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Uncovering the shared lipidomic markers of subclinical osteoporosis-atherosclerosis comorbidity: The Young Finns Study

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ABSTRACT

Background: Osteoporosis and atherosclerosis are complex multifactorial diseases sharing common risk factors and pathophysiological mechanisms suggesting that these are comorbidities. Omics studies identifying joint molecular markers associated with these diseases are sparse.

Subjects and methods: Using liquid chromatography-tandem mass spectrometry, we quantified 437 molecular lipid species from the Young Finns Study cohort (aged 30–45 years and 57% women) and performed lipidome-wide multivariate analysis of variance (MANOVA) with early markers for both diseases. Carotid intima-media thickness for atherosclerosis measured with ultrasound and bone mineral density from distal radius and tibia for osteoporosis measured with peripheral quantitative computed tomography were used as early markers of the diseases.

Results: MANOVA adjusted with age, sex and body mass index, identified eight statistically significant (adjusted *p*-value (p_{adj}) < 0.05) and 15 suggestively significant (p_{adj} < 0.25) molecular lipid species associated with the studied markers. Similar analysis adjusted additionally for smoking habit, physical activity and alcohol consumption identified four significant and six suggestively significant molecular lipid species. These most significant lipid classes/species jointly associated with the studied markers were glycerolipid/*TAG(18:0/18:0/18:1)*, glycerophospholipid/*PC(40:3)*, sphingolipid/*Gb3(d18:1/22:0)*, and sphingolipid/*Gb3(d18:1/24:0)*.

Conclusion: Our results support the osteoporosis-atherosclerosis comorbidity hypothesis and present potential new joint lipid biomarkers for these diseases.

1. Introduction

Osteoporosis and atherosclerosis are both complex multifactorial diseases contributing to significant disease burden worldwide with serious morbidities and death [1,2]. The diseases were considered independent conditions sharing common risk factors such as ageing,

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smoking habit, low physical activity and alcohol intake [3]. However, several studies have identified common pathophysiological mechanisms involving inflammatory cytokines, lipid oxidation products, vitamin D and K deficiency [4–6].

The diseases also share molecular pathways involving bone and vascular mineralization and estrogen deficiency [5,6]. Similarly, *osteoprotegerin*, *matrix-gla protein*, and *apolipoprotein E* have been associated with both atherosclerosis and bone loss [3]. Hence, there is indication of common cellular and molecular process involved in the pathogenesis of both the diseases. Therefore, in-depth understanding of common underlying molecular mechanisms is essential for joint prevention and therapeutics of the diseases.

Lipids play important role in human health and disease. Consequently, dyslipidemia is associated with wide range of diseases [7]. Abnormalities in lipids are risk factors for both osteoporosis [8] and atherosclerosis [9]. For example, elevated total serum cholesterol, triglycerides and low-density lipoprotein (LDL) cholesterol are known risk factors for atherosclerosis [10]. Elevated level of cholesterol [11] and oxidised LDL [12] inhibit osteoblastic differentiation. Statins, cholesterol-lowering drugs for individuals who are at a high risk of cardiovascular disease, have potent positive effects on bone formation [13]. Therefore, biological and epidemiological evidence on dyslipidaemia support the notion that osteoporosis and atherosclerosis are comorbid diseases. However, most of the existing literature is focussed on large lipid classes such as triglycerides, cholesterol and free fatty acids. Deeper insights in lipids and their role in the comorbidities require understanding of dyslipidemia at molecular species level because different molecular weight and lipid component composition might have different biological effects [14,15].

Lipidomics involves identification of the entire spectrum of cellular lipids (lipidome) in biological systems. Lipidome-wide association study of a disease phenotype can identify marker lipid species of the disease that can potentially explain its developmental process. In our recent study, we investigated entire spectrum of lipidomics data to identify networks of lipid modules associated jointly with early markers of both osteoporosis and atherosclerosis [16]. The study was based on modular system-level approach where lipid species were first clustered based on their co-abundance and the statistical analysis was done with the clusters also called as modules. While such system-level approach provides more statistical power for identifying novel lipidomic modules and hub lipid markers within the modules, it can potentially miss to identify individual lipid species that do not cluster well with other lipid species perhaps due to their independent biological role in osteoporosis-atherosclerosis comorbidity. Therefore, in this complementary study, we aim to investigate osteoporosis-atherosclerosis comorbidity hypothesis by performing lipidome-wide multivariate association analysis of early markers of both the diseases to identify molecular lipid species with potentially distinct biological role in the comorbidity.

