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Osteoarthritis

Lipidomics unravels lipid changes in osteoarthritis articular cartilage

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ARTICLE INFO

ABSTRACT

Objectives: Osteoarthritis (OA) is linked to disrupted lipid metabolism. We aimed to profile the lipid composition of human articular cartilage, investigate OA-associated lipidome changes, and explore biological effects.

Methods: Lipidomic profiling and computational analyses were performed on human articular chondrocytes (hACs) from non-OA (n = 13) and OA (n = 14) hips. Lipid changes were confirmed in the destabilisation of the medial meniscus (DMM) mouse model. The effect of specific lipids was evaluated by *in vitro* supplementation and gene silencing.

Results: We identified 573 lipid species covering 11 lipid classes in hACs. OA and non-OA hACs showed distinct lipid profiles. Most ceramides and dihydroceramides were increased, while cholesteryl esters, diacylglycerols, triacylglycerols, sphingomyelins, hexosylceramides, and lactosylceramides were predominantly decreased in OA chondrocytes. Most upregulated lipids in OA contained C18:1, C20:4, or C22:4 side chains. Many downregulated lipids contained C18:2 or odd-chain C17:0. Lipid profiling of articular cartilage from the DMM mouse model paralleled changes in OA hACs, including odd-chain C17:0 reduction. Further analysis showed that deficiency in enzyme 2-hydroxyacyl-CoA lyase 1 (HACL1), responsible for odd-chain fatty acid synthesis, leads to accumulation of 2-hydroxy C18:0, precursor of C17:0, which results in a shift in hACs from an anabolic to a catabolic state.

Conclusions: Our study maps the hAC lipid composition and highlights changes in lipid profiles associated with OA. Dysregulation of certain lipids, especially odd-chain fatty acids, linked to a deficiency in the enzyme HACL1, leads to pathological changes. This understanding opens potential avenues for therapies aimed at targeting lipid imbalances to slow down or treat OA.

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WHAT IS ALREADY KNOWN ON THIS TOPIC

- Osteoarthritis (OA) is a widespread, yet uncurable, joint disease characterised by cartilage degradation.
- Several risk factors for OA, such as ageing, obesity, and metabolic syndrome, are strongly associated with disrupted lipid metabolism. Prior proteomic analyses and gene expression studies in cartilage of OA patients have revealed dysregulations in lipid-related pathways. However, the lipidome of articular cartilage has not been systematically characterised, and how the lipid profile changes in OA remains poorly defined.

WHAT THIS STUDY ADDS

- We mapped the lipid profile of human articular cartilage and identified OA-associated lipid changes. We found that specific lipid alterations in classes, species, and side chain composition are associated with human OA.
- We validated our findings in a mouse model of OA. Our data indicate that lipidome changes in articular cartilage from osteoarthritic mice largely mirror the changes observed in human osteoarthritic cartilage.
- Our detailed fatty acid analysis highlighted a marked reduction in odd-chain fatty acid C17:0 associated with OA. We identified that deficiency in the enzyme 2-hydroxyacyl-CoA lyase 1 (HACL1), responsible for odd-chain fatty acid synthesis, leads to accumulation of 2-hydroxy C18:0, precursor of C17:0, causing detrimental effects on chondrocytes.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Our study provides a detailed resource on the lipidome of articular cartilage and its changes in OA.
- HACL1-mediated dysregulation of odd-chain fatty acids is linked to pathological changes in human articular chondrocytes, suggesting opportunities for therapeutic targeting.

INTRODUCTION

Osteoarthritis (OA), the most common chronic joint disease, affects over 500 million people globally, emerging as a leading cause of disability and source of persistent pain, thereby imposing a substantial socioeconomic burden [1]. Current treatment strategies for OA are very limited, offering only relief of symptoms and, in severe cases, requiring joint replacement surgery. Disease-modifying or curative approaches are entirely lacking, underscoring an urgent medical need. OA is primarily characterised by progressive degradation of the articular cartilage, often accompanied by increased joint-associated bone remodelling and varying degrees of inflammation [2]. The articular cartilage, crucial for normal joint mobility, consists of articular chondrocytes embedded within a self-produced extracellular matrix (ECM) mainly composed of type 2 collagen fibres and proteoglycan aggrecan [3]. In OA, molecular alterations in chondrocytes lead to the synthesis of an ECM of inferior biomechanical quality and increased production of tissue-destructive enzymes, together exacerbating tissue damage and contributing to functional impairment [3].

