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Lipidomic architecture shared by subclinical markers of osteoporosis and atherosclerosis: The Cardiovascular Risk in Young Finns Study

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ABSTRACT

Background: Studies have shown that osteoporosis and atherosclerosis are comorbid conditions sharing common risk factors and pathophysiological mechanisms. Understanding these is crucial in order to develop shared methods for risk stratification, prevention, diagnosis and treatment. The aim of this study was to apply a system-level bioinformatics approach to lipidome-wide data in order to pinpoint the lipidomic architecture jointly associated with surrogate markers of these complex comorbid diseases.

Subjects and methods: The study was based on the Cardiovascular Risk in Young Finns Study cohort from the 2007 follow-up (n = 1494, aged 30–45 years, women: 57%). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to analyse the serum lipidome, involving 437 molecular lipid species. The subclinical osteoporotic markers included indices of bone mineral density and content, measured using peripheral quantitative computer tomography from the distal and shaft sites of both the tibia and the radius. The subclinical atherosclerotic markers included carotid and bulbus intima media thickness measured with high-resolution ultrasound. Weighted co-expression network analysis was performed to identify networks of densely interconnected lipid species (i.e. lipid modules) associated with subclinical markers of both osteoporosis and atherosclerosis. The levels of lipid species (lipid profiles) of each of the lipid modules were summarized by the first principal component termed as module eigenlipid. Then, Pearson's correlation (r) was calculated between the module eigenlipids and the markers. Lipid modules that were significantly and jointly correlated with subclinical markers of both osteoporosis and atherosclerosis were considered to be related to the comorbidities. The hypothesis that the eigenlipids and profiles of the constituent lipid species in the modules have joint effects on the markers was tested with multivariate analysis of variance (MANOVA).

Results: Among twelve studied molecular lipid modules, we identified one module with 105 lipid species significantly and jointly associated with both subclinical markers of both osteoporosis (r = 0.24, p-value = 2×10^{-20}) and atherosclerosis (r = 0.16, p-value = 2×10^{-10}). The majority of the lipid species in this module belonged to the glycerolipid (n = 60), glycerophospholipid (n = 13) and sphingolipid (n = 29) classes. The module was also enriched with ceramides (n = 20), confirming their significance in cardiovascular outcomes and suggesting their joint role in the comorbidities. The top three of the 37 statistically significant (adjusted p-value < 0.05) lipid species jointly associated with subclinical markers of both osteoporosis and atherosclerosis within the module were all triacylglycerols (TAGs) – TAG(18:0/18:0/18:1) with an adjusted p-

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value of 8.6×10^{-8}, TAG(18:0/18:1/18:1) with an adjusted p-value of 3.7×10^{-6}, and TAG(16:0/18:0/18:1) with an adjusted p-value of 8.5×10^{-6}.

Conclusion: This study identified a novel lipid module associated with both surrogate markers of both subclinical osteoporosis and subclinical atherosclerosis. Alterations in the metabolism of the identified lipid module and, more specifically, the TAG related molecular lipids within the module may provide potential new biomarkers for testing the comorbidities, opening avenues for the emergence of dual-purpose prevention measures.

1. Introduction

Cardiovascular diseases and osteoporosis are both widely prevalent disorders, inducing serious morbidities, bone fractures and death [1–4]. Evidence indicates that there is a similar pathophysiological mechanism underlying both diseases [5]. Several association studies have linked bone measures with atherosclerosis-related measures, such as echogenic calcified atherosclerotic plaques, pulse wave velocity and coronary artery calcification [3,6–8]. Using human atherosclerotic plaque transcriptomics and confocal microscopic analysis, we have shown that advanced atherosclerotic lesions express a variety of markers related with osteoclastogenesis, osteoblastogenesis and calcification and that they involve osteoclast-like cells [9]. Furthermore, genetic polymorphism of apolipoprotein E, a key regulator of serum lipid levels [10] and atherosclerosis [11], has also been shown to be associated with bone structural traits [12]. Various studies have also revealed a positive biological effect of statin, a cholesterol-lowering drug used for the prevention of cardiovascular diseases, to be effective on bone density [13,14]. However, there are also studies that reveal no significant association between the bone and vascular markers [15–17]. Although, they share the same biomarkers and risk factors – for example, oestrogen deficiency, vitamin D abnormalities, dyslipidaemia, smoking, physical inactivity, intake of dietary calcium, dietary saturated fat, oxidative stress and genetic factors [18–21] – the nature and the mechanism involved remains elusive.

Lipidomics offers a tool to investigate the systemic lipid profiles produced in the body's cells, tissues and organs, as well as their interactions with other molecular and cellular components [22]. An altered lipidome has been shown to be associated with several clinical conditions [23,24]. Understanding these alterations can provide useful insight into the development process of the diseases. A study investigating the shared underlining mechanism of atherosclerosis and osteoporosis comorbidity by utilizing lipidomics data is lacking. Therefore, the objective of the present study was to perform a system-level analysis of lipidomics data to identify networks of lipid species associated jointly with subclinical markers of both osteoporosis and atherosclerosis.