2. Materials and methods

2.1. Study population

The Cardiovascular Risk in Young Finns Study (YFS) is a Finnish prospective multi-centre longitudinal study investigating cardiovascular risk factors from childhood to adulthood [17]. The study was initiated in 1980 with 3596 children and adolescents aged 3–18 years. The participants were randomly selected from the areas of five university hospital catchment areas in Finland and have been followed for over 40 years. Out of 2200 participants who attended the 27-year follow-up in 2007, we included those for whom measurements of early markers of both osteoporosis and atherosclerosis as well as lipidomic data were available. The present study is, thus, based on 1545 participants (57% were females), aged 30–45 years from the 2007 follow-up, with one atherosclerotic and two osteoporotic early markers, as summarized in Table 1. The YFS was approved by the 1st ethical committee of the Hospital District of Southwest Finland and by local ethical committees (1st Ethical Committee of the Hospital District of Southwest Finland, Regional Ethics Committee of the Expert Responsibility area of Tampere University Hospital, Helsinki University Hospital Ethical Committee of Medicine, The Research Ethics Committee of the Northern Savo Hospital District and Ethics Committee of the Northern Ostrobothnia Hospital District). The study protocol of each study phase corresponded to the proposal by the World Health Organization. All subjects gave written informed consent, and the study was conducted in accordance with the Helsinki declaration. At prior YFS follow-ups, informed consent of every participant under the age of 18 years was obtained from a parent and/or legal guardian.

Table 1
Multivariate analysis of variance (MANOVA) models analyzed in the study.

MANOVA model 1	$(CIMT + DTTrD) \sim lipid$
	$(CIMT + DRTrD) \sim lipid$
MANOVA model 2	$(CIMT + DTTrD) \sim lipid + age + sex + body\ mass\ index$
	$(CIMT + DRTrD) \sim lipid + age + sex + body\ mass\ index$
MANOVA model 3	$(CIMT + DTTrD) \sim lipid + age + sex + body\ mass\ index + smoking + alcohol\ consumption + physical\ activity$
	$(CIMT + DRTrD) \sim lipid + age + sex + body\ mass\ index + smoking + alcohol\ consumption + physical\ activity$

2.2. Measurement of early markers of osteoporosis

Trabecular bone densities (TrD, mg/cm^3) of tibia and radius were used as early markers of osteoporosis. Peripheral quantitative computed tomography (pQCT) was performed at the distal and the diaphysis sites of the radius and tibia. The same pQCT device was used in all five centres (XCT 2000R, Stratec, Medizintechnik GmbH, Pforzheim, Germany). The study is based on tomographic slices taken from the distal part (a trabecular-rich bone site) of the weight-bearing tibia (30% and 5% from the distal endplate of the tibia, respectively) and of the nonweight-bearing radius (30% and 4% from the distal endplate of the radius, respectively) according to standard procedures [18]. Precision of the pQCT methods in this multicentre study was evaluated by performing repeated scans of volunteers in each centre before starting and after completing the measurements. Radius and tibia were measured among 39 women and men twice with repositioning. Reproducibility (coefficient of variation, CV%) was 0.5% for distal tibia TrD and 1.6% for distal radius TrD [18].

2.3. Measurement of early markers of atherosclerosis

Carotid intima-media thickness (CIMT) was used as early marker of atherosclerosis. An ultrasound imaging device with a high-resolution system (Sequoia 512, Acuson) including 13.0 MHz linear array transducers was used for CIMT measurement by trained sonographers following a standardized protocol. The image was focused on the posterior (far) wall, and images were recorded from the angle showing the greatest distance between the lumen–intima interface and the media–adventitia interface. A scan including the beginning of the carotid bifurcation and the common carotid artery was recorded and stored in digital format on optical discs for subsequent off-line analysis. All scans were analyzed by one reader blinded to the participants' details. The best-quality end-diastolic frame was selected. Several measurements of the common carotid far wall were taken approximately 10 mm proximally and mean CIMT was used as the outcome. For reproducibility of the CIMT measurements, we re-examined 60 participants 3 months after the initial visit (2.5% random sample). The between-visit CV% of CIMT measurements was 6.4%. For reproducibility of the CIMT image analysis, 113 scans were re-analyzed by a second observer; CV% was 5.2%.

