Journal of Glycomics & Lipidomics
ISSN: 2153-0637
OPEN ACCESS Freely available online
Research Article

# Alterations in Lipoprotein Liposomes in Postmenopausal Women with Osteoporosis

**Aimei Shi[1], Kai Su[1], Yu-Che Ou[2,3], Wei-Chung Cheng[4,5], Lumin Chen[1,4], Wen-Jen Lin[5], Yao-Ching Hung[4,5], Wei-Chun Chang[4,5*], Wen-Lung Ma[4,5,6*]**

*[1]Department of Obstetrics and Gynecology, BenQ Medical Center, Nanjing Medical University, Suzhou, Jiangsu Province, China; [2]Department of Obstetrics and Gynecology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan; [3]Department of Obstetrics and Gynecology, Chiayi Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Chiayi, Taiwan; [4]Department of Obstetrics and Gynecology, Sex Hormone Research Center, Research Center for Tumor Medicine, and Chinese Medicine Research and Development Center, China Medical University Hospital, Taichung, Taiwan; [5]Graduate Institute of Biomedical Sciences, Graduate Institution of Cancer Biology, and Graduate Institute of Public Health, China Medical University, Taichung, Taiwan; [6]Department of Nursing, Asia University, Taichung, Taiwan*

## ABSTRACT

Osteoporosis is considered as an aging disease that affects the quality of life. Menopause is an important risk factor for osteoporosis in women because of systemic alterations in metabolism. The changes in the levels of Total Cholesterol (TC), Triglyceride (TG), and lipoproteins in blood are postulated to be associated with osteoporosis. The current study examined systemic lipid profiles in post-menopausal women with and without osteoporosis. BMI (Body Mass Index) and levels of total cholesterol and LDL (Low-Density Lipoprotein) were found to be lower in those with osteoporosis. There was no significant difference in the levels of TG and HDL (High-Density Lipoprotein) among the study subjects. With high throughput lipid profiling technology, the lipidomes of LDL and HDL in 205 postmenopausal women were determined. The LDL lipidomes of patients with osteoporosis showed reduced levels of ceramide, decreased number of hydroxyl groups, and increased number of double bonds, compared to normal individuals. For HDL lipidomes the levels of Lyso Phosphatidyl Ethanolamine (LPE) were increased, and the number of double bonds was decreased in patients with osteoporosis. These results suggest a change in systemic lipid metabolism in post-menopausal women with osteoporosis.

**Keywords:** Menopause; Osteoporosis; Low-Density Lipoprotein; High-Density Lipoprotein; Lipidome; Ceramide; Lyso Phosphatidyl Ethanolamine

## INTRODUCTION

Mobility maintenance is an important aspect of health care in the elderly [1,2]. Adverse health conditions such as Osteoporosis (OP) and Osteoarthritis (OA) greatly affect mobility [3]. OA and OP are also associated with aging, metabolic alterations, and inflammation [4]. OP is a systemic skeletal disease with decreased bone mass and deteriorated microarchitecture of bone leading to increased fragility and risk for fracture [4]. Osteoporosis and fracture are most prevalent in postmenopausal women [5] Aspray and Hill mainly due to the decline in estrogen production [6, 7]. During the perimenopausal period; bone loss is accelerated; and bone micro-architecture is altered in many sites Goltzman [8]. Bone marrow adiposity is one of the important factors that contribute to OP [9]. Bone marrow adipocytes arise from mesenchymal stem cells and share their origin with osteoblasts [10]. The shift in the distributions of bone marrow adipocytes is associated with increased fracture risk in postmenopausal women [11]. Among the systemic alterations in post-menopausal women changes in the levels of adipokines e.g. visfatin have been associated with obesity, insulin resistance, and type II diabetes Franco-Trepat et al. [12] suggesting a role in lipid homeostasis. In 2017; Casado-Diaz et al. found that dietary olive oil extract could stimulate osteoblasogenesis and reduce bone marrow adiposity by reducing lipid oxidation Casado-Diaz et al. [13]. They also found reduced lipid peroxidation and oxidized Low-Density Lipoprotein (LDL) in postmenopausal women. Furthermore; serum from individuals who consumed such extracts was found to increase osteoblastogenesis and reduce adipogenesis *in vitro*. These

**Correspondence to:** Wei-Chun Chang, Department of Medicine, School of Medicine, China Medical University, Taichung, Taiwan, Telephone: +886-975681032; E-mail: wei66@seed.net.tw, Wen-Lung Ma, Department of Medicine, School of Medicine, China Medical University, Taichung, Taiwan, Telephone: +886-975681032; E-mail: maverick@mail.cmu.edu.tw

**Received:** February 03, 2021; **Accepted:** February 19, 2021; **Published:** April 20, 2021

**Citation:** Shi A, Su K, Ou YC, Cheng WC, Chen L, Lin WJ, et al. (2021) Alterations in Lipoprotein Lipidomes in Postmenopausal Women with Osteoporosis, Taiwan. J Glycomics Lipidomics. 10:145.

**Copyright:** © Shi A, et al. (2021) This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

observations suggest that some systemic diseases may be alleviated by controlling lipid metabolism.

Two major lipoproteins; LDL and HDL have been linked to postmenopausal OP. Yamauchi et al. [14] examined approximately 200 patients and found that high serum LDL-cholesterol level is a risk factor for non-vertebral fractures independent of bone turnover, bone mass, vitamin D insufficiency or frail status in postmenopausal women. However; Maghbooli et al. [15] examined approximately 500 patients and found no correlation between LDL levels and postmenopausal OP. On the contrary; Ersoy et al. [16] examined 400 patients and found that total cholesterol and LDL levels were lower in postmenopausal women. In 2018; Maghbooli et al. [15] found that HDL levels is negatively associated with postmenopausal OP. Another study of 15,000 patients revealed that high HDL-cholesterol level is an independent risk factor for bone loss; especially in menopausal women Jiang et al. [17].

These discrepancies in the association between lipoproteins and postmenopausal OP may be due to differences in the expression of LDL and HDL receptors (SR-B1: scavenger receptor-B1, express in liver and vessel endothelium) or genetic polymorphisms in lipoprotein receptor genes [18, 19, 20]. Since lipoproteins are the lipid carriers variations in lipid species in lipoproteins may reflect systemic lipid metabolism. In this study; we used a high throughput lipid profiling technology and determined the species and amounts of lipids associated with LDL and HDL in postmenopausal women with and without OP.

## MATERIALS AND METHEDS

### Clinical samples and data collection

This study was approved by the Internal Review Board (IRB) of both Nanjing Medical University (S2019-05-01). Patient data during the period of 2016-2018 were obtained by chart review. A total of 205 postmenopausal women with (125) or without (80) osteoporosis were included in the study (Table 1 Nanjing Medical University). Data collected included Bone Mineral Density (BMD) and levels of HDL, LDL, cholesterol and triglyceride. The lipidome of lipoproteins patient sample were from Chang-Gong Memorial Hospital cohort (IRB #2016-1075B0).