The lipidomics data in this study involved 437 molecular lipids generated with liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique from the serum of 1494 participants. Traditional individual molecule-wise statistical methods are limited in their ability to provide a holistic system-level picture. We, therefore, performed a signed weighted lipid co-expression network analysis to identify networks of lipid species (modules) jointly associated with subclinical markers of both osteoporosis and atherosclerosis [25].

2. Material and methods

2.1. Study subjects

The Cardiovascular Risk in Young Finns Study (YFS) is a prospective multi-centre follow-up study investigating cardiovascular risk factors from childhood to adulthood [26]. 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 hospitals in Finland (Turku, Tampere, Helsinki, Kuopio and Oulu) and have been followed for nearly 40 years. The present study is based on 1494 participants aged 30–45 from the 2007 follow-up, with four atherosclerotic and six osteoporotic markers, as summarized in Table 2.

2.2. Measurement of surrogate markers of subclinical atherosclerosis

Carotid and bulbus intima-media thickness (IMT) were used as surrogate markers of subclinical atherosclerosis. An ultrasound imaging device with a high-resolution system (Sequoia 512, Acuson) including 13.0 MHz linear array transducers was used for IMT 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 analysed 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 to derive the maximal carotid IMT. To assess the reproducibility of the IMT measurements, we re-examined 60 participants 3 months after the initial visit (2.5% random sample). The between-visit coefficient of variation of IMT measurements was 6.4%. To assess the reproducibility of the IMT image analysis, 113 scans were re-analysed by a second observer, and the coefficient of variation was 5.2%. The mean and maximum carotid and bulbus IMT was used in the study.

2.3. Measurement of surrogate markers of subclinical osteoporosis

Two trained researchers in each study centre performed the peripheral quantitative computed tomography (pQCT) bone measurements from both 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, Pforzheim, Germany). The tomographic slices were taken from the shaft (a cortical-rich bone site) and 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 our standard procedures [27]. For the shaft regions, the analysed bone traits were total area (ToA, mm^2), cortical area (CoA, mm^2), and cortical density (CoD, mg/cm^3). For the distal parts of the radius and tibia, the measured bone traits were ToA (mm^2), CoA (mm^2) and trabecular density (TrD, mg/cm^3). The range of in vivo precision of the used pQCT-measured traits ranged from 0.5% (CoD of the radial shaft) to 4.4% (CoA of the distal radius). Mineral content was calculated as 0.2 × (area/100) × density. The measured indices are demonstrated in Table 2.

2.4. Health and life style data

The physical activity index was calculated as metabolic equivalents (METs) by combining information on the frequency, intensity and duration of physical activity including leisure-time physical activity and commuting to the workplace (MET h/wk). One MET corresponds to the energy consumption of one kilocalorie per kilogram of weight per hour at rest [28]. Alcohol consumption was measured by asking participants to report their alcohol consumption during the previous week. One unit is equivalent to 14 g of alcohol [29].

2.5. Lipidome-wide analysis

Lipidome quantification for the stored serum samples was performed at Zora Biosciences Oy (Espoo, Finland). Lipid extraction was based on a previously described method [30]. In brief, 10 µl of 10 mM 2,6-di-tert-butyl-4-methylphenol (BHT) in methanol was added to 10 µl of the 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). The samples were mixed and sonicated in a water bath for 10 min, followed by a 40-min incubation and centrifugation (15 min at 5700 $\times g$). 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. Then, 100 µl of methanol was added to the samples before the extracts were centrifuged for 5 min at 3500 $\times g$, and finally the supernatants were transferred to the analysis plate for mass spectrometric (MS) analysis. The MS analyses have also been described in detail previously [31]. The analyses were performed on a hybrid triple quadrupole/linear ion trap mass spectrometer (QTRAP 5500, AB Sciex, Concord, Canada) equipped with ultra-high-performance liquid chromatography (UHPLC) (Nexera-X2, Shimadzu, Kyoto, Japan). Chromatographic separation of the lipidomic screening platform was performed on an 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 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

The lipid profiles were $\log_e$ transformed to correct for skewness. We used signed weighted co-expression network analysis implemented in R statistical software [25] to identify groups of densely interconnected lipid species, hereafter referred to as lipid modules. The analysis pipeline is illustrated in Fig. 1. Pearson's correlation coefficients (r) were calculated for all pairwise comparisons of lipid species across all participants. The correlation matrix was transformed to an adjacency matrix by raising it to the power of 5, chosen based on scale-free topology criteria (Fig. S1). The power transformation reduces noise by supressing low correlations and emphasizing stronger correlations between lipid species. The power term is chosen in a manner that leads to