2.4. Health and lifestyle data

Weekly metabolic equivalent hours (MET-h/wk) calculated from information on the frequency, intensity and duration of physical activity including leisure-time physical activity and commuting to the workplace were used as an index for physical activity. One MET corresponds to the energy consumption of one kilocalorie per kilogram of weight per hour at rest [19]. Information on alcohol consumption was based on participants self-report on their alcohol consumption during the previous week where one unit is equivalent to 14 g of alcohol [20].

2.5. Plasma lipidomic profiling

Lipidome quantification for the stored plasma samples was performed at Zora Biosciences Oy (Espoo, Finland). Lipid extraction was based on a previously described method [21]. In brief, 10 μl of 10 mM 2,6-di-tert-butyl-4-methylphenol (BHT) in methanol was added to 10 μl of sample, followed by 20 μl of internal standards (Avanti Polar Lipids Inc., Alabaster, AL) and 300 μl of chloroform:methanol (2:1, v:v) (Sigma-Aldrich GmbH, Steinheim, Germany). Samples were mixed and sonicated in a water bath for 10 min, followed by a 40-min incubation and centrifugation (15 min at 5700 ×*g*). The upper phase was transferred and evaporated under nitrogen. Extracted lipids were resuspended in 100 μl of water saturated butanol and sonicated in a water bath for 5 min. 100 μl of methanol was added to the samples before the extracts were centrifuged for 5 min at 3500 ×*g*, and finally the supernatants were transferred to the analysis plate for mass spectrometric (MS) analysis. Details of MS analyses have also been described in detail previously [22]. The analyses were performed on a hybrid triple quadrupole/linear ion trap mass spectrometer (QTRAP 5500, AB Sciex, Concords, Canada) equipped with ultra-high-performance liquid chromatography (UHPLC) (Nexera-X2, Shimadzu, Kyoto, Japan). Chromatographic separation of the lipidomic screening platform was performed on Acquity BEH C18, 2.1 × 50 mm id. 1.7 μm column (Waters Corporation, Milford, MA, USA). The data were collected using a scheduled multiple reaction monitoring algorithm and the data were processed using Analyst and MultiQuant 3.0 software (AB Sciex). The heights of the peaks obtained from the MS analysis were normalized with the internal standard of the lipid classes.

2.6. Biostatistical analysis

All the statistical analyses were performed using the R environment for statistical computing, version 3.6.1 [23]. Skewness in the values for body mass index (BMI), physical activity, alcohol consumption and lipidome data were corrected with log2 transformation. Multivariate analyses of variance (MANOVA) with early markers of osteoporosis and atherosclerosis as outcomes and lipidome as predictors were performed using *car* R package [24]. Analyses were done without any covariates (MANOVA model 1) and for two different sets of covariates: one adjusted for age, sex, BMI (MANOVA model 2) and the other adjusted for three additional covariates: smoking habit, physical activity and alcohol consumption (MANOVA model 3). However, as lipidome is known to be affected by age, sex and BMI [25,26], we focus on the results from MANOVA models 2 and 3 in the main manuscript and present results from MANOVA model 1 as Supplementary material. Under each of the MANOVA models, different outcome combinations, for one atherosclerotic and two osteoporosis early markers were analyzed (Table 1). The analysis was also done separately for males and females. In addition, association analysis of lipidome with early markers of osteoporosis and atherosclerosis separately was done with linear regression analyses. For multiple testing correction, appropriate number of independent tests was estimated using the eigenvalues of the correlation matrix using the Matrix Spectral Decomposition (matSpDlite) software [27]. Adjusted *p*-values (p_{adj}) were calculated with Bonferroni method. We report lipid species with $p_{adj} < 0.05$ as significant. Considering the exploratory nature of this study, we also report those lipid species with $p_{adj} < 0.25$ as suggestively significant [28].

3. Results

3.1. Study population characteristics

Population characteristics and measured early markers of osteoporosis and atherosclerosis of the study are shown in Table 2. Number of diseases are based on self-reports [18].