OA is a complex multifactorial disease. Several important risk factors for OA such as ageing, obesity, and metabolic syndrome are strongly associated with dysregulations in lipid metabolism [4]. In line with this, proteomic analyses [5,6] and gene expression studies [7,8] in cartilage of OA patients have revealed dysregulations in lipid-related pathways [5–8]. Despite these accumulating observations that suggest an important role for lipids in OA, the composition of the chondrocyte's lipidome remains poorly defined, and the specific lipid changes within OA cartilage are largely unknown.

Lipids represent a diverse array of water-insoluble molecules such as fatty acids, triacylglycerols (TAG), phospholipids, and cholesterols, playing pivotal roles as constituents of cellular membranes, energy sources, and signalling molecules [9]. Despite these important biological functions, lipid research has not attained the same level of scientific attention as studies focusing on proteins, RNA, and DNA. This discrepancy partly arises from the extensive variety of lipid species and the high structural complexity of lipids, and their lack of genetic encoding, necessitating the use of direct chemical analysis methods for scientific investigations. However, recent advances in technology have facilitated lipidomics, enabling more comprehensive investigations into the structure and function of lipids within cells and tissues [10,11]. Such technologies offer unprecedented opportunities to understand the role of lipids in the pathogenesis of OA.

Here, using targeted shotgun lipidomics, we mapped the lipid landscape of human articular cartilage and its disease-associated changes in osteoarthritic patients. Importantly, we validated these findings in a standardised surgical mouse model of the disease. Our data reveal significant changes in odd-chain fatty acids within the chondrocyte lipidome in both humans and mice with OA, suggesting a novel molecular mechanism driving disease progression. These findings highlight potential avenues for therapeutic intervention by targeting lipid dysregulation, with potential to modify the disease course and to improve patient outcomes.

METHODS

Extended materials and methods are provided in the supplementary materials.

RESULTS

Lipidomic profiling of articular chondrocytes reveals alterations in lipid classes and species associated with human OA

To map the lipid profile of human articular cartilage and identify OA-associated lipid changes, we performed a comprehensive lipidomics analysis of human articular chondrocytes isolated from OA patients (n = 14) and non-osteoarthritic controls (n = 13) (Fig 1A; Supplementary Table S1). This analysis covered 13 lipid classes: free fatty acids (FFA), sterol lipids (cholesteryl esters [CE]), glycerolipids (including diacylglycerols [DAG] and TAG), glycerophospholipids (including phosphatidylethanolamines [PE], phosphatidylcholines [PC], lysophosphatidylethanolamines [LPE], and lysophosphatidylcholines [LPC]), and sphingolipids (including sphingomyelins [SM], ceramides [CER], dihydroceramides [DCER], hexosylceramides [HCER], and lactosylceramides [LCER]). Representative structures of these lipid classes [12] are presented in Supplementary Figure S1. The result of data quality control indicated that most lipids had no missing values (Supplementary Fig S2A) and technical reproducibility was examined prior to full data analysis (Supplementary Fig S2B). Lipid species from the LPE and LPC classes were excluded from further analyses because quality control standards were not met for these groups. After data processing and normalisation, we identified 573 lipid species from 11 lipid classes across the articular chondrocytes of different individuals in both groups (Supplementary Table S2). Most identified lipid species belonged to the TAG class (73.5% of total identified