### LDL and HDL extraction

Extraction of LDL and HDL from serum was performed as previously described [21, 22, 23]. Briefly; serum from 20 ml whole blood was subjected to density gradient centrifugation. Lipoproteins in the density range of 1.019-1.063 (LDL) and 1.063-1.21 (HDL) were collected and dialyzed in a buffer containing 20 mM Tris-HCl and 0.5 mM EDTA (pH 8.0).

### Lipid profiling

The shotgun lipidomic study was performed as described previously [24]. Lipids were extracted from HDL and LDL with methyl tert-butyl ether and methanol [1, 25]. Control samples were spiked with lipid class-specific internal standards prior to extraction. After drying and resuspending in Mass Spectrum (MS) acquisition buffer; lipid extracts were subjected to mass spectrometric analysis. To determine the exact acyl chain (e.g. fatty acid) composition lipid molecules were fragmented in the MS/MS mode. For determination of lipid species; single MS was performed without fragmentation of lipid molecules; and the sums of carbon atoms and double bonds in the hydrocarbon moieties were determined. Lipid species were annotated according to their molecular composition as follows: NAME <sum of all carbon atoms in hydrocarbon moiety>;<sum of all double bonds in hydrocarbon moiety>;<sum of all hydroxyl groups>. For example; PI 34:1; 0 denotes a Phosphatidy Iinositol (PI) with 34 carbon atoms; one double bond; and 0 hydroxyl group. In the case of Sphingolipids; SM 34:1; 2 denotes a Sphingo Myelin (SM) with 34 carbon atoms; 1 double bond; and 2 hydroxyl groups on the Ceramide backbone.

Annotation of lipid subspecies included additional information on the identity of their acyl moieties and their Stereospecific Numbering (sn). For example; PI 18:1; 0_16:0; 0 denotes phosphatidylinositol with octadecenoic (18:1; 0) and hexadecanoic (16:0; 0) fatty acids; the exact position (sn-1 or sn-2) of each of these two fatty acids on the glycerol backbone cannot be discriminated (the underscore "_" separates the two acyl chains). In contrast; PC O18:1;0/16:0;0 denotes an ether-linked Phosphatidyl Choline (PC) with an alkyl chain of 18 carbon atoms and 1 double bond (O-18:1;0). The ether group is linked to the sn-1 position of the glycerol backbone; and a hexadecanoic acid (16:0;0) is connected *via* an ester bond to the sn-2 position of the glycerol backbone (the slash "/" separates the two chains and indicates the snpositions of the two molecules (ether and hexadecanoic acid) on the glycerol backbone).

Identified lipid molecules were quantified by normalization to a lipid-class specific internal standard. The amounts in p-moles of individual lipid molecules (species or of subspecies) of a given lipid class were summed to yield the total amount of the lipid class or normalized to the total lipid amount yielding mol percentage.

For determination of the total double bond index the quantities of the lipid species containing the same number of double bonds were summed and normalized to the total amount of the given lipid class; the values were expressed as mol percentage of the lipid class. To determine the total carbon length index; the quantities of the lipid species containing the same number of carbon atoms in the hydrocarbon moiety were summed and normalized to the total amount of the given lipid class and the values were expressed as mol. percentage of the lipid class. For determination of the total hydroxylation index the quantities of the lipid species containing the same number of hydroxyl groups were summed and normalized to the total amount of the given lipid class; and the values were expressed as mol percentage of the lipid class.Bioinformatic analysis of lipidomes.

**Differential expression:** The differences in HDL and LDL lipid profiles between postmenopausal women without osteoporosis (referred to as non-OP individual) those with postmenopausal osteoporosis (referred to as OP patients) were determined as described previously [26, 27]. Differences between normal and osteoporosis group were analysed by two-tailed Student's t-tests; and a p-values <0.05 was considered significant. All statistical analyses were performed with R on the mol percentage-transformed lipid dataset.

**Hierarchical clustering**: After identifying statistically significant lipid species through differential expression analysis; hierarchical clustering (h-clustering) was performed [28]. Each lipid was scaled to a mean of 0 and a standard deviation of 1 before hierarchical clustering and heatmap plotting. "Kendall" or "binary" distance was applied between each sample; and clustering was achieved by the "average" or "ward" linkage method in the h-clustering function of R.

**Lipid enrichment analysis**: With the gene pathway enrichment

concept; significantly elevated and decreased lipid species of a lipid class were analyzed for double bond or hydroxyl group enrichment [29]. The p value of the Fisher's exact test was calculated for each feature; and a value <0.05 was considered significant.

## RESULTS

The goal of this study was to investigate differences in lipid profiles in postmenopausal women with OP and in those without OP (non-OP). A total of 205 individuals of similar ages (non-OP, 59.7, OP, 60.4) were investigated; including 125 OP and 80 non-OP individuals (Table 1). The mean BMI of non OP was slightly higher than that of OP (41.6 *vs.* 39.5) (Figure 1A, p=0.008). Cholesterol (Figure 1B, p=0.019) and LDL (Figure 1C, p=0.032) levels were also higher in non-OP than in OP groups. Triglyceride (Figure 1D, TG, referring to VLDL level, p=0.083) and HDL (Figure 1E, p=0.572) levels were comparable among the two groups. This data indicating the study population is similar with other cohorts [16].

With this study population; lipidomes of LDL (Figure 2 and Figure 3) and HDL (Figure 4 and Figure 5) of 11 OP (BMD T-score < -2.5) 10 non-OP (BMD T-score >-2.0) individuals were examined. A profound difference in LDL lipidome profiles was observed between non-OP (green) and OP (red) groups (Figure 2). By enrichment analysis; ceramide was found to be the most significantly down regulated (reduced) lipid species; and LPE and PE were the most upregulated (elevated) lipid species in LDL of OP patients (Figure 3A). The overall number of double bond (-C=C-) in the lipids associated with LDL (referred to as LDL lipids) was also increased in OP compared to non-OP groups (Figure 3B). The number of hydroxyl group (-OH) in LDL lipids was decreased in OP group (Figure 3C).

For HDL lipids, Lyso Phosphatidyl Ethanolamine (LPE) was the most significantly upregulated lipid species in OP patients (Figure 5A). The number of double bond in HDL lipids was lower in OP than in non-OP group (Figure 5B). There was no significant difference in the number of hydroxyl group between OP and non-OP groups (Figure 5C).