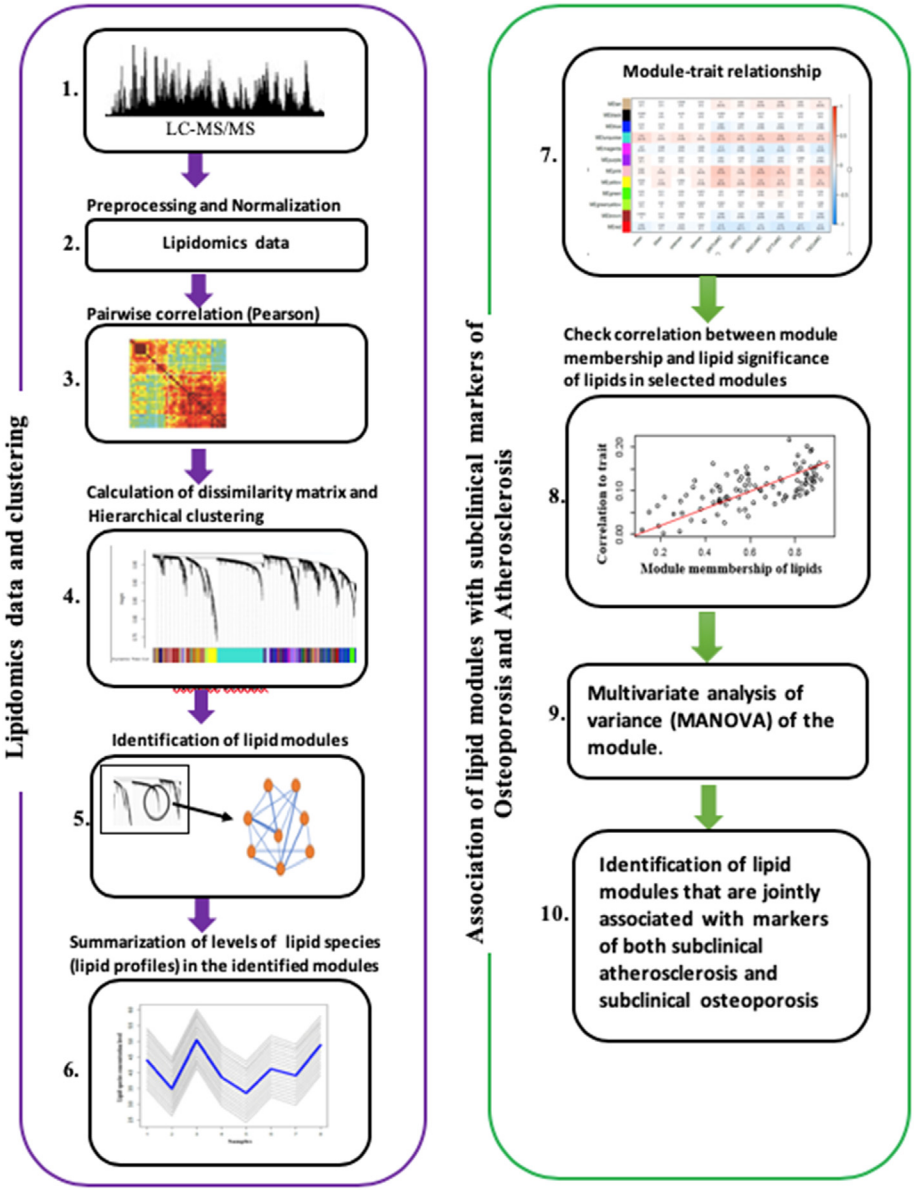

Fig. 1. Weighted co-expression network analysis pipeline. 1) Lipidomics data generated with liquid chromatography-tandem mass spectrometry technique (LC-MS/MS). 2) Levels of lipid species. 3) Correlation matrix based on the pairwise correlation (Pearson) of the lipid species. 4) Hierarchical clustering of the dissimilarity matrix generated from the correlation matrix. 5) Identification of lipid species modules based on clustering. 6) Summarization of the levels of constituent lipid species in the modules by calculating their first principal component called as eigenlipid. 7) Correlation between eigenlipids (as representative of modules) and markers of subclinical osteoporosis and atherosclerosis. 8) Examination of the correlation between module membership and the lipid significance of lipid species in the selected modules as a quality check of the modules.

a scale-free network topology because most of the biological networks are expected to be approximately scale-free. The resulting adjacency matrix was used to generate a Topological Overlap Matrix (TOM). The TOM is a pairwise similarity matrix of the lipid species that considers topological similarity among lipid species. For example, a high TOM implies that a pair of lipid species shares several neighbour lipid species with similar levels. The TOM was transformed into a dissimilarity matrix. Average linkage hierarchical clustering of the dissimilarity matrix was performed to generate a hierarchical clustering tree of lipid species. Lipid modules that are weighted networks of lipid species were identified with a dynamic tree-cutting algorithm. The lipid profiles in each module were summarized by the module eigenlipid (ME), which is defined as the first principal component of the modules' lipid profiles. Association analysis was performed by calculating Pearson's correlation coefficients (r) between the modules and the studied markers. Multivariate analyses of variance (MANOVA) were conducted for significant modules and their constituent member lipid species in order to test the hypothesis that the eigenlipid and profiles of the constituent lipid species in the modules have joint effects on markers of both subclinical osteoporosis and subclinical atherosclerosis. All the multivariate analyses were adjusted for age and sex. All statistical analyses and data processing were performed using the statistical package R version 3.4.3 [32].