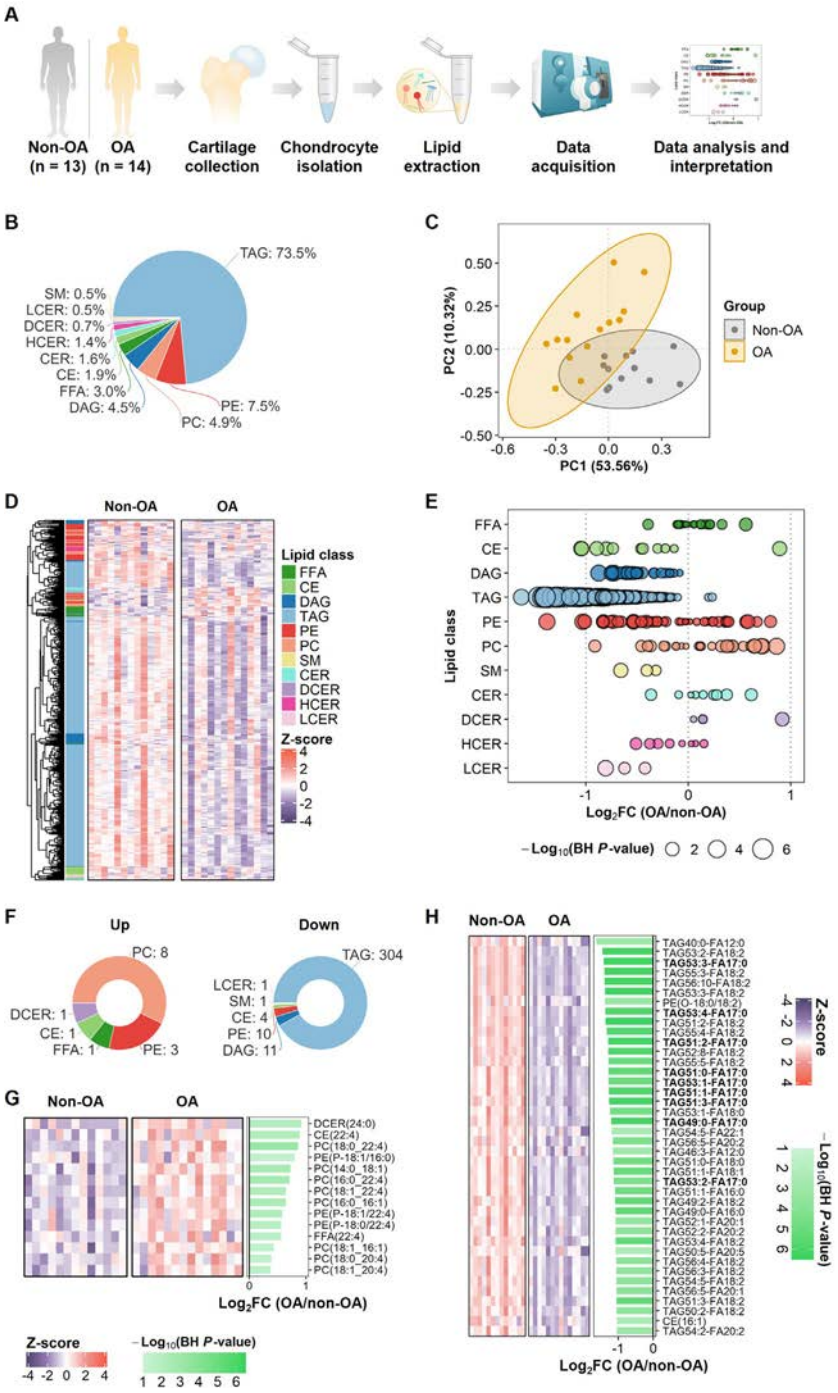

Figure 1. Changes in lipid classes and species associated with human osteoarthritis. (A) Overview of the lipidomics analysis of human articular chondrocytes (hACs). (B) Pie chart indicating the proportions of identified lipid species within each lipid class in hACs. (C) Principal component analysis (PCA) showing clustering of OA and non-OA patients. Each dot represents 1 patient. (D) Heatmap displaying *z*-scored abundance of lipid species in hACs of OA and non-OA patients. Hierarchical clustering of lipid species was performed. (E) Log_2 fold changes of lipid species summarised by lipid classes in hACs of OA vs non-OA patients. Each bubble indicates a lipid species. The bubble size represents the statistical significance level. (F) Donut plot indicating the number of significantly up- and downregulated lipid species within each lipid class (false discovery rate [FDR] < 0.05). (G, H) Heatmap displaying *z*-scored abundance of significantly (G) increased and (H) top-40 decreased lipid species in hACs of OA compared with non-OA patients (FDR < 0.05). The bar plot shows the log_2 fold changes of lipid species in hACs of OA vs non-OA patients. The bar colour represents the significance level. BH, Benjamini-Hochberg; CE, cholesteryl esters; CER, ceramides; DAG, diacylglycerols; DCER, dihydroceramides; FC, fold change; FFA, free fatty acids; HCER, hexosylceramides; LCER, lactosylceramides; OA, osteoarthritis; PC, phosphatidylcholines; PE, phosphatidylethanolamines; SM, sphingomyelins; TAG, triacylglycerols.

lipids), followed by PE (7.5%), PC (4.9%), DAG (4.5%), FFA (3.0%), CE (1.9%), CER (1.6%), HCER (1.4%), DCER (0.7%), LCER (0.5%), and SM (0.5%) classes (Fig 1B).

