santos-Lozano JM, Serra-Majem L, Bullo M, Toledo E, Barragan R, Fito M, Gea A, Salas-Salvado J. Dietary fat intake and risk of cardiovascular disease and all-cause mortality in a population at high risk of cardiovascular disease. Am J Clin Nutr. 2015; 102:1563–1573. DOI: 10.3945/ajcn.115.116046 [PubMed: 26561617]
14. Jakobsen MU, O'Reilly EJ, Heitmann BL, Pereira MA, Bälter K, Fraser GE, Goldbourt U, Hallmans G, Knekt P, Liu S, Pietinen P, Spiegelman D, Stevens J, Virtamo J, Willett WC, Ascherio A. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. Am J Clin Nutr. 2009; 89:1425–32. DOI: 10.3945/ajcn.2008.27124 [PubMed: 19211817]
15. Forouhi NG, Koulman A, Sharp SJ, Imamura F, Kröger J, Schulze MB, Crowe FL, Huerta JM, Guevara M, Beulens JWJ, van Woudenbergh GJ, Wang L, Summerhill K, Griffin JL, Feskens EJM, Amiano P, Boeing H, Clavel-Chapelon F, Dartois L, Fagherazzi G, Franks PW, Gonzalez C, Jakobsen MU, Kaaks R, Key TJ, Khaw KT, Kühn T, Mattiello A, Nilsson PM, Overvad K, Pala V, Palli D, Quiros JR, Rolandsson O, Roswall N, Sacerdote C, Sanchez MJ, Slimani N, Spijkerman AMW, Tjonneland A, Tormo MJ, Tumino R, van der ADL, van der Schouw YT, Langenberg C, Riboli E, Wareham NJ. Differences in the prospective association between individual plasma phospholipid saturated fatty acids and incident type 2 diabetes: The EPIC-InterAct case-cohort study. Lancet Diabetes Endocrinol. 2014; 2:810–818. DOI: 10.1016/S2213-8587(14)70146-9 [PubMed: 25107467]
16. De Goede J, Verschuren WMM, Boer JMA, Kromhout D, Geleijnse JM. N-6 and n-3 fatty acid cholesteryl esters in relation to incident stroke in a Dutch adult population: A nested case control study. Nutr Metab Cardiovasc Dis. 2013; 23:737–743. DOI: 10.1016/j.numecd.2012.03.001 [PubMed: 22633188]
17. Minihane AM, Armah CK, Miles EA, Madden JM, Clark AB, Caslake MJ, Packard CJ, Kofler BM, Lietz G, Curtis PJ, Mathers JC, Williams CM, Calder PC. Consumption of Fish Oil Providing Amounts of Eicosapentaenoic Acid and Docosahexaenoic Acid That Can Be Obtained from the Diet Reduces Blood Pressure in Adults with Systolic Hypertension: A Retrospective Analysis. J Nutr. 2016; 146:516–23. DOI: 10.3945/jn.115.220475 [PubMed: 26817716]

18. Erkkilä AT, Lehto S, Pyörälä K, Uusitupa MIJ. n-3 Fatty acids and 5-y risks of death and cardiovascular disease events in patients with coronary artery disease. Am J Clin Nutr. 2003; 78:65–71. [accessed November 17, 2016] http://ajcn.nutrition.org/cgi/content/long/78/1/65. [PubMed: 12816772]

19. Miettinen TA, Naukkarinen V, Huttunen JK, Mattila S, Kumlin T. Fatty-acid composition of serum lipids predicts myocardial infarction. Br Med J (Clin Res Ed). 1982; 285:993–6. DOI: 10.1136/bmj.285.6347.993

20. Scherer M, Montoliu I, Qanadli SD, Collino S, Rezzi S, Kussmann M, Giusti V, Martin FPJ. Blood plasma lipidomic signature of epicardial fat in healthy obese women. Obesity. 2015; 23:130–137. DOI: 10.1002/oby.20925 [PubMed: 25400283]

21. Gorden DL, Ivanova PT, Myers DS, McIntyre JO, VanSaun MN, Wright JK, Matrisian LM, Brown HA. Increased diacylglycerols characterize hepatic lipid changes in progression of human nonalcoholic fatty liver disease; comparison to a murine model. PLoS One. 2011; 6:e22775.doi: 10.1371/journal.pone.0022775 [PubMed: 21857953]

22. Arendt BM, Ma DWL, Simons B, Noureldin SA, Therapondos G, Guindi M, Sherman M, Allard JP. Nonalcoholic fatty liver disease is associated with lower hepatic and erythrocyte ratios of phosphatidylcholine to phosphatidylethanolamine. Appl Physiol Nutr Metab. 2013; 38:334–340. DOI: 10.1139/apnm-2012-0261 [PubMed: 23537027]