**Table 1:** Demography of study population.

| | | mean | SD | * p-value |
|---|---|---|---|---|
| patient number | 205 | 59.9 | 2.8 | |
| age | Normal (n=80) | 59.7 | 2.8 | 0.3267 |
| | Osteoporosis (n=125) | 60.4 | 2.7 | |
| BMI | | 40.4 | 5.5 | |
| | Normal (n=80) | 41.6 | 5.4 | 0.0076 |
| | Osteoporosis (n=125) | 39.5 | 5.5 | |
| weight | | 66.1 | 12 | |
| | Normal (n=80) | 66.6 | 7.9 | n.s. |
| | Osteoporosis (n=125) | 65.7 | 14.8 | |
| height | | 160.2 | 8 | |
| | Normal (n=80) | 161.4 | 7.8 | n.s. |
| | Osteoporosis (n=125) | 159.4 | 8 | |
| HDL (mg/dL) | | 1.4 | 0.5 | |
| | Normal (n=80) | 1.5 | 0.6 | 0.572 |
| | Osteoporosis (n=125) | 1.3 | 0.4 | |
| LDL (mg/dL) | | 3.1 | 0.6 | |
| | Normal (n=80) | 3.5 | 1 | 0.003 |
| | Osteoporosis (n=125) | 3.2 | 0.7 | |
| TG (mg/dL) | | 0.8 | 0.1 | |
| | Normal (n=80) | 1 | 0.5 | 0.0828 |
| | Osteoporosis (n=125) | 1.4 | 0.5 | |
| Cholesterol (mg/dL | | 5.8 | 0.7 | |
| | Normal (n=80) | 5.5 | 1 | 0.0193 |
| | Osteoporosis (n=125) | 4.8 | 1 | |

*unpaired t-test comparing OP *vs.* non-OP.

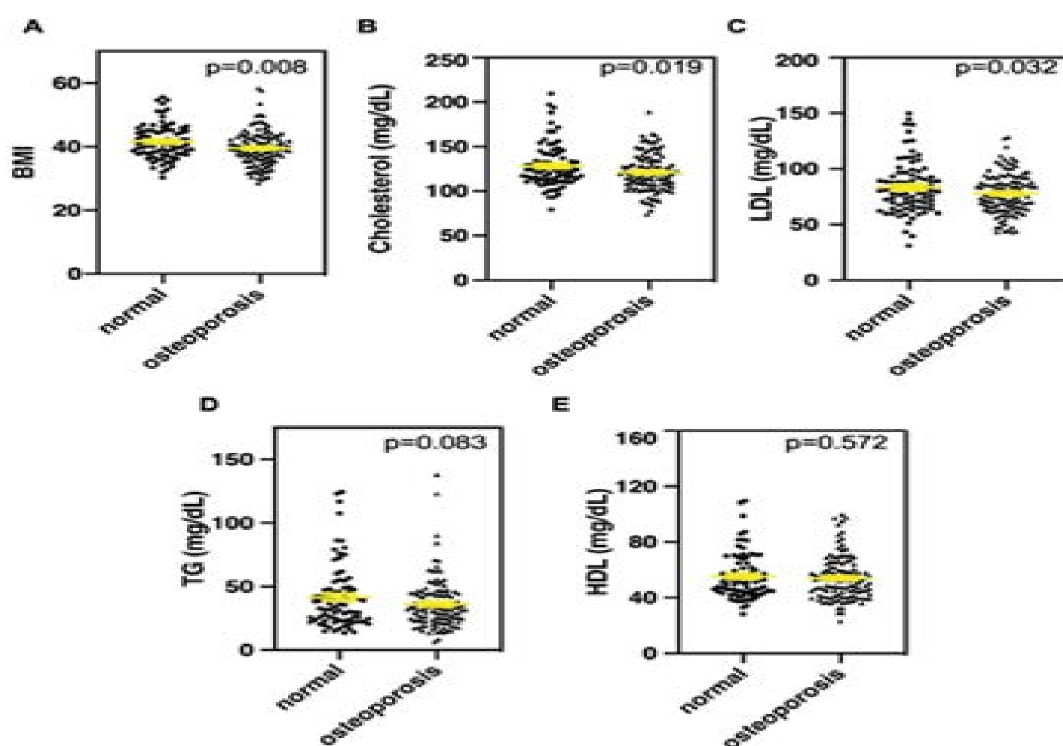


**Figure 1:** Parameters associated with post-menopausal OP patients; A): Body Mass Index (BMI); B): Cholesterol levels (mg/dL). C): LDL levels (mg/dL). D): Triglyceride (TG) levels (mg/dL). E): HDL levels (mg/dL).

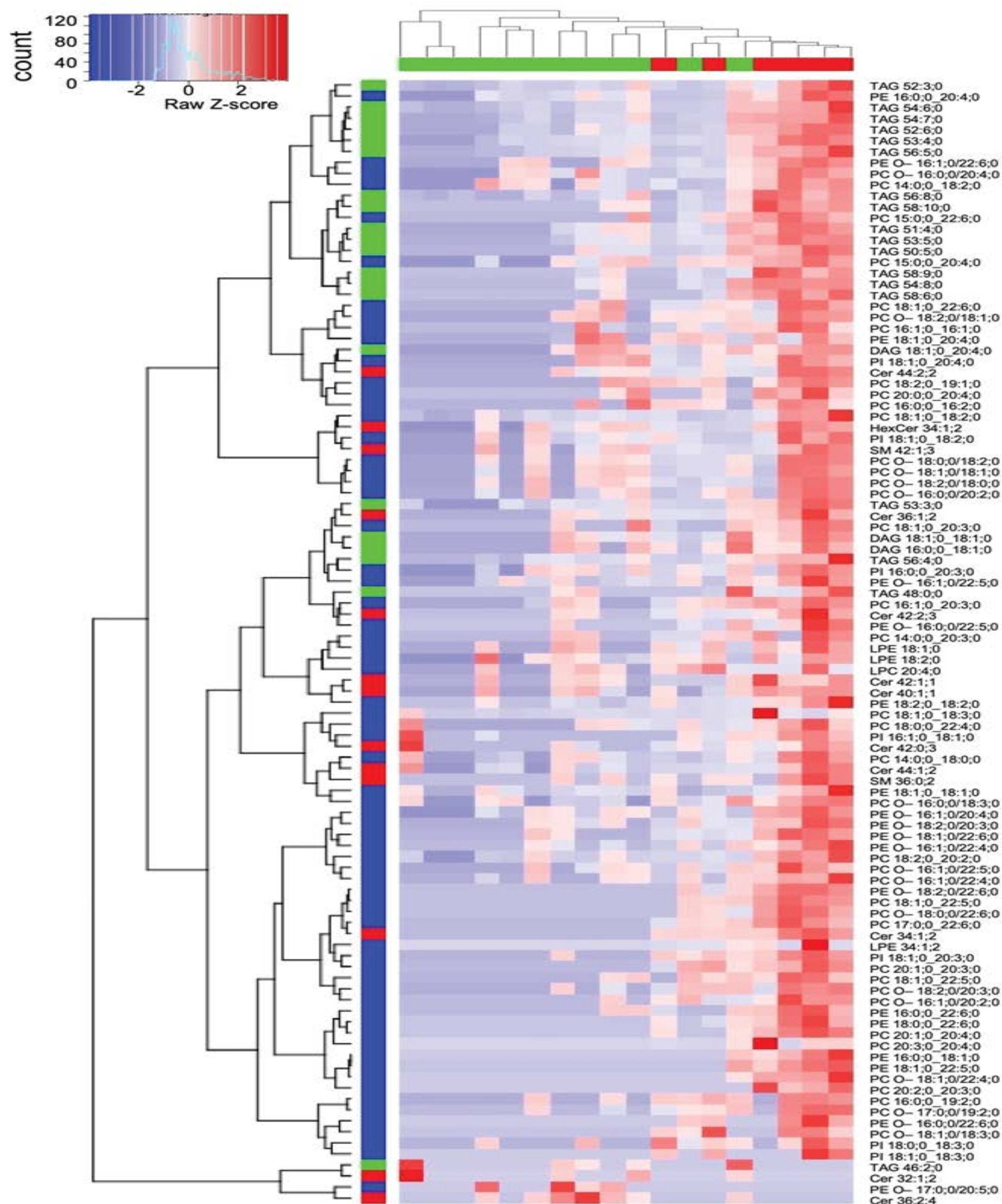


**Figure 2:** Hierarchical lipidome profiles of LDL samples from post- menopausal OP and non-OP individuals. Heatmap representing levels of various lipid species in LDL samples from OP patients (red color bar on top of the heatmap) and non-OP individuals (green color bar on top of the heatmap) is shown. The blue (lower level) and red color (lower level) intensity spectrum represents fold change in the amount of each lipid species compared to the average normal level.