3.2. MANOVA and joint associations of lipid species with early markers of osteoporosis and atherosclerosis

MANOVA model 2 adjusted for age, sex and BMI identified 8 significant ($p_{adj} < 0.05$) and 15 suggestively significant ($p_{adj} < 0.25$) lipid species belonging to four different lipid classes that are jointly associated with the early markers of both diseases (Fig. 1, Tables S11, S14). The four different classes are glycerolipid, glycerophospholipid, sphingolipid and fatty acyl. Glycerolipids include acylglycerols, glycerophospholipids contain a phosphate group esterified to one of the glycerol hydroxyl groups, sphingolipids contain long-chain nitrogenous base and fatty acyls are the most fundamental category as they are building block of complex lipids [29]. As expected, lipids within a class are more correlated among each other than with lipids from other classes (Fig. S1). Among both significant and suggestively significant lipid species, 18 were associated with CIMT and DTTrD and the remaining 5 were associated with CIMT and DRTrD. Only two lipid species *TAG(18:0/18:0/18:1)* and *SM(42:3)* were common for both of

Table 2
Population characteristics and measured early markers of osteoporosis and atherosclerosis of the Young Finns Study cohort. Data are mean (± Standard Deviation) or proportions (%).

	Men	Women
Number of subjects	669 (43%)	876 (57%)
Age, years	38 (±5)	38 (±5)
Body mass index, kg/m^2	27 (±4)	25 (±5)
Total cholesterol (mmol/l)	5.2 (±0.9)	4.9 (±0.8)
LDL cholesterol (mmol/l)	3.3 (±0.8)	3.0 (±0.7)
HDL cholesterol (mmol/l)	1.2 ± (0.3)	1.4 ± (0.3)
Triglycerides (mmol/l)	1.5 ± (0.7)	1.2 (±0.6)
Serum glucose (mmol/l)	5.5 (±0.7)	5.2 (±0.7)
Insulin (IU/l)	10 (±26)	8.5 (±8.5)
C-reactive protein (mg/l)	1.7 (±4.7)	2 (±3.4)
Systolic blood pressure (mmHg)	125 (± 13)	116 (±14)
Diastolic blood pressure (mmHg)	78 (±11)	73 (±11)
Participants with hypertension (%)	40/662 (6%)	45/876 (5%)
Alcohol consumption, units/day	1.4 (±2)	0.6 (±0.7)
Physical activity index (MET-h/wk)	20 (±20)	20 (±22)
Daily smoking, %	129/669 (19%)	119/876 (14%)
Daily calcium intake (mg)	1364 (±606)	1188 (±504)
Daily vitamin D intake (μg)	8.4 (±4.6)	7.4 (±3.6)
Family risk factor for coronary heart disease (%)	109/669 (16%)	156/876 (18%)
Participants with osteoporosis (%)	3/664 (0.5%)	7/874 (0.8%)
Participants with bone fractures (%)	297/647 (46%)	280/866 (32%)
Participants with family history for osteoporosis (%)	25/642 (4%)	61/858 (7%)
Usage of corticosteroids at least once a month (%)	16/647 (3%)	61/865 (7%)
Carotid intima-media thickness (CIMT) (average, mm)	0.65 (±0.11)	0.61 (±0.09)
Participants with CIMT >1 mm	5/669 (0.7%)	0/876
Distal radius trabecular bone mineral density (DRTrD) (mg/cm^3)	247 (±31)	207 (±28)
Distal tibia trabecular bone mineral density (DTTrD) (mg/cm^3)	255 (±33)	229 (±30)

these two early marker combinations shown in Fig. 1. While the most significantly associated lipid species with CIMT and DTTrD was *TAG (18:0/18:0/18:1)*, for CIMT and DRTrD, the most significantly associated species was *Gb3(d18:1/22:0)*. Similar association analyses with MANOVA model 3 (with a stricter model) adjusted with three additional covariates, smoking habit, physical activity and alcohol consumption, identified 4 significant ($p_{adj} < 0.05$) and 6 suggestively significant ($p_{adj} < 0.25$) lipid species belonging to three different lipid classes that were jointly associated with the early markers of both the diseases (Fig. 2, Tables S12, S15). All the 10 lipid species were also present in the results from MANOVA model 2. MANOVA analyses with model 1 with CIMT-DRTrD and CIMT-DTTrA as outcomes identified 202 and 154 significant ($p_{adj} < 0.05$) lipid species respectively (Tables S10, S13).

3.3. MANOVA and sex-specific joint associations of lipid species with early markers of osteoporosis and atherosclerosis

Sex-stratified associations between lipid species and early markers of osteoporosis and atherosclerosis were investigated with MANOVA model adjusted with age, BMI, smoking habit, physical activity and alcohol consumption. *TAG(18:0/18:0/18:1)* and *PC(39:6)* were identified to be female specific and *SM(42:3)* and *SM(44:3)* were identified to be male specific with suggestive statistical significance threshold ($p_{adj} < 0.25$). Note that these lipid species were also found in general analysis above.