23. Li Z, Agellon LB, Allen TM, Umeda M, Jewell L, Mason A, Vance DE. The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis. Cell Metab. 2006; 3:321, 331.doi: 10.1016/j.cmet.2006.03.007 [PubMed: 16679290]

24. Razquin C, Martinez JA, Martinez-Gonzalez MA, Mitjavila MT, Estruch R, Marti a. A 3 years follow-up of a Mediterranean diet rich in virgin olive oil is associated with high plasma antioxidant capacity and reduced body weight gain. Eur J Clin Nutr. 2009; 63:1387–1393. DOI: 10.1038/ejcn.2009.106 [PubMed: 19707219]

25. Casas R, Sacanella E, Urpi-Sarda M, Chiva-Blanch G, Ros E, Martínez-González M-AÁ, Covas M-II, Lamuela-Raventos RM, Salas-Salvadó J, Fiol M, Arós F, Estruch R, Urpí-Sardà M, Chiva-Blanch G, Ros E, Martínez-González M-AÁ, Covas M-II, Ma Lamuela-Raventos Rosa, Salas-Salvadó J, Fiol M, Arós F, Estruch R. The effects of the Mediterranean diet on biomarkers of vascular wall inflammation and plaque vulnerability in subjects with high risk for cardiovascular disease. A randomized trial. PLoS One. 2014; 9:e100084.doi: 10.1371/journal.pone.0100084 [PubMed: 24925270]

26. Tresserra-Rimbau A, Guasch-Ferré M, Salas-Salvadó J, Toledo E, Corella D, Castañer O, Guo X, Gómez-Gracia E, Lapetra J, Arós F, Fiol M, Ros E, Serra-Majem L, Pintó X, Fitó M, Babio N, Martínez-González MA, Sorli JV, López-Sabater MC, Estruch R, Lamuela-Raventós RM. Intake of Total Polyphenols and Some Classes of Polyphenols Is Inversely Associated with Diabetes in Elderly People at High Cardiovascular Disease Risk. J Nutr. 2016; 146:767–777. DOI: 10.3945/jn.115.223610

27. Quiñones M, Miguel M, Aleixandre A. Beneficial effects of polyphenols on cardiovascular disease. Pharmacol Res. 2013; 68:125–131. DOI: 10.1016/j.phrs.2012.10.018 [PubMed: 23174266]

28. Estruch R, Martinez-Gonzalez MA, Corella D, Salas-Salvado J, Ruiz-Gutierrez V, Covas MI, Fiol M, Gomez-Gracia E, Lopez-Sabater MC, Vinyoles E, Aros F, Conde M, Lahoz C, Lapetra J, Saez G, Ros E. Investigators PS. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Ann Intern Med. 2006; 145:1–11. [PubMed: 16818923]