## DISCUSSION

In this study; we examined lipid profiles in sera from postmenopausal OP and non-OP patients. The levels of cholesterol and LDL were found to be significantly lower in OP than in non-OP individuals; but those of HDL and TG were comparable between the two groups. Lipidome examination revealed a difference in lipid species, e.g. decreased levels of ceramide in LDL and increased levels of Lyso Phosphatidyl Ethanolamine (LPE) in HDL in patients with OP. The number of double bonds was increased in LDL lipids; but was decreased in HDL lipids in patients with OP. The number of hydroxyl groups was decreased in LDL lipids; but was not changed in HDL lipids in patients with OP. These results suggest that these alterations can be a biomarker for OP. A recent study using metabolome profiling technology in ovariectomyinduced osteoporosis mouse model shown that lipid metabolism in bone was profoundly been alterated [30]. Among the lipids, fatty acyls, glycerolipids, glycerophospholipids, sphingolipids and sterols showed robust changes. Lipids metabolic disorders caused by changes in the hormone levels in OVX are closely related to the imbalance between bone resorption and formation and may underlie the development of post-menopausal osteoporosis. In this study; we found that the loading context of lipid species and the lipid chemical properties in LDL and HDL, e.g. double bond or hydroxyl group; are biomarkers for postmenopausal OP. Considering the physiological roles and the metabolism of LDL and HDL in the body the alternation of lipoprotein lipid loading context could be a trajectory of systemic metabolic status. We would like to discuss our study in the follow perspectives.

**Systemic lipid metabolism vs. lipoprotein loading context: Cause or result?** Bone is considered as an endocrine organ that can affect the metabolism of the whole body. Osteoblasts express receptors and catabolic enzymes to take up and metabolize fatty acids

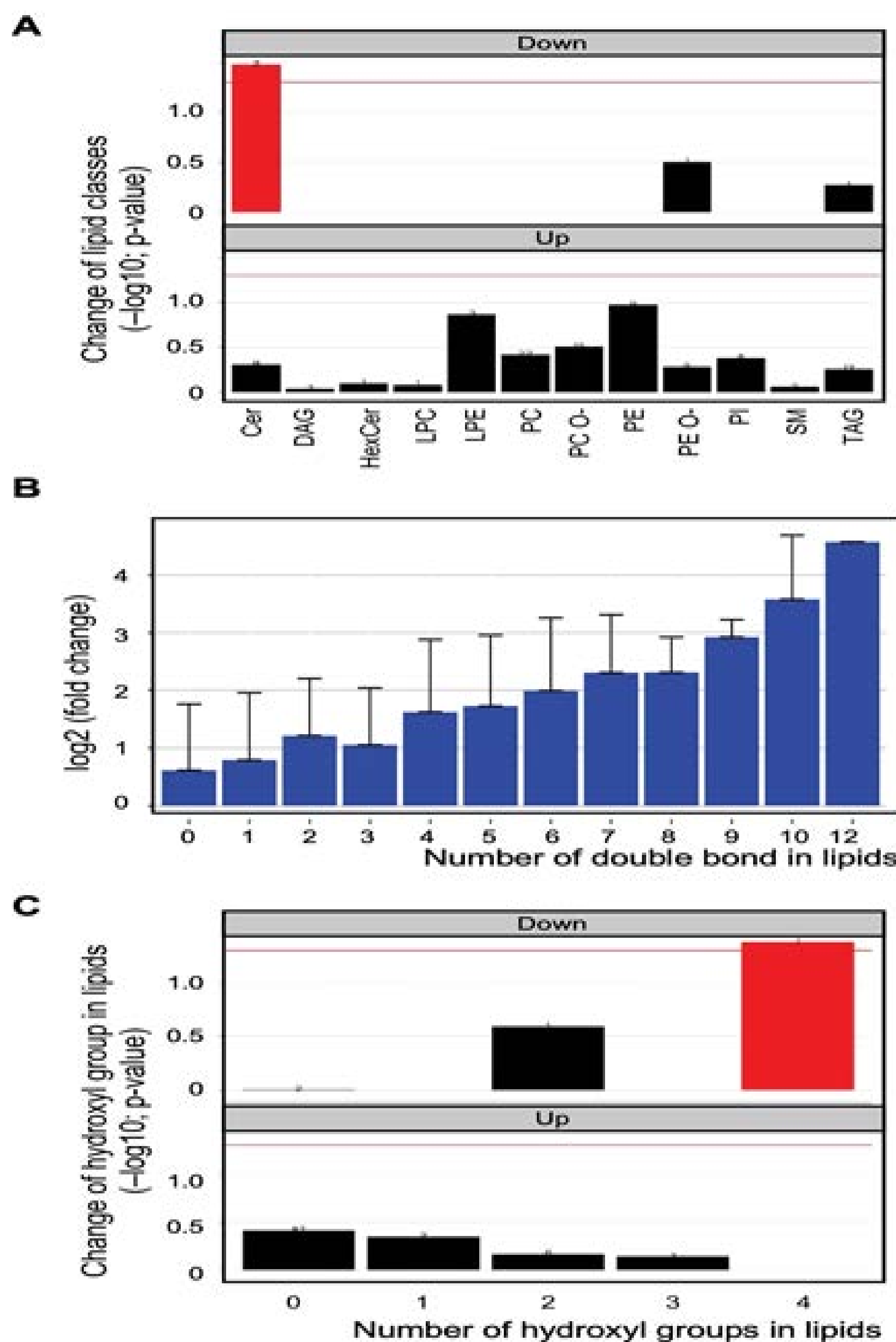


**Figure 3:** LDL lipidome bioinformatics; A): Enriched lipid classes included Cerceramide, DAG: Diacylglycerol, HexCer: Hexosyceramide, LPC: Lyso-Phosphatidylcholine, LPE: Lyso-Phosphatidylethanolamine, PC: Phosphatidylcholine, PC O-: ether-link Phosphatidylcholine. PE: Phatidylethanolamine, PE O-: ether-link Phatidylethanolamine, PI: Phosphatidylinositol, SM: Sphingomyelin, TAG: Triacylglycerol. The upper panel shows the LDL lipid classes that were downregulated; and the lower panel shows showing the LDL lipid classes those that were upregulated in OP patients compared to non-OP individuals. Y-axis indicates-log10 (p-value). Red colored bar indicates significant alteration; B): Double bond number in on LDL lipids of OP patients divided by that of non-OP individual. Y-axis indicates fold change in the number of double bonds as log2 values; C): Number of hydroxyl group number in LDL lipids of OP patients divided by that of non-OP individuals. The red line represents the significant level of change as – log10 (p-value). Red colored bars indicate significant alteration.