3.4. Associations of lipid species with early markers of osteoporosis and atherosclerosis separately

Associations between lipid species and early markers of atherosclerosis (CIMT) or osteoporosis (DTTrD, DRTrD) separately were analyzed with linear regression models similar to that of MANOVA models except that only one marker (either for osteoporosis or atherosclerosis) was used as outcome in each model (Table 1). There were 130 lipid species associated with CIMT with model 1 (without any covariates) with p.adj < 0.25 (Table S1). However, only three (*TAG(18:0/18:0/18:1)*, *AcylCarnitine(16:1) and SM(31:2)*) remained significant after adjusting for age, sex and BMI with model 2 (Table S2) and two (*TAG(18:0/18:0/18:1)*, *AcylCarnitine(16:1)*) remained significant after adjusting additionally for alcohol consumption, smoking and physical activity in model 3 (Table S3). Similarly, 142 and 200 lipid species were associated with early markers of osteoporosis, DTTrD and DRTrD respectively with model 1 (Tables S4, S7). However, with model 3 only four lipid species (*PC(40:3), PC(36:7), PC(36:1), PC(38:3)*) were associated with DTTrD (Table S6) and only three (*Gb3(d18:1/22:0)*, *Gb3(d18:1/24:0)*, *PC(40:3)*) with DRTrD (Table S9). These findings are similar to those obtained from MANOVA with the osteoporosis and atherosclerosis related outcomes combined as described in Section 3.2.

4. Discussion

To the best of our knowledge, this is the first lipidome-wide association study investigating the joint associations between molecular lipid species and early markers of both osteoporosis and atherosclerosis. Using lipidomics data, we identified molecular lipid species that are jointly associated with early markers of both the studied diseases. We identified eight plasma molecular lipid species jointly and significantly associated with the studied early markers of osteoporosis (trabecular bone density determined with pQCT) and atherosclerosis (carotid intima-media thickness determined with ultrasound).