Next, we aimed to determine differences in lipid classes and species between osteoarthritic and non-osteoarthritic human articular chondrocytes. Principal component analysis (PCA) separated the disease and control samples (Fig 1C). The heatmap depicting the relative abundance of lipid species revealed lipidomic signatures that distinguish between the 2 groups (Fig 1D). Hierarchical clustering analysis of lipid species showed that TAG, DAG, glycerophospholipid (PC and PE), CE, and FFA species clustered together respectively (Fig 1D). Differential analysis of individual lipid species showed that lipids within the CE, DAG, TAG, SM, HCER, and LCER classes were predominantly decreased, with TAG showing the largest effect (Fig 1E). In contrast most FFA, CER, and DCER species were increased in osteoarthritic vs non-osteoarthritic chondrocytes (Fig 1E). A total of 345 lipid species were significantly changed (false discovery rate [FDR] < 0.05), with 14 lipids increased and 331 lipids decreased in the osteoarthritic samples (Fig 1F; Supplementary Table S3). In detail, upregulated lipids consisted of 8 PC, 3 PE, 1 FFA, 1 CE, and 1 DCER species, while downregulated lipids included 304 TAG, 11 DAG, 10 PE, 4 CE, 1 SM, and 1 LCER species (Fig 1F). Most upregulated lipids contained, as side chains, C18:1 (eg, oleic acid), C20:4 (eg, arachidonic acid), or C22:4 (eg, adrenic acid) (FDR < 0.05; Fig 1G). The top-40 downregulated lipid species in osteoarthritic chondrocytes highlighted markedly low levels of lipids (mostly TAG) that contained C18:2 (eg, linoleic acid) or C17:0 (eg, margaric acid), an odd-chain fatty acid (FDR < 0.05; Fig 1H). The presence of TAG with odd-chain fatty acids was further confirmed through high resolution mass spectrometry-based lipid analysis. We

specifically examined TAG with odd-chain fatty acids and ether-linked TAG with highly similar molecular masses. For example, TAG 16:0_17:0_16:1 and TAG-O 18:0_16:0_16:1 would present with $[M+NH_4]^+$ molecular ions at 836.7709 and 836.7904 Da, respectively (Supplementary Fig S3A,B). We could confirm these structures by tandem mass spectrometry analysis and further validate our finding of decreased TAG with the odd-chain fatty acid, not ether-linked TAG, in OA samples (Supplementary Fig S3C,D). This observation was striking because odd-chain fatty acids have low abundance and are generally considered to be insignificant or nonfunctional in humans [13]. However, an inverse association between circulating odd-chain fatty acids and disease risk has been reported, for instance, in coronary heart disease and type 2 diabetes [14,15], suggesting that these rare lipids may play a role in disease pathogenesis. Overall, the enrichment of the indicated fatty acid chains in differentially regulated lipid classes and species in diseased chondrocytes shows that specific lipid alterations are associated with human OA.

Lipidomics profiling of articular cartilage from mice with surgically induced OA validates lipid insights in human osteoarthritic cartilage