Kushwaha et al. [31] Polymorphisms in the genes encoding LDL-Related Proteins (LRPs) are frequently observed. For example; a retrospective study of approximately 500 postmenopausal OP patients on the polymorphism of the LRP5 gene revealed the association of A1330V mutation (rs3736228) with decreased BMD at three skeletal sites [32]. Another study of 100 Mexican postmenopausal OP patients examined LRP5 SNP and found a positive correlation between V667M (rs4988321) variation and BMD. Agued, et al. [33] In addition to genetic variations of LRPs; the expression of LDL receptors (LDLR) is also related to bone homeostasis. Using LDLR knockout mice Okayasu, et al. [34] found that LDLR deletion results in a suppression in osteoblastic differentiation but an enhancement in the differentiation of as to osteoclast Okayasu et al. also found that LDLR deletion in osteoclast resulting in increased bone resorption. These observations are consistent with our finding that LDL lipid species instead of LDL amount; are associated with postmenopausal OP.

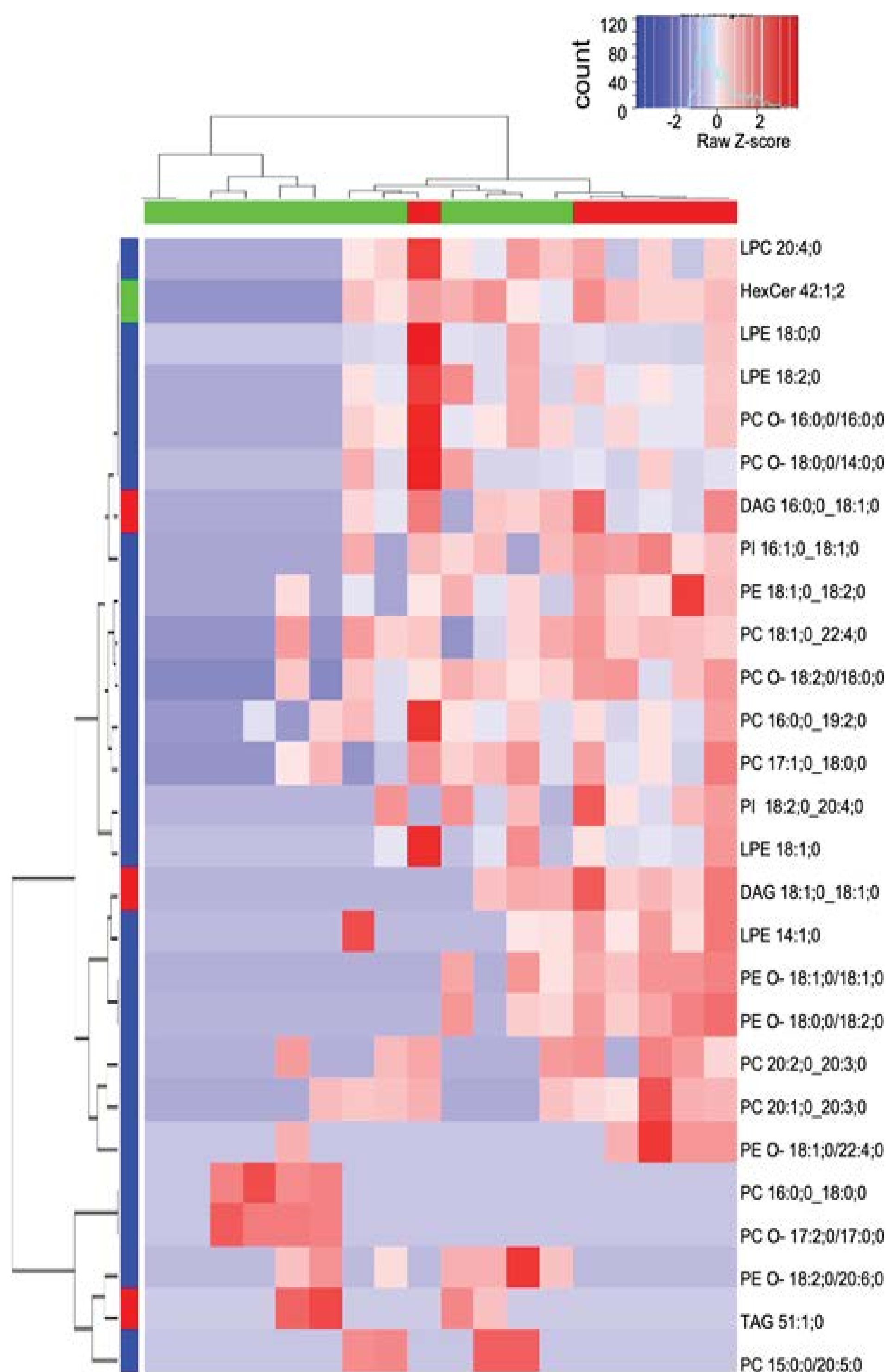


**Figure 4:** Hierarchical lipidome profiles of HDL samples from post- menopausal OP and non-OP individuals. Heatmap representing levels of various lipid species in HDL samples from OP (red color bar on top of the heatmap) and non-OP individuals (green color bar on top of the heatmap) is shown. The blue (lower level) and red color (lower level) intensity represents fold change in quantity of each lipid species compared to the average normal level.

HDL and bone pathophysiology are highly associated. Reduced HDL levels have been associated with the inflammatory microenvironment that affects the differentiation and function of osteoblasts [35]. Perturbation in HDL metabolism favors adipocyte differentiation and restrains osteoblastic differentiation through the modification of bone-related chemokines. HDL compositions are quite variable and may contain 45 percentage-55 percentage apoproteins, 26 percentage-32 percentage phospholipids, 15 percentage-20 percentage esterified cholesterol, 3 percentage-5 percentage free cholesterol and approximately 5 percentage triglycerides Tsompanidi et al. [36]. It is speculated that the protein content in HDL may affect its lipid loading ability [37]. The major protein component of HDL has been shown to vary among APOA1 (related to the biogenesis and functions of HDL [38]. Apolipoprotein E (APOE) Kypreos and Zannis [39] and Apolipoprotein CIII (APOCIII) Kypreos [40]. The structure and function of APOA1-HDL are different from those of APOE-HDL [41]. This provide important insight that the discrepancies of correlation of HDL amount to postmenopausal OP. Our data suggested that variations in HDL lipids, e.g. high LPE and low double bond could be a marker for postmenopausal OP.