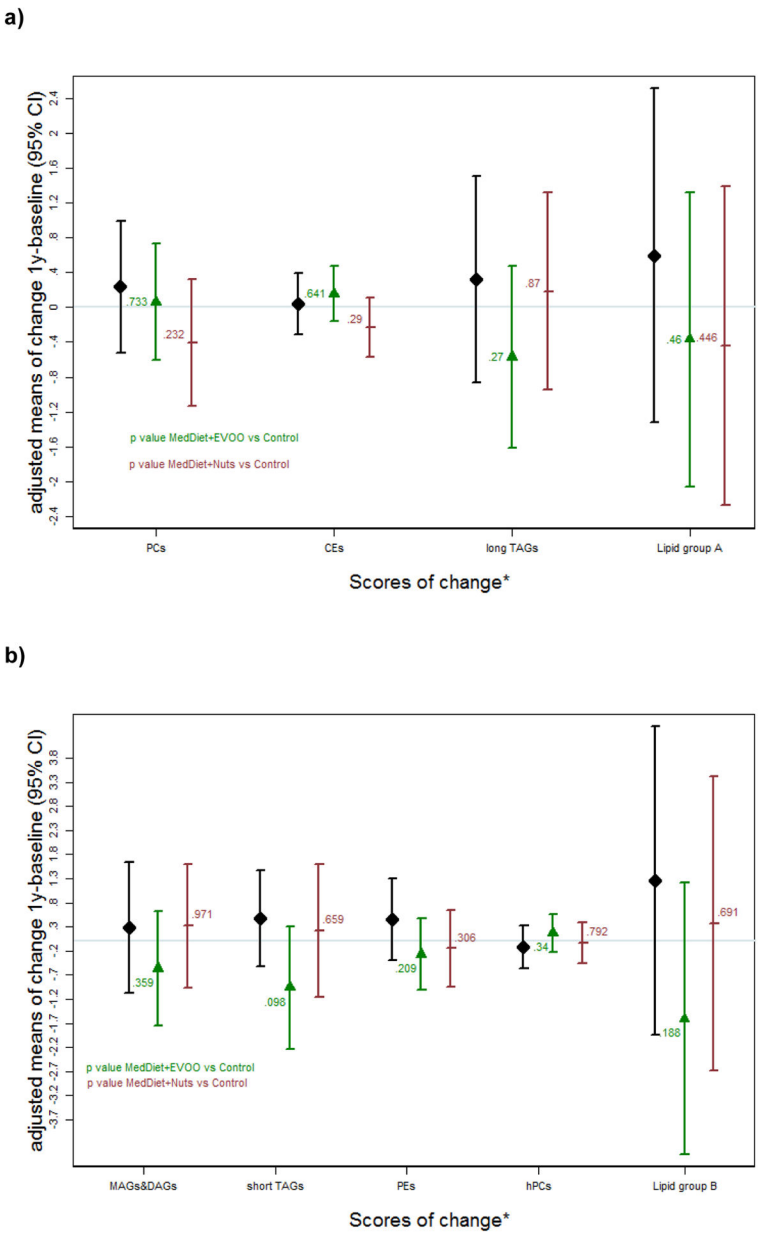

Figure 1.

Lipid scores changes depending on the intervention group (control group as reference for each score): a) scores included in lipid group A and b) scores included in group B

*Mean scores of changes adjusted for its respective baseline score, age, sex, BMI, smoking habit, family history of CVD, leisure time physical activity.

Table 1

Baseline characteristics of the study participants according to the outcome status.

Variables	Subcohort (n=790*)	CVD Cases (n=230)
Age	67.2 (5.9)	69.5 (6.5)
BMI	29.8 (3.6)	29.6 (3.7)
LTPA (METS-min/day)	258 (258)	237 (238)
Women (%)	57.1	39.6
Familiar history of CHD (%)	24.9	19.1
Type 2 diabetes (%)	47.1	64.8
Dyslipidemia (%)	73.5	58.3
Hypertension (%)	83.7	82.6
Smoking		
Non smokers (%)	62.3	45.2
Smokers (%)	12.3	20
Former smokers (%)	25.4	34.8
Total energy intake (Kcal/day)	2334 (615)	2365 (687)
Adherence to MedDiet†	8.82 (1.9)	8.43 (1.81)
Intervention group		
Control (%)	29.7	36.1
MedDiet+EVOO (%)	37.1	35.7
MedDiet+Nuts (%)	33.2	28.3

*Including 37 overlapping cases

†14-items questionnaire

LTPA: Leisure time physical activity, CHD: Coronary Heart Disease

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Table 2

Association between baseline lipid factors (PCA extracted) and CVD risk (adjusted for age, sex, BMI, smoking habit, family history of CVD, leisure time physical activity and intervention group)

	Quartiles of factors				Linear trend	Per SD
	Q1	Q2	Q3	Q4		
Factor 2 *	Ref.	1.02 (0.59–1.74)	0.57 (0.32–1.03)	0.68 (0.38–1.22)	**0.010**	**0.75 (0.62–0.91)**
Factor 9 *	Ref.	1.07 (0.63–1.84)	0.92 (0.53–1.60)	0.60 (0.33–1.08)	**0.028**	**0.80 (0.68–0.95)**
Factor10 *	Ref.	0.76 (0.47–1.25)	**0.54 (0.31–0.93)**	**0.49 (0.28–0.87)**	**0.024**	**0.80 (0.68–0.95)**
Factor 1 *	Ref.	0.94 (0.53–1.67)	**1.84 (1.03–3.30)**	1.67 (0.95–2.90)	**0.010**	1.17 (0.98–1.40)
Factor 11 *	Ref.	**2.20 (1.20–4.03)**	**3.08 (1.70–5.58)**	**3.27 (1.75–6.09)**	**<0.001**	**1.42 (1.21–1.67)**
Factor 12 *	Ref.	1.01 (0.58–1.78)	1.37 (0.77–2.42)	**1.48 (0.84–2.60)**	**0.032**	**1.41 (1.17–1.71)**
Factor 15 *	Ref.	1.10 (0.60–2.04)	1.53 (0.84–2.79)	**1.81 (1.04–3.15)**	**0.032**	**1.20 (1.02–1.41)**

*Additionally adjusted for the rest of factors (1–15)

Table 3

Baseline characteristics and medication use among the study participants according to extreme quartiles of lipid group A.