The lipid profile of patients can be influenced by genetic factors [16,17] and by lifestyle and environmental cues, such as diet [18] and physical exercise [19]. We therefore sought to verify our findings obtained in human cartilage by using a standardised animal model of OA in which genetic, environmental, and dietary factors are strictly controlled. To this end, we used the surgical destabilisation of the medial meniscus (DMM) model to induce OA in mice, characterised by gradually progressing disease development driven by altered joint biomechanics [20]. We first analysed the lipidome in articular cartilage from DMM-operated mice and sham controls 12 weeks after surgery (n = 5 animals per group) (Fig 2A). Data quality control revealed that most lipids had no missing values (Supplementary Fig S2C), and technical reproducibility of lipid screening was examined before subsequent data analysis (Supplementary Fig S2D). In mouse articular cartilage, we identified 762 lipid species covering 13 lipid classes, and most identified lipids were TAG (Fig 2B; Supplementary Table S4), similar to our observations in human cartilage (Fig 1B). Sham control and DMM mouse samples were not completely separated by PCA, but most variation was explained by PC1 (Fig 2C). The heatmap of relative abundance of lipid species evidenced a distinct lipidomic signature between the groups and, mirroring the signature of human OA cartilage, most lipids were decreased in the articular cartilage of osteoarthritic mice (Fig 2D). Hierarchical clustering analysis of lipid species showed the clustering of TAG and glycerophospholipids (PC and PE) (Fig 2D). Differential analysis of individual lipid species indicated that HCER and LCER were increased in articular cartilage of osteoarthritic mice compared with sham controls, while most FFA, CE, DAG, TAG, PE, PC, LPE, LPC, SM, and CER species were reduced (Fig 2E). Upregulated lipids included CE(20:2), LCER(24:0), and TAG36:0-FA12:0 (FDR < 0.05; Fig 2F,G; Supplementary Table S5). Downregulated lipids included 465 TAG and 13 DAG as the main decreased lipid classes (FDR < 0.05; Fig 2F; Supplementary Table S5). Most of top-40 decreased lipid species in articular cartilage from mice with OA contained fatty acid chains including C14:0 (eg, myristic acid), C16:0 (eg, palmitic acid), odd-chain C17:0 (eg, margaric acid), C20:0 (eg, arachidic acid), or C20:1 (eg, gadoleic acid) (FDR < 0.05; Fig 2H). Altogether, these data indicate that lipidome changes in articular cartilage from osteoarthritic mice largely mirror the changes observed in human osteoarthritic cartilage, in particular the reduction in overall TAG levels and the decrease in TAG species that contain odd-chain fatty acid C17:0.

We further analysed the time course changes in lipid species and classes 2, 4, and 12 weeks after the induction of OA. No lipid species were significantly altered at 2 and 4 weeks after surgery in contrast to the changes detected at 12 weeks (Supplementary Fig S4A). At 4 weeks, the total levels of most lipid classes showed a transient increase in osteoarthritic mice compared with the sham control, with LPC and SM showing the most pronounced changes (Supplementary Fig S4B). After 12 weeks, most lipid classes were decreased, with TAG displaying the most substantial reduction (Supplementary Fig S4B).

Specific shifts in fatty acid side chain composition in articular cartilage are associated with human OA

We then performed a deeper analysis of the fatty acid side chain composition of the different lipids in human articular chondrocytes and the changes therein that are associated with OA. Fatty acids are aliphatic chains with different numbers of carbons and double bonds, contributing to the diversity in lipids by varying chain length and degree of unsaturation, respectively. We analysed fatty acid side chains in 3 dimensions: (1) individual species (Fig 3A-C), (2) length (Fig 3D-F), and (3) degree of unsaturation (Fig 3G-I). For each dimension, we analysed the data at 3 different levels: (1) the number of measured fatty acid chains within the different lipid classes, (2) the fold change of specific fatty acid chains in osteoarthritic chondrocytes compared with non-osteoarthritic chondrocytes, and (3) the fold change of specific fatty acid chains that are statistically significantly different between osteoarthritic chondrocytes and non-osteoarthritic chondrocytes presented by lipid class. Twenty-six different fatty acid chains were detected in human articular chondrocytes (Fig 3A). C16:0, C18:0, C18:1, and C18:2 showed the highest counts in our analysis (Fig 3A). In osteoarthritic chondrocytes, fatty acid chains C12:0, C18:0, C18:2, and odd-chain C17:0, and to a lesser extent C15:0, were among those predominantly reduced compared with non-osteoarthritic chondrocytes (Fig 3B). The analysis of the significantly dysregulated fatty acid chains in osteoarthritic chondrocytes provided more details about their type and distribution among lipid classes (Fig 3C). Most significantly decreased fatty acid side chains in osteoarthritic chondrocytes were found in the TAG class of lipids (Fig 3C). Only C14:0, C16:0, C16:1, C18:0, C18:1, C20:4, C22:4, and C24:0 were significantly increased in specific species, mainly of the PC and PE classes (Fig 3C).