3. Results

3.1. Study population characteristics

The characteristics of the study population are shown in Table 1. The disease incidences are based on self-reports [27]. The measured markers of subclinical osteoporosis and atherosclerosis are shown in Table 2.

3.2. Association between surrogate markers of subclinical osteoporosis and atherosclerosis

The surrogate markers of subclinical osteoporosis had a weak but significant (p-value < 0.01) positive correlation with those of subclinical atherosclerosis (Fig. 2).

3.3. Identification of lipid modules

An adjacency matrix was generated from the correlation matrix of the molecular lipid species using a soft-thresholding power of five with the *WGCNA* R package. The threshold was chosen based on a network topology analysis. The network resembled a scale-free graph, with $r^2 > 0.80$, when the correlation matrix was raised to the power of five (Fig. S1). The hierarchical clustering of the TOM dissimilarity matrix defined 12 modules, containing 6–105 highly correlated lipid species (Fig. S2). The lipid modules were named according to colour for downstream analysis, as shown in Fig. 3.

3.4. Module trait relationships and identification of the most significant modules

Pearson's correlation between the module eigenlipids (MEs) and the studied markers was calculated. Three modules (turquoise, pink and yellow) were found to be significantly associated with several of both the osteoporotic and the atherosclerotic markers (Fig. 3). The turquoise module was significantly associated with the carotid-IMT-related variables *imtav* ($r = 0.16$, p-value $= 2 \times 10^{-10}$) and *imtmax* ($r = 0.16$, p-value $= 1 \times 10^{-9}$). The same module was also significantly associated with all of the pQCT bone measurements, the closest association being with *DTToMC* ($r = 0.24$, p-value $= 2 \times 10^{-20}$). The pink and yellow modules were significantly associated with both bulbus IMT variables *bbav* (pink: $r = 0.10$, p-value $= 7 \times 10^{-5}$, yellow: $r = 0.11$, p-value $= 1 \times 10^{-5}$) and *bbmax* (pink: $r = 0.10$, p-value $= 8 \times 10^{-5}$, yellow: $r = 0.12$, p-value $= 7 \times 10^{-6}$). The same modules were also significantly associated with five of the six pQCT-based subclinical osteoporosis indices. The exact lipid content of the most significant turquoise module is listed in Table S1 and explained under Section 3.6.

3.5. Lipid significance (LS) and module membership (MM)

LS is defined as the correlation between the module's member lipids and the study marker. MM is defined as the correlation between the eigenlipid and the other member lipids. An ideal module is the one where LS and MM are highly correlated suggesting that the lipids that are highly correlated with the biological marker of interest are also the important member of the analysed module [25]. Among the three significant modules (Fig. 4), the joint turquoise module has a highly significant correlation between LS and MM with respect to both subclinical atherosclerotic (*imtav*; $r = 0.66$, p-value $= 1.9 \times 10^{-14}$) and subclinical osteoporotic (*DTToMC*; $r = 0.64$, p-value $= 2 \times 10^{-13}$) markers (Fig. 3). The yellow module has a highly significant correlation between LS and MM only with respect to the subclinical atherosclerotic marker (*bbmax*; $r = 0.49$, p-value $= .00013$), whereas the pink module has no significant correlation between LS and MM with respect to any of the studied markers (data not shown).

3.6. Lipid species distribution in the joint turquoise module for subclinical osteoporotic and atherosclerosis markers

There were 105 lipid species in the joint turquoise module for subclinical markers of osteoporosis and atherosclerosis. The majority of the lipid species belonged to the classes of glycerolipid, glycerophospholipid and sphingolipid (Fig. 5A). The glycerolipid class included 19 diacylglycerol and 41 triacylglycerol (TAG) lipid species (Fig. 5B). The glycerophospholipid class had seven phosphatidylcholine lipid species, and the sphingolipid class was enriched with 20 ceramide species (Fig. 5B).

Table 1
Population characteristics of the Cardiovascular Risk in Young Finns Study cohort. Data are expressed as mean ± SD or percentages.

	Men	Women
Number of subjects	646 (43%)	848 (57%)
Age, years	38 ± 5	38 ± 5
Body mass index, kg/m^2	26.5 ± 3.9	25.1 ± 4.7
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	1.5 ± 0.3
Triglycerides (mmol/l)	1.6 ± 0.9	1.2 ± 0.6
Serum glucose (mmol/l)	5.5 ± 0.6	5.2 ± 0.7
Insulin (IU/l)	9.9 ± 26.3	8.3 ± 8.6
C-reactive protein (mg/l)	1.6 ± 4.7	2.0 ± 3.5
Systolic blood pressure (mmHg)	125.2 13.1	116 ± 13.4
Diastolic blood pressure (mmHg)	78.3 ± 10.9	72.8 ± 10.7
Alcohol consumption, units/day	1.4 ± 1.9	0.6 ± 0.7
Physical activity index (MET h/wk)	20.4 ± 22.2	19.4 ± 20.1
Daily smoking, %	129/641 (20%)	121/843 (14%)
Daily calcium intake (mg)	1371 ± 602	1190 ± 483
Daily vitamin D intake (μg)	8.4 ± 4.5	7.3 ± 3.5
Family risk factor for Coronary Heart Disease (%)	107/646 (16.6%)	140/847 (16.5%)
Participants with osteoporosis (%)	3/641 (0.5%)	8/845 (1%)
Participants with epilepsy (%)	5/624 (0.8%)	7/835 (0.8%)
Participants with Crohn's disease (%)	5/625 (0.8%)	9/836 (1.1%)
Participants with Anorexia (%)	0	8/836 (1%)
Usage of corticosteroids at least once a month (%)	13/624 (2.1%)	54/837 (6.5%)