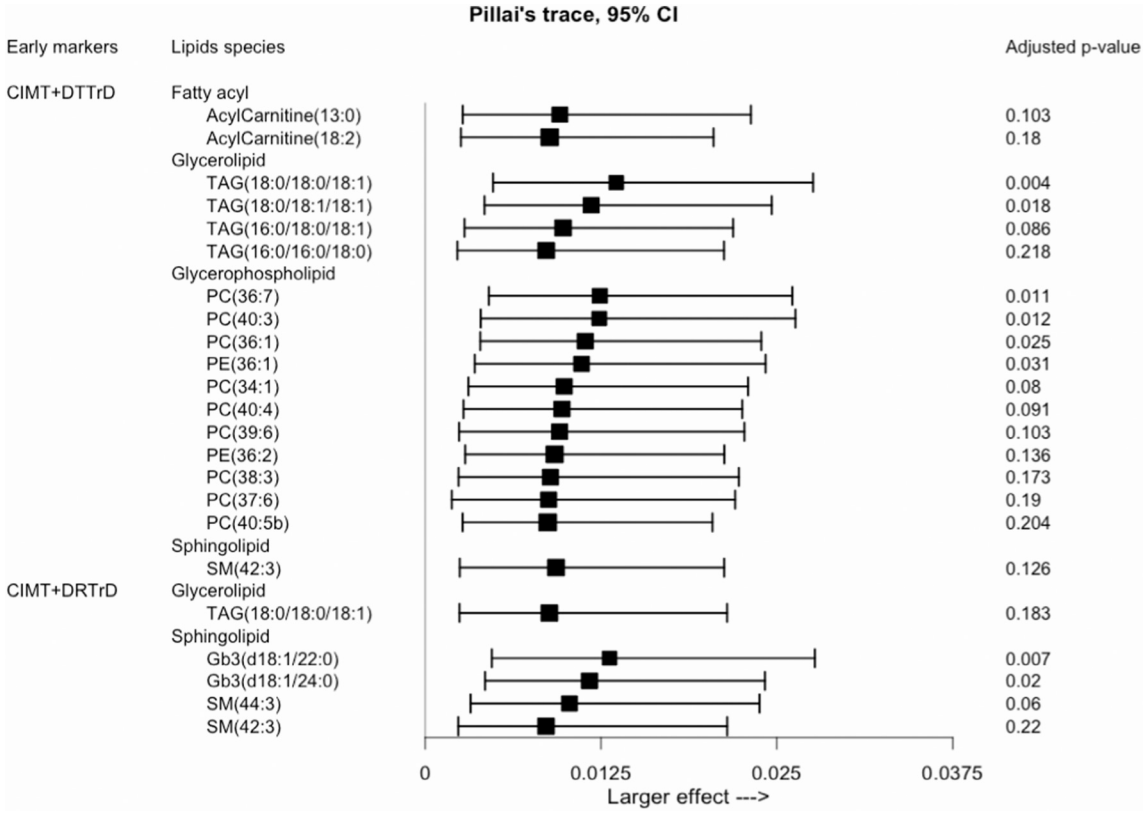

Fig. 1. Forest plot of the multivariate lipidome-wide association study of early markers of osteoporosis and atherosclerosis adjusted for age, sex and body mass index. Analysis was performed for two different outcomes based on combination of early markers of atherosclerosis, carotid intima media thickness (CIMT); and osteoporosis, distal tibia's trabecular bone (DTTrD) and distal radius's trabecular bone (DRTrD). The second column represents lipid species and the classes they belong to. Pillai's trace is the test statistic in multivariate analysis of variance (MANOVA) ranging from 0 to 1. Confidence interval of the test statistic was calculated from 100 bootstraps of the original data. *P*-values were adjusted using Bonferroni's method.