We next interrogated differences in fatty acid chain length between osteoarthritic and control human articular chondrocytes. Long-chain fatty acids (LCFA) were the most abundant fatty acids found in articular chondrocytes, with fewer very-long-chain fatty acids (VLCFA) and medium-chain fatty acids (MCFA) showing the lowest number (Fig 3D). Specifically, the most abundant fatty acids were 18- and 16-carbon LCFA (Supplementary Fig S5A). Moreover, most MCFA, LCFA, and VLCFA were downregulated in OA (Fig 3E), especially 17- and 18-carbon LCFA (Supplementary Fig S5B). Additionally, among significantly regulated fatty acids, most downregulated MCFA, LCFA, or VLCFA were TAG; all the increased LCFA were species of the PE or PC class, while increased VLCFA were PC, PE, DCER, CE, or FFA (Fig 3F; Supplementary Fig S5C).

Next, we analysed differences in fatty acid chain unsaturation between osteoarthritic and control human articular chondrocytes. Higher counts of saturated fatty acids (SFA) and

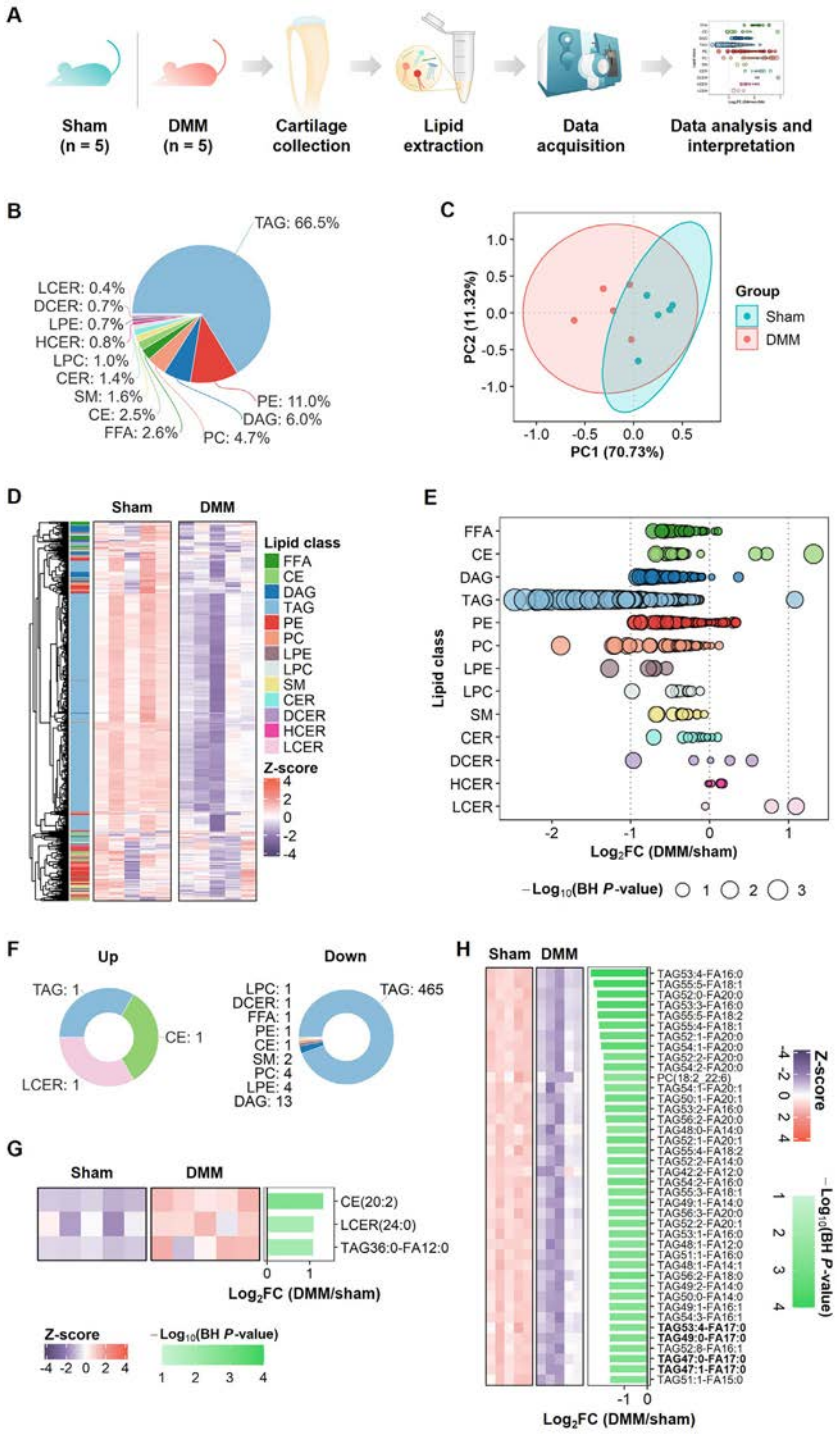

Figure 2. Changes in lipid classes and species associated with mouse osteoarthritis. (A) Overview of the lipidomics analysis of mouse articular cartilage. (B) Pie chart indicating the proportions of identified lipid species within each lipid class in articular cartilage. (C) PCA showing clustering of sham and DMM mice. Each dot represents 1 mouse. (D) Heatmap displaying *z*-scored abundance of lipid species in articular cartilage from sham and DMM mice. Hierarchical clustering of lipid species was performed. (E) Log_2 fold changes of lipid species summarised by lipid classes in articular cartilage of DMM vs sham mice. Each bubble indicates a lipid species. The bubble size represents the statistical significance level. (F) Donut plot indicating the number of significantly up- and downregulated lipid species within each lipid class (FDR < 0.05). (G,H) Heatmap displaying *z*-scored abundance of significantly (G) increased and (H) top-40 decreased lipid species in articular cartilage of DMM compared with sham mice (FDR < 0.05). The bar plot shows the log_2 fold changes of lipid species in articular cartilage of DMM vs sham mice. The bar colour represents the significance