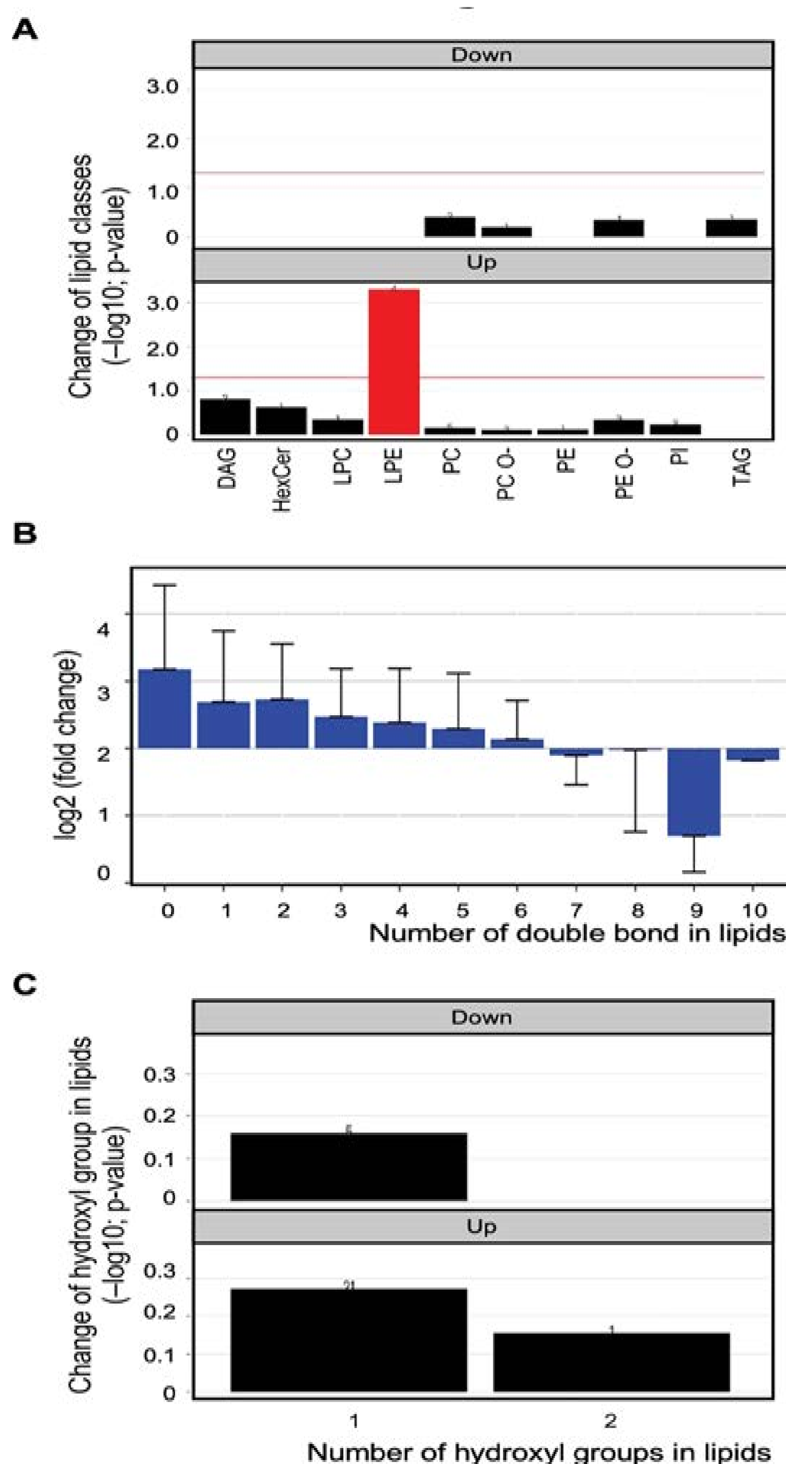


**Figure 5:** HDL lipidome bioinformatics; A): Enriched lipid classes included DAG: diacylglycerol, HexCer: hexosyceramide, LPC: lyso- phosphatidylcholine, LPE: lyso-phosphatidylethanolamine, PC: phosphatidylcholine, PC O-: ether-link phosphatidylcholine, PE: phatidylethanolamine, PE O-: ether-link phatidylethanolamine, PI: phosphatidylinositol, TAG: triacylglycerol. The upper panel shows the HDL lipid classes that were downregulated, and the lower panel shows those that were upregulated in OP patients compared to non-OP individuals. Y-axis indicates significance level of changes in lipid quantity as -log10 (p-value). Red colored bar indicates significant alteration. B. Double bond number in on HDL lipids of OP patients divided by that of non-OP individual. Y-axis indicates fold change in the number of double bond as log2 values. C. Number of hydroxyl group in HDL lipids of OP patients divided by that of non- OP individuals. The red line represents the significant level of change as -log10 (p-value). Red colored bars indicate significant alteration.

### Significance of loading oxidized lipid in LDL and HDL particle

LDL is the most abundant lipoprotein in the body. It carries cholesterol and long chain fatty acids that reflect systemic lipid metabolic status. LDL is produced in multiple organs, e.g., liver, muscle, adipocyte, and bone cells. HDL is the second most abundant lipoprotein that carries cholesterol or phospholipids to the liver. Its receptor is SR-B1 which is not known to be expressed in bone cells. During the menopausal period estrogen levels are greatly decreased leading to increased oxidative stress Manolagas et al. [42] and risk of atherosclerosis and OP. It has been shown that Lyso Phosphatidyl Choline (LPC), which is the major phospholipid component of LDL could increase cytotoxicity in rodent osteoblast cells [43]. The peroxisome Proliferator-Activated Receptor gamma (PPARg) which is responsible for sensing lipid oxidation in bone