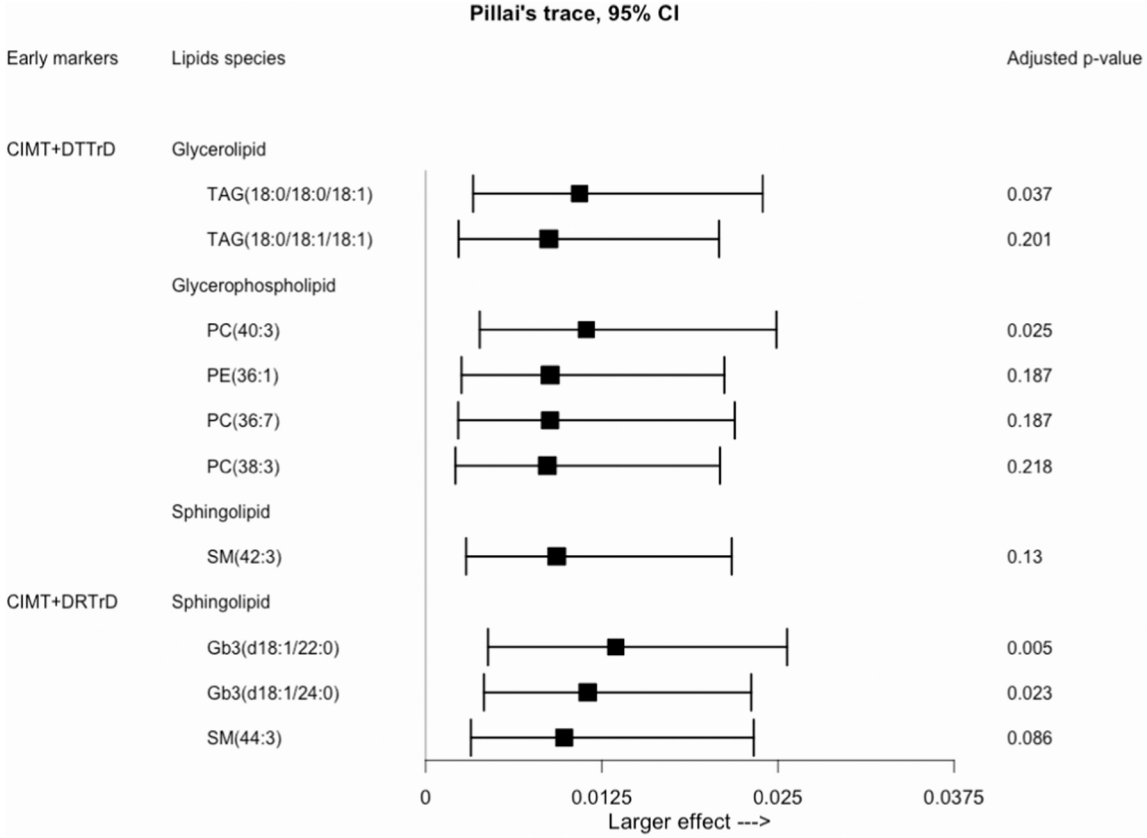

Fig. 2. Forest plot of the multivariate lipidome-wide association study of early markers of osteoporosis and atherosclerosis adjusted for age, sex, body mass index, smoking habit, physical activity and alcohol consumption. Analysis was performed for two different outcomes based on combination of early markers of atherosclerosis, carotid intima media thickness (CIMT); and osteoporosis, total mineral density of the distal tibia's trabecular bone (DTTrD) and distal radius's trabecular bone (DRTrD). The second column represents lipid species and the classes they belong to. Pillai's trace is the test statistic in multivariate analysis of variance (MANOVA) ranging from 0 to 1. Confidence interval of the test statistic was calculated from 100 bootstraps of the original data. *P*-values were adjusted using Bonferroni's method.

For early markers of osteoporosis, we focused mainly on the bone mineral density measured from metabolically active trabecular-rich distal site of weight-bearing tibia (DTTrD) and non-weight-bearing radius (DRTrD). Based on our results, mostly glycerolipids and glycerophospholipids are jointly associated with DTTrD and CIMT. On the other hand, mostly sphingolipids seem to be associated with DRTrD and CIMT. We speculate that these differences might be attributed to the differences in weight-bearing functionality between radius and tibia. The four glycerolipids associated with DTTrD and CIMT: *TAG(18:0/18:0/18:1)*, *TAG(18:0/18:1/18:1)*, *TAG(16:0/18:0/18:1)* and *TAG (16:0/16:0/18:1)* differ from each other in the structure of their fatty acyls side chains. Elevated triacylglyceride (TAG) level is independent risk factor for cardiovascular disease after controlling for low-density lipoprotein and high-density lipoprotein cholesterol [30]. For example, *TAG(54:2)*, triacylglyceride with fatty acyls of 54 carbons and 2 double bonds has been shown to be a strong predictor for cardiovascular disease [31]. Similarly, *TAG(56:6)*, triacylglyceride with fatty acyls of 56 carbons and 6 double bonds, has been shown to be associated with development of cardiovascular disease in type 2 diabetes mellitus [32]. TAG might promote atherosclerotic plaque through endothelial dysfunction, inflammation and thrombosis mechanisms via atherogenic remnant particles and apo C-III, a proinflammatory and proatherogenic protein [33]. Various studies have shown positive correlation between TAG and bone mineral density [34–37]. Dragojevič et al. reported that osteoporotic tissue cells exhibit higher osteoclastogenesis and lower TAG metabolism. This leads to speculation that perhaps TAG plays protective role in osteoporosis [38].

Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are glycerophospholipids with choline and ethanolamine head respectively. PCs are major components of cell membrane and play role in cell signalling, food and energy storage and glycerophospholipid metabolism [39]. PEs are enriched in the inner leaflet of cell membrane and are involved in protein biogenesis, oxidative phosphorylation, membrane fusion, mitochondrial stability and autophagy [39]. Lysophosphatidylcholines, a metabolite of PCs, are reportedly significantly increased in the plasma of osteoporotic mice and is likely to increase reactive oxygen species which leads to oxidative stress damage [40,41]. This increase in oxidative stress may facilitate extensive bone loss and bone fragility and thereby exacerbating the process of osteoporosis [42]. Furthermore, oxidative stress may promote endothelial dysfunction, thereby promoting a vascular inflammatory response which leads to the progression of atherosclerosis [43]. Studies have shown that PCs with lower levels of carbon atoms might be associated with cardiovascular disease [44]. For example, *PC(34:1),* a phosphatidylcholine with 34 carbons and 1 double bonds is related to ischemia [45]. Also, PEs and PCs along with TAGs have been shown to be associated with osteoporosis [46].

Sphingomyelins, *SM (44:3)* and *SM (43:3)* and globotriasoylceramides, *Gb3(d18:1/24:0)* and *Gb3 (d18:1/22:0)* are sphingolipids that were significantly associated with the early markers of both osteoporosis and atherosclerosis. Sphingolipids are involved in important biological processes such as proliferation, migration and apoptosis [47]. Sphingosine 1-phosphate (S1P), an intermediate in sphingolipid metabolism, is a potent mediator of bone homeostasis and acts as a coupling factor between osteoclast and osteoblast activity [48,49]. S1P, however, possesses both protective and harmful effects in the pathogenesis of