level. BH, Benjamini-Hochberg; CE, cholesteryl esters; DAG, diacylglycerols; DCER, dihydroceramides; DMM, destabilisation of the medial meniscus; FC, fold change; FDR, false discovery rate; FFA, free fatty acids; LCER, lactosylceramides; LCFA, long-chain fatty acids; MCFA, medium-chain fatty acids; MUFA, monounsaturated fatty acids; OA, osteoarthritis; PC, phosphatidylcholines; PCA, principal component analysis; PE, phosphatidylethanolamines; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SM, sphingomyelins; TAG, triacylglycerols; VLCFA, very-long-chain fatty acids.

polyunsaturated fatty acids (PUFA) were detected compared to monounsaturated fatty acids (MUFA) in these cells (Fig 3G; Supplementary Fig S5D). Most SFA, MUFA, and PUFA were decreased in osteoarthritic chondrocytes (Fig 3H), particularly those with 0-2 double bonds (Supplementary Fig S5E). One hundred forty-six of 249 SFA, 85 of 161 MUFA, and 146 of 260 PUFA were significantly changed in osteoarthritic human articular chondrocytes, mainly in species from the TAG, DAG, PE, and PC classes (Fig 3I). The analysis of significantly regulated fatty acids grouped by unsaturation degree exhibited similar changes among SFA, MUFA, and PUFA, as reduced fatty acids were mainly species from the TAG class and increased lipids were mainly PC or PE (Fig 3I; Supplementary Fig S5F). In summary, the observed shifts in the fatty acid side chains of lipids from osteoarthritic human articular chondrocytes, at the 3 dimensions including individual species, chain length, and unsaturation, document the fatty acid spectrum in articular chondrocytes and demonstrate that specific changes in the lipidome are associated with human OA.

Fatty acid side chain analysis of articular cartilage from mice with surgically induced OA validates identifies shifts in human osteoarthritic cartilage

We then examined whether identified lipid side chain shifts in osteoarthritic human articular chondrocytes are also observed in the standardised DMM mouse model of the disease. We first profiled the fatty acid composition at 12 weeks after DMM induction. Twenty-seven different fatty acid chains were detected in mouse articular cartilage (Fig 4A). C16:0, C18:0,