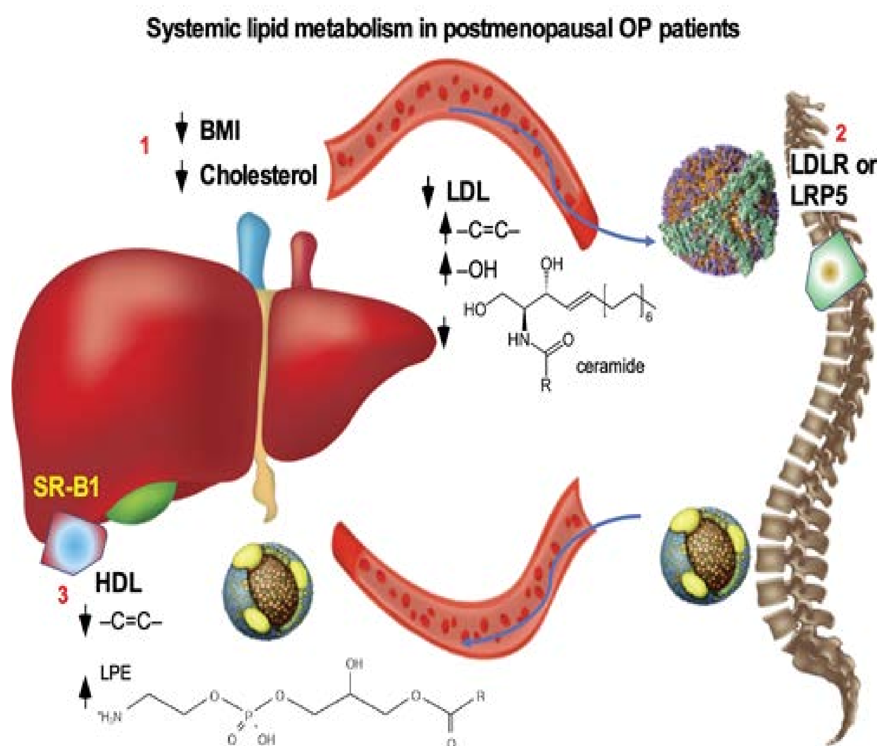


**Figure 6:** Hypothetical model of systemic lipid metabolism. BMI and serum cholesterol levels were decreased in postmenopausal OP. Total LDL levels were decreased and characteristics of LDL lipids were also changed including increased number of double bonds and hydroxyl groups and decreased ceramide levels. Changes in the expression of LDLR or LRP5 genetic variations might contribute to bone homeostasis or micro-architecture remodeling. The lipid oxidative stress clearance lipoprotein HDL was also altered with decreased number of double bond and increased LPE levels. These changes may be related to the pathogenesis of postmenopausal OP.

cells could contribute to menopausal menopause-associated bone decrease.

Low BMD is related to atherosclerosis in postmenopausal women Hmamouchi et al. [44]. Whether HDL is associated with low LDL and cholesterol levels leading to postmenopausal OP is debatable. It has been shown that phospholipid oxidation indicated by increased number of hydroxyl group or double bond in lipids can affect pro-inflammatory cytokine production in bone cells Tseng et al. [45] this may explain the high comorbidity of atherosclerosis and OP. Based on our data and those in the literatures; we proposed a hypothetical model to explain the relationship between lipid metabolism and OP (Figure 6). In postmenopausal OP patients; systemic lipid metabolism is altered; leading to decreased BMI and levels of circulating cholesterol and LDL. Alterations in lipids associated with LDL also occurred; including increased number of double bonds and hydroxyl groups in and decreased levels of ceramide. The variation in the levels of LDLR and/or genetic polymorphism of LRP5 may affect the uptake of LDL. The oxidation of lipid or HDL results in reduced number of double-bonded lipids and increased levels of LPE which is proinflammatory and thus increasing the risk of OP in postmenopausal women.

In conclusion; we performed lipidome analyses of LDL and HDL in patients with postmenopausal OP. The discovery in this study provide a possibility of using alterations in LDL and HDL lipid profiles as a biomarker for pathophysiological insight to the pathogenesis and a value to develop a bio signature of postmenopausal OP.

## ACKNOWLEDGEMENTS

We thank Lipotype GmbH (Dresden, Germany) and Taiwan-Bio Active Lipid Ltd. Co. (Taiwan) for technical support in lipoprotein lipidome profiling and bioinformatics analyses.

## FUNDING STATEMENT

This work was supported by the Taiwan Ministry of Science and Technology (MOST108-2314-B-039-043-MY3, MOST108-2320-B-039-017, MOST108-2314-B-039-042-MY3 and MOST106-2221-E-039-011-MY3); the National Health Research Institute (NHRI-EX109-10705BI) and China Medical University/Hospital (CMU107-S-05, CMU108-MF-33,DMR-108-079, DMR-108-080, DMR-CELL-1806, DMR-109-086, DMR-109- 084).

## AVAILABILITY OF DATA AND MATERIALS

All datasets are available from the corresponding author upon request.

## REFERENCES

1. Stanghelle B, Bentzen H, Giangregorio L, Pripp AH, Bergland A. Associations between health-related quality of life, physical function and pain in older women with osteoporosis and vertebral fracture. BMC Geriatr 2019;19:298.

2. Greco EA, Pietschmann P, Migliaccio S. Osteoporosis and sarcopenia increase frailty syndrome in the elderly. Frontiers in endocrinology. Front Endocrinol (Lausanne) 2019;10: 255.

3. Ebeling PR, Cicuttini F, Scott D, Jones G. Promoting mobility and healthy aging in men: a narrative review. Osteoporos Int;. 2019;30:1911-1922.

4. García-Gómez MC, Vilahur G. Osteoporosis and vascular calcification: A shared scenario. Clin Investig Arterioscler. 2020;32:32-41.

5. Aspray TJ, Hill TR. Osteoporosis and the ageing skeleton. Biochem. and Cell Biol. of Ageing. 2019:453-476.

6. Li L, Wang Z. Ovarian aging and osteoporosis. Adv Exp Med Biol 2018;199-215.

7. Cannarella R, Barbagallo F, Condorelli RA, Aversa A, La Vignera S, Calogero AE. Osteoporosis from an endocrine perspective: The role of hormonal changes in the elderly. J Clin Med. 2019;8:1564.

8. D. Goltzman. The Aging Skeleton. Adv Exp Med Biol. 2019;153-160.

9. Woods GN, Ewing SK, Sigurdsson S, Kado DM, Eiriksdottir G, Gudnason V, et al. Greater bone marrow adiposity predicts bone loss in older women. J Bone Miner Res. 2020;35:326-332.

10. Bolamperti S, Signo M, Spinello A, Moro G, Fraschini G, Guidobono F, et al. GH prevents adipogenic differentiation of mesenchymal stromal stem cells derived from human trabecular bone *via* canonical Wnt signaling. Bone. 2018;112:136-144.

11. Stringer B, Waddington R, Sloan A, Houghton A, Stone M, Russell G, et al. Serum from postmenopausal women directs differentiation of human clonal osteoprogenitor cells from an osteoblastic toward an adipocytic phenotype. Calcif Tissue Int 2007 Apr;80:233-243.

12. Franco-Trepat E, Guillán-Fresco M, Alonso-Pérez A, Jorge-Mora A, Francisco V, Gualillo O, Gómez R. Visfatin connection: present and future in osteoarthritis and osteoporosis. J Clin Med. 2019 ;8:1178.

13. Casado-Díaz A, Túnez-Fiñana I, Mata-Granados JM, Ruiz-Méndez MV, Dorado G, Romero-Sánchez MC, et al. Serum from postmenopausal women treated with a by-product of olive-oil extraction process stimulates osteoblastogenesis and inhibits adipogenesis in human mesenchymal stem-cells (MSC). Exp Gerontol. 2017;90:71-78.

14. Yamauchi M, Yamaguchi T, Nawata K, Tanaka KI, Takaoka S, Sugimoto T. Increased low-density lipoprotein cholesterol level is associated with non-vertebral fractures in postmenopausal women. Endocrine. 2015 ;48:279-286.

15. Maghbooli Z, Khorrami-Nezhad L, Adabi E, Ramezani M, Asadollahpour E, Razi F, et al. Negative correlation of high-density lipoprotein-cholesterol and bone mineral density in postmenopausal Iranian women with vitamin D deficiency. Menopause. 2018;25:458-464.

16. Ersoy GS, Simsek EE, Vatansever D, Kasikci HO, Keser B, Sakin O. Lipid profile and plasma atherogenic index in postmenopausal osteoporosis. North Clin Istanb.2017;4:237.