	Lipid group A-Q1 (n=274)	Lipid group A-Q4 (n=244)
Age	68.8 (6.1)	66.7 (6.3)
BMI	29.7 (3.9)	29.5 (3.2)
LTPA (METS-min/day)	230 (220)	256 (244)
Women (%)	46	58
Familiar history of CHD (%)	22	25
Type 2 diabetes (%)	62	48
Dyslipidemia (%)	57	84
Hypertension (%)	81	84
Smoking		
Non smokers (%)	55	64
Smokers (%)	13	13
Former smokers (%)	32	23
Adherence to MedDiet[†]	8.57 (1.82)	8.93 (1.69)
Total energy intake (Kcal/day)	2390 (664)	2293 (630)
Intervention group		
Control (%)	30	35
MedDiet+EVOO (%)	35	38
MedDiet+Nuts (%)	35	27
Statins (%)	28	48
Lipid lowering drugs (%)	5	4
Insulin (%)	6	4
Other oral antidiabetics (%)	41	29
Antiplatelet agents (%)	28	20
ACE inhibitors/AIIRA (%)	55	48
Diuretics (%)	22	19
Other antihypertensives (%)	31	25
Hormone replacement therapy (%[*])	2	2

[*] Calculated only for women

[†] 14-items questionnaire

LTPA: Leisure time physical activity, CHD: Coronary Heart Disease

Table 4

Baseline characteristics and medication use among the study participants according to extreme quartiles of lipid group B.

	Lipid group B-Q1 (n=242)	Lipid group B-Q4 (n=270)
Age	68.5 (5.8)	67.2 (6.4)
BMI	28.8 (3.8)	30.6 (3.7)
LTPA (METS-min/day)	271 (234)	246 (282)
Women (%)	48	57
Familiar history of CHD (%)	26	20
Type 2 diabetes (%)	44	59
Dyslipidemia (%)	66	74
Hypertension (%)	84	83
Smoking		
Non smokers (%)	55	62
Smokers (%)	17	14
Former smokers (%)	29	25
Adherence to MedDiet†	8.83 (1.79)	8.66 (1.81)
Total energy intake (Kcal/day)	2284 (611)	2333 (668)
Intervention group		
Control (%)	32	30
MedDiet+EVOO (%)	37	38
MedDiet+Nuts (%)	31	33
Statins (%)	37	34
Lipid lowering drugs (%)	2	7
Insulin (%)	5	6
Other oral antidiabetics (%)	28	38
Antiplatelet agents (%)	24	18
ACE inhibitors/AIIRA (%)	49	53
Diuretics (%)	24	23
Other antihypertensives (%)	25	29
Hormone replacement therapy (%*)	3	1

*Calculated only for women

†14-items questionnaire

LTPA: Leisure time physical activity, CHD: Coronary Heart Disease

Table 5

Association between scores at baseline and CVD risk (adjusted for age, sex, BMI, smoking, family history of CVD, leisure-time physical activity and intervention group).

	N of metabolites	Quartiles of scores				Linear trend	Per SD
		Q1	Q2	Q3	Q4		
1. PC * score	10	Ref.	0.74 (0.49–1.12)	0.72 (0.47–1.11)	0.62 (0.38–1.02)	**0.036**	**0.78 (0.65–0.93)**
2. CE † score	4	Ref.	0.77 (0.51–1.16)	0.71 (0.45–1.10)	**0.59 (0.37–0.94)**	**0.012**	**0.71 (0.59–0.86)**
3. Long TAG ‡ score	14	Ref.	0.75 (0.49–1.15)	**0.56 (0.34–0.90)**	0.93 (0.60–1.44)	0.472	**0.84 (0.71–1.00)**
Lipid group A (1+2+3)	28	Ref.	0.99 (0.66–1.49)	**0.52 (0.32–0.84)**	0.68 (0.43–1.07)	**0.015**	**0.76 (0.63–0.92)**
4. MAG&DAG score	19	Ref.	1.00 (0.62–1.61)	1.50 (0.96–2.34)	1.56 (0.97–2.50)	**0.026**	**1.31 (1.11–1.55)**
5. Short TAG § score	15	Ref.	1.35 (0.86–2.13)	1.22 (0.76–1.96)	**1.72 (1.09–2.73)**	**0.037**	1.16 (0.98–1.38)
6. PE score	10	Ref.	0.86 (0.55–1.36)	1.05 (0.67–1.65)	**1.59 (1.02–2.49)**	0.066	**1.26 (1.05–1.51)**
7. hPC score	4	Ref.	0.68 (0.42–1.11)	0.98 (0.61–1.60)	1.24 (0.81–1.90)	0.105	**1.30 (1.07–1.57)**
Lipid group B (4+5+6+7)	48	Ref.	1.41 (0.89–2.23)	1.13 (0.70–1.83)	**2.10 (1.32–3.33)**	**0.006**	**1.32 (1.10–1.58)**

*PC, LysoPC and PC-plasmalogens containing ≥5 double bonds,

†CE containing ≥5 double bonds,

‡TAG with 54 or more carbon atoms and containing ≥5 double bonds,

§TAG with a low number of double bounds (≤4) and <50C.