Table 2
Surrogate markers of both subclinical osteoporosis and subclinical atherosclerosis with their descriptive statistics among the study participants, expressed as mean ± SD.

Description (unit)	Abbreviations	Mean (± SD)
Subclinical atherosclerosis		
Carotid intima-media thickness (average, mm)	*imtav*	0.6 ± 0.1
Carotid intima-media thickness (maximum, mm)	*imtmax*	0.7 ± 0.2
Bulbus intima-media thickness (average, mm)	*bbav*	0.8 ± 0.1
Bulbus intima-media thickness (maximum, mm)	*bbmax*	0.8 ± 0.1
Subclinical osteoporosis		
Total mineral density of the distal radius's trabecular bone (mg/cm^3)	*DRTrD*	224.4 ± 36.1
Total mineral density of the distal tibia's trabecular bone (mg/cm^3)	*DTTrD*	240.3 ± 34.1
Total mineral content of the distal radius (mg)	*DRToMC*	243.6 ± 64.2
Total mineral content in the radial shaft's cortical bone (mg)	*RSCoMC*	214.2 ± 44.9
Total mineral content in the distal tibia (mg)	*DTToMC*	602.1 ± 126.9
Total mineral content in the tibia shaft's cortical bone (mg)	*TSCoMC*	646.4 ± 110.5

3.7. Multivariate analysis of the turquoise module and its constituent lipid species with subclinical markers of osteoporosis and atherosclerosis

In multivariate analysis of variance (adjusted with age and sex), average carotid intima media thickness (*imtav*) for subclinical atherosclerosis and total mineral content in the distal tibia (*DTToMC*) for subclinical osteoporosis were chosen as outcomes because they obtained the maximum correlation and the minimum p-value in a module–trait relationship analysis (Fig. 3). There was a statistically significant joint association between the turquoise eigenlipid and the markers of subclinical osteoporosis and atherosclerosis, F(2, 1489) = 12.50, p-value = 4.1×10^{-6}, Pillais' Trace = 0.01. The turquoise eigenlipid had a statistically significant positive association with both markers (p-value with *imtav*: 2.7×10^{-6} and p-value with *DTToMC*: 0.03) in separate regression analyses.

Multivariate analysis of all the member lipid species in the turquoise module with *imtav* and *DTToMC* as outcomes, identified 37 lipid species that were jointly associated with the markers, with a Bonferroni-adjusted p-value of < 0.05 (Table S1). The three most significant joint biomarkers of both osteoporosis and atherosclerosis were TAG (18:0/18:0/18:1), TAG (18:0/18:1/18:1) and TAG (16:0/18:0/18:1), with adjusted p-values of 8.6×10^{-8}, 3.7×10^{-6}, and 8.5×10^{-6}, respectively.

In separate regression analyses of each member lipid species and *imtav*, 36 out of the 37 lipid species were found to be positively associated, with a Bonferroni-adjusted p-value of < 0.05 (Table S2). Similarly, regression analyses of each lipid species with *DTToMC* were also performed. All the 37 lipid species that were found to be jointly associated with markers of subclinical osteoporosis and atherosclerosis were positively associated with *DTToMC*; 16 of these were nominally significant (p-value < 0.05), but none of the lipid species reached a Bonferroni-adjusted p-value of 0.05 (Table S3).

4. Discussion

To the best of our knowledge, this is the first lipidome-wide system-level association study investigating the joint lipid architecture of surrogate markers of both subclinical osteoporosis and subclinical atherosclerosis. We performed lipidomics analysis to identify modules of lipid species that are significantly and jointly associated with the markers' of both of the studied comorbidities. We identified a shared module that is significantly associated with subclinical markers of both osteoporosis (pQCT bone measurements) and atherosclerosis