17. Jiang J, Qiu P, Wang Y, Zhao C, Fan S, Lin X. Association between serum high-density lipoprotein cholesterol and bone health in the general population: a large and multicenter study. Arch Osteoporos. 2019;14:36.

18. Méndez JP, Rojano-Mejía D, Coral-Vázquez RM, Coronel A, Pedraza J, Casas MJ, et al. Impact of genetic variants of IL-6, IL6R, LRP5, ESR1 and SP7 genes on bone mineral density in postmenopausal Mexican-Mestizo women with obesity. Gene. 2013;528:216-20.

19. Horváth P, Balla B, Kósa JP, Tóbiás B, Szili B, Kirschner G, Győri G, Kató K, Lakatos P, Takács I. Strong effect of SNP rs4988300 of the LRP5 gene on bone phenotype of Caucasian postmenopausal women. J Bone Miner Metab. 2016;34:79-85.

20. Shen WJ, Asthana S, Kraemer FB, Azhar S. Scavenger receptor B type 1: expression, molecular regulation, and cholesterol transport function. J Lipid Res. 2018;59:1114-1131.

21. Seidel D, Alaupovic P, Furman RH. A lipoprotein characterizing obstructive jaundice. I. Method for quantitative separation and identification of lipoproteins in jaundiced subjects. J Clin Invest. 1969;48:1211-1223.

22. Chen CH, Jiang T, Yang JH, Jiang W, Lu J, Marathe GK, Pownall HJ, Ballantyne CM, et al. Low-density lipoprotein in hypercholesterolemic human plasma induces vascular endothelial cell apoptosis by inhibiting fibroblast growth factor 2 transcription. Circulation. 2003;107:2102-1208.

23. Shiou YL, Lin HT, Ke LY, Wu BN, Shin SJ, Chen CH, Tsai WC, Chu CS, Lee HC. Very low-density lipoproteins of metabolic syndrome modulates STIM1, suppresses store-operated calcium entry, and deranges Myofilament proteins in atrial Myocytes. J Clin Med. 2019;8:881.

24. Surma MA, Herzog R, Vasilj A, Klose C, Christinat N, Morin-Rivron et al. An automated shotgun lipidomics platform for high throughput, comprehensive, and quantitative analysis of blood plasma intact lipids. Eur J Lipid Sci Technol. 2015;117:1540-1549.

25. Klose C, Surma MA, Simons K. Organellar lipidomics-background and perspectives. Curr Opin Cell Biol. 2013;25:406-413.

26. Chew WS, Torta F, Ji S, Choi H, Begum H, Sim X, et al. Large-scale lipidomics identifies associations between plasma sphingolipids and T2DM incidence. JCI insight. 2019;4(13).

27. Grzybek M, Palladini A, Alexaki VI, Surma MA, Simons K, Chavakis T, et al. Comprehensive and quantitative analysis of white and brown adipose tissue by shotgun lipidomics. Mol Metab.2019;22:12-20.

28. Herrero J, Valencia A, Dopazo J. A hierarchical unsupervised growing neural network for clustering gene expression patterns. Bioinformatics. 2001;17:126-136.

29. Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 2009;37:1-3.

30. Zhao H, Li X, Zhang D, Chen H, Chao Y, Wu K, et al. Integrative bone metabolomics—lipidomics strategy for pathological mechanism of postmenopausal osteoporosis mouse model. Sci Rep. 2018;8: 16456.

31. Kushwaha P, Wolfgang MJ, Riddle RC. Fatty acid metabolism by the osteoblast. Bone. 2018;115:8-14.

32. Canto-Cetina T, Polanco Reyes L, González Herrera L, Rojano-Mejía D, Coral-Vázquez RM, Coronel A, et al. Polymorphism of LRP5, but not of TNFRSF11B, is associated with a decrease in bone mineral density in postmenopausal maya-mestizo women. Am J Hum Biol. 2013;25:713-718.

33. Agueda L, Bustamante M, Jurado S, Garcia-Giralt N, Ciria M, Saló G, et al. A haplotype-based analysis of the LRP5 gene in relation to osteoporosis phenotypes in Spanish postmenopausal women. J Bone Miner Res. 2008;23:1954-1963.

34. Okayasu M, Nakayachi M, Hayashida C, Ito J, Kaneda T, Masuhara M, et al. Low-density lipoprotein receptor deficiency causes impaired osteoclastogenesis and increased bone mass in mice because of defect in osteoclastic cell-cell fusion. J Biol Chem. 2012; 287: 19229-19241.

35. Papachristou NI, Blair HC, Kypreos KE, Papachristou DJ. High-density lipoprotein (HDL) metabolism and bone mass. J Endocrinol. 2017;233: R95-R107.

36. Tsompanidi EM, Brinkmeier MS, Fotiadou EH, Giakoumi SM, Kypreos KE. HDL biogenesis and functions: role of HDL quality and quantity in atherosclerosis. Atherosclerosis. 2010;208:3-9.

37. Zakiev E, Rached F, Lhomme M, Darabi-Amin M, Ponnaiah M, Becker PH, et al. Distinct phospholipid and sphingolipid species are linked to altered HDL function in apolipoprotein AI deficiency. J Clin Lipidol. 2019;13:468-480.

38. Georgiadou D, Chroni A, Vezeridis A, Zannis VI, Stratikos E. Biophysical analysis of apolipoprotein E3 variants linked with development of type III hyperlipoproteinemia. PLoS One. 2011;6:e27037.

39. Kypreos KE, Zannis VI. Pathway of biogenesis of apolipoprotein E-containing HDL in vivo with the participation of ABCA1 and LCAT. Biochemical J. 2007;403:359-367.

40. Kypreos KE. ABCA1 promotes the de novo biogenesis of apolipoprotein CIII-containing HDL particles in vivo and modulates the severity of apolipoprotein CIII-induced hypertriglyceridemia. Biochemistry. 2008;47:10491-10502.

41. Filou S, Lhomme M, Karavia EA, Kalogeropoulou C, Theodoropoulos V, Zvintzou E, et al. Distinct roles of apolipoproteins A1 and E in the modulation of high-density lipoprotein composition and function. Biochemistry. 2016;55:3752-62.

42. Manolagas SC. From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis. Endocr Rev. 2010;31:266-300.

43. Fallah A, Pierre R, Abed E, Moreau R. Lysophosphatidylcholine-induced cytotoxicity in osteoblast-like MG-63 cells: Involvement of transient receptor potential vanilloid 2 (TRPV2) channels. Mol Membr Biol. 2013;30:315-326.

44. Hmamouchi I, Allali F, Khazzani H, Bennani L, Mansouri LE, Ichchou L, et al. Low bone mineral density is related to atherosclerosis in postmenopausal Moroccan women. BMC Public Health. 2009;9:388.

45. Tseng W, Lu J, Bishop GA, Watson AD, Sage AP, Demer L, Tintut Y. Regulation of interleukin-6 expression in osteoblasts by oxidized phospholipids. J Lipid Res. 2010;51:1010-1016.