atherosclerosis [50,51]. S1P maintains the endothelial integrity by promoting endothelial barrier function [50], whereas also induce inflammation and thrombosis in atherosclerosis [51]. Sphingomyelins, the most common sphingolipids in mammalian cells and tissues are independently associated with coronary artery disease [52]. Sphingomyelins are also crucial for mineralization process in healthy bones [53]. Fabry disease, an X-linked inborn error of glycosphingolipid catabolism, leads to accumulation of globotriaosylceramide in body fluids. Increase in globotriaosylceramide is associated with osteopenia and accelerated bone resorption of the lumbar spine and femoral neck [54]. Globotriaosylceramide also induces oxidative stress, inflammation [55] and endothelial dysfunction [56] in Fabry patients.

This study complements our previous system-level lipidomics based osteoporosis-atherosclerosis study [16] by identifying thirteen additional novel lipid species (*AcylCarnitine.13.0.*, *AcylCarnitinr.18.2.*, *PC(36:1)*, *PC(36:7)*, *PC(40:3)*, *PC(34.1)*, *PC(39.6)*, *PC(37.6)*, *PE(36:2)*, *Gb3(d18:1/22:0)*, *Gb3(d18:1/24:0)*, *SM(42:3),SM(44:3)*) jointly associated with early markers of both the diseases. The study also replicated the three most significant lipid species identified by the system-level approach (*TAG(18:0/18:0/18:1)*, *TAG(18:0/18:1/18:1)*, *TAG(16:0/18:0/18:1)*) confirming their potential joint role in osteoporosis-atherosclerosis comorbidity.

There were certain limitations in our study. The study was based on a relatively young population cohort with an early phase of cardiovascular disease and osteoporosis and very few clinically diagnosed cases. However, we believe that our results unveiled suggestive joint associations between the molecular lipid species and early markers of the diseases that warrants further research in a case–control setting that includes participants with clinically diagnosed osteoporosis and atherosclerosis. Sex-stratified associations between lipid species and early markers of osteoporosis and atherosclerosis remained undetected with statistical significance because of the lack of sufficient statistical power. Furthermore, all study participants are of European origin. Further research in a case–control setting with populations of different ethnicities are needed.

5. Conclusion

Our results from lipidome-wide multivariate association analysis of early markers of osteoporosis and atherosclerosis support the osteoporosis-atherosclerosis comorbidity hypothesis. Specifically, this study identified *TAG(18:0/18:0/18:1)*, *PC(40:3)*, *Gb3(d18:1/22:0) and Gb3(d18:1/24:0)* as the most significant molecular lipid species jointly associated with early markers of both the diseases. These new molecules may provide potential new biomarkers for the prediction of these comorbid conditions.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2021.116030.

Data availability statement

The dataset supporting the conclusions of this article were obtained from the Cardiovascular Risk in Young Finns Study which comprises health related participant data. The use of data is restricted under the regulations on professional secrecy (Act on the Openness of Government Activities, 612/1999) and on sensitive personal data (Personal Data Act, 523/1999, implementing the EU data protection directive 95/46/EC). Due to these restrictions, the data cannot be stored in public repositories or otherwise made publicly available. Data access may be permitted on a case-by-case basis upon request only. Data sharing outside the group is done in collaboration with YFS group and requires a data-sharing agreement. Investigators can submit an expression of interest to the chairman of the publication committee Professor Mika Kähönen (Tampere University, Finland) or Professor Terho Lehtimäki (Tampere University, Finland).

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Ethical approval

The study was approved by the ethical committee of the Hospital District of Southwest Finland on 20 June 2017 (ETMK:68/1801/2017), and all participants have given an informed written consent. Data protection will be handled according to current regulations.

CRediT authorship contribution statement

Binisha H. Mishra: Conceptualization, Investigation, Writing – original draft. **Pashupati P. Mishra:** Supervision, Data curation, Writing – review & editing. **Nina Mononen:** Writing – review & editing. **Mika Hilvo:** Writing – review & editing. **Harri Sievänen:** Writing – review & editing. **Markus Juonala:** Writing – review & editing. **Marika Laaksonen:** Writing – review & editing. **Nina Hutri-Kähönen:** Writing – review & editing. **Jorma Viikari:** Writing – review & editing. **Mika Kähönen:** Writing – review & editing. **Olli T. Raitakari:** Writing – review & editing. **Reijo Laaksonen:** Writing – review & editing. **Terho Lehtimäki:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

None.

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