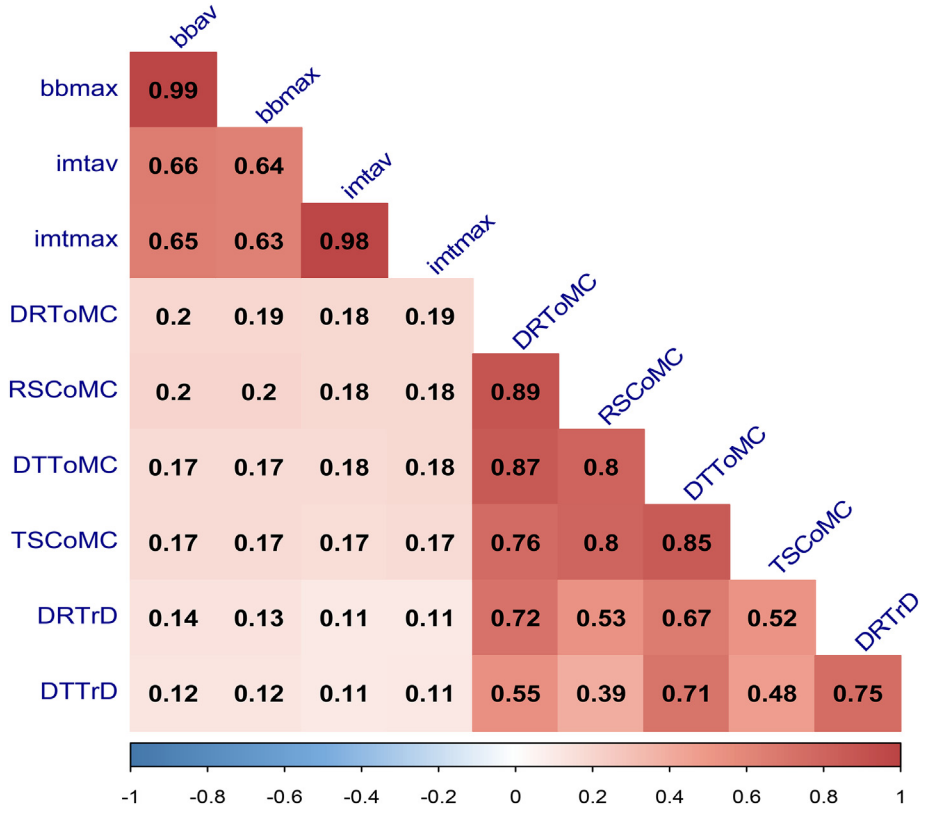

Fig. 2. Pearson's correlation coefficients (r) between surrogate markers of subclinical osteoporosis and atherosclerosis. All correlations are statistically significant (p-value < 0.01). The abbreviations in this figure are explained in Table 2.

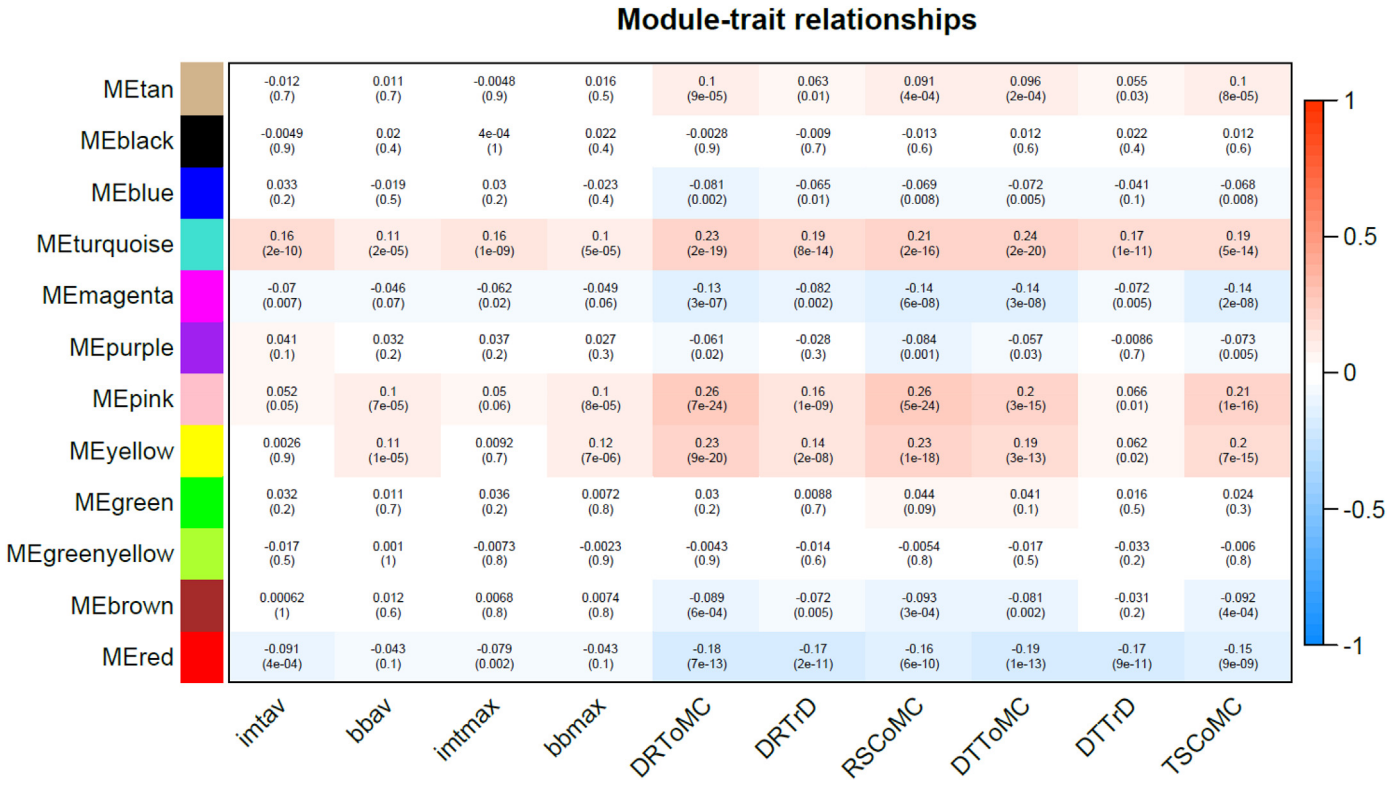

Fig. 3. Module–surrogate marker relationships. The rows correspond to the different modules and their eigenlipids (ME). The columns correspond to the measured subclinical osteoporotic and atherosclerotic markers of the study. The values in the cells represent Pearson's correlation coefficients (r), with the associated p-values in parentheses. The modules are named according to colour and the correlation coefficients have a colour-coding shown in the colour legend (between −1 and +1) on the right side of the figure. The abbreviations for the subclinical osteoporotic and atherosclerotic markers in the column names are explained in Table 2. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

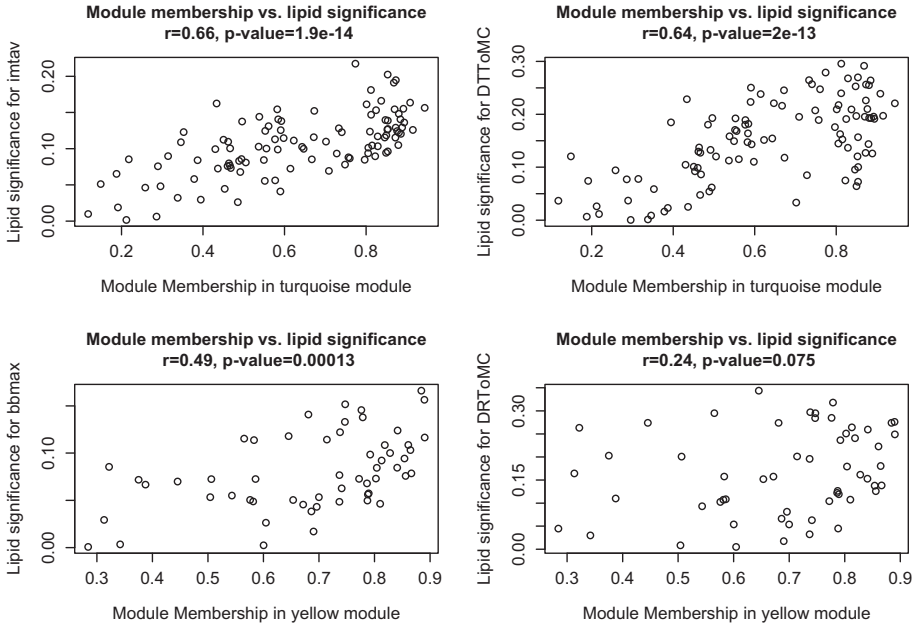

Fig. 4. Scatter plots of lipid significance (LS) vs module membership (MM) in the turquoise and the yellow modules. The left panel corresponds to subclinical atherosclerotic markers and the right panel to subclinical osteoporotic markers. Abbreviations: imtav, carotid intima media thickness (average); bbmax, bulbus intima media thickness (maximum); DTToMC, total mineral content in distal tibia; DRToMC, total mineral content of the distal radius.

(ultrasound carotid IMT).

Whether osteoporosis and atherosclerosis are independent conditions that only share common risk factors, such as aging, or also constitute comorbid conditions with a similar pathophysiological mechanism is an active field of research [19,33]. Several studies have shown an association between decreased bone mass density and increased carotid IMT in different study groups [34–36]. Other studies have suggested an association between osteoporosis and cardiovascular mortality [37–40]. Similarly, one study suggested that defects in bone mineralization and arterial calcification have a similar pathogenesis [41].

In contrast to most of the published findings, we identified weak, but statistically significant positive correlations between surrogate markers of these two diseases. The positive correlations might be due to

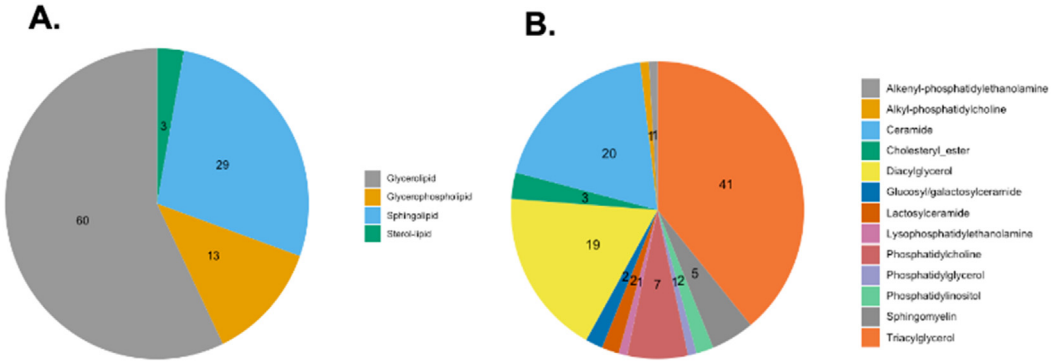

Fig. 5. Distribution of lipids classes (A) and constituent lipids (B) in the joint turquoise module for subclinical markers of osteoporosis and atherosclerosis.

the relatively younger age of the study participants who have not yet developed the clinical manifestations of the diseases. Thus, the positive associations might arise from the shared biological mechanisms between bone and vascular tissue during their normal growth and development. Similar results have been published elsewhere [42]. We speculate that the dynamics of the lipid molecules that are associated with both bone-related and vascular markers change during adverse conditions leading to the comorbidity. Knowledge of the lipid molecules that are associated with the surrogate markers of both the diseases is crucial for identifying alterations in molecular dynamics that take place during the disease. This will not only confirm whether or not the diseases are comorbid but can also potentially improve the risk stratification, prevention, diagnosis and treatment of the diseases.

The majority of the lipid species in the most significant joint module belonged to the glycerolipid, glycerophospholipid and sphingolipid classes. Within glycerophospholipids, one of the phosphatidylcholine lipid species, namely lysophosphatidylcholine (LPC), is a pro-inflammatory lipid that is generated by various pathological activities and is a major component of oxidized low-density lipoprotein (LDL) [43]. Oxidized LDL is known to be a potential factor for the co-occurrence of vascular calcification with the loss of bone mass [44]. Studies have shown that oxidized LDL promotes atherosclerosis via a chemotactic and proliferative mechanism on monocytes by stimulating their adhesion into the endothelial cells and by initiating the formation of foam cells [45]. Oxidized-LDL has also been shown to proliferate and stimulate the migration of smooth muscle cells into the tunica media, which stimulates the production of collagen, thus contributing the fibrous lining in the atherosclerosis plaque [46]. Studies have also suggested that oxidized LDL inhibits osteoblastic differentiation and bone formation and promotes osteoblast cell death [47,48]. A recent study suggested that there is a causal effect of LDL cholesterol on bone mass density [49]. However, clinical findings related to oxidized LDL in the context of cardiovascular diseases have been controversial [50–52].

The identified joint module includes high-risk cardiovascular ceramides among 20 other ceramides, which confirms their previously shown association with cardiovascular outcomes [53,54] and suggests their potential role in subclinical osteoporosis as well. Ceramides are responsible for the activation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) that causes the apoptosis of bone cells [55]. A study has demonstrated an association between ceramides and trabecular bone density in mice [56]. In addition, sphingomyelins have been found to be decreased in the bone tissue of mice with osteoporosis [57]. Ceramides also promote lipoprotein infiltration into the vessel wall by acting as a key signalling molecule [58].

An earlier study has shown a significantly increased level of cholesterol ester in the arterial wall of atherosclerotic lesions [59]. An elevated level of triglycerides is known to be an important biomarker in the development of cardiovascular disease [60]. Lipoproteins that carry triglycerides in the blood stream accumulate in the artery wall intima and are taken up by macrophages to form foam cells that contribute to the build-up of plaque along the walls of artery [61]. The triglyceride metabolism in bone tissue has been shown to diminish in subjects with osteoporosis, when compared with the healthy controls [62]. Furthermore, a study with middle-aged women in Japan revealed that patients with hypertriglyceridemia had reduced bone resorption and were at risk of fractures [63]. Furthermore, among the 37 joint lipid species identified herein by multivariate analysis of variance as being significantly associated with both osteoporosis and atherosclerosis, the top three were triglycerides namely TAG(18:0/18:0/18:1), TAG(18:0/18:1/18:1) and TAG(16:0/18:0/18:1). A previous study has shown an association between TAG(18:0/18:1/18:1) and cardiovascular disease [64]. Furthermore, TAG(16:0/18:0/18:1) has been linked to a faster progression of type 2 diabetes [65] which is a risk factor for both cardiovascular disease [66] and bone fractures [67].

This study is limited to the subclinical phase of atherosclerosis and osteoporosis, as it is based on a relatively young cohort population with very few diagnosed cases of cardiovascular disease and osteoporosis. Therefore, further research on lipidome-wide associations with clinical comorbidities in a case–control setting is crucial. Furthermore, as all of the participants of this study are of Caucasian origin, studies with populations of different ethnicities are needed.

5. Summary and conclusion

Several earlier studies have shown that osteoporosis and atherosclerosis are comorbid conditions, emphasizing that these conditions should be investigated in detail to identify common risk factors and joint molecular mechanisms and to develop common methods for risk stratification, prevention, diagnosis and treatment. In the present study, we identified a lipidome module, with its specific molecular lipids, that was significantly associated with surrogate markers of the subclinical phase of both osteoporosis and atherosclerosis. Alteration in the metabolism of the identified lipid species might contribute to the comorbid conditions and yield new possibilities for their dual-based prevention methods.

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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.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2019.115160.

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