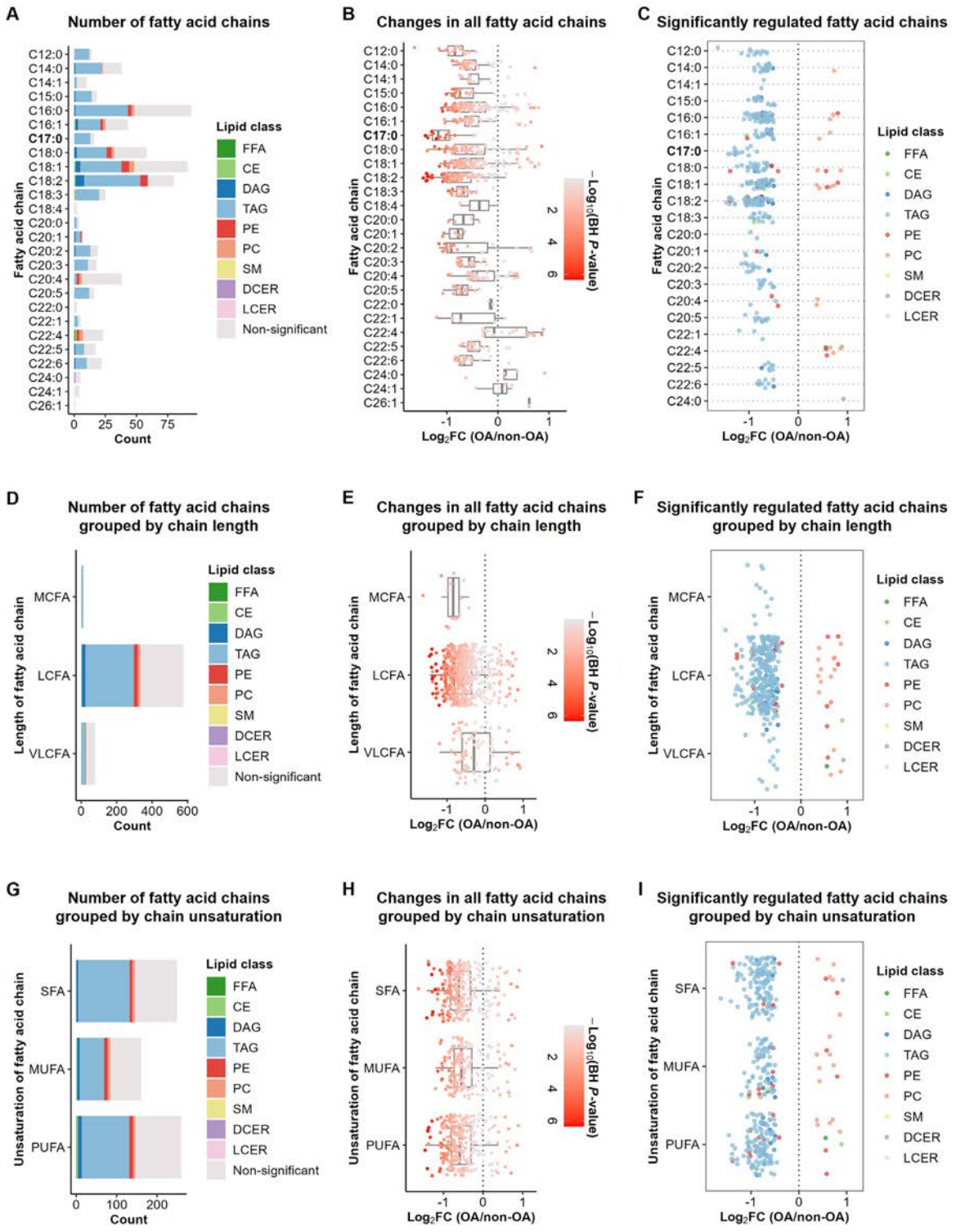

Figure 3. Changes in fatty acid chain composition associated with human osteoarthritis. (A) Number of fatty acid chains identified in hACs by lipid class (bar colour). (B) Log_2 fold changes of individual fatty acid chains in OA vs non-OA hACs. Each dot represents 1 fatty acid chain. The dot colour indicates the statistical significance level. (C) Log_2 fold changes of significantly altered fatty acid chains in OA vs non-OA hACs (FDR < 0.05) by lipid class (dot colour). (D) Number of fatty acid chains categorised by chain length in hACs per lipid class (bar colour). (E) Log_2 fold changes of fatty acid chains categorised by chain length in OA vs non-OA hACs. Each dot represents 1 fatty acid chain. The dot colour indicates the significance level. (F) Log_2 fold changes of significantly altered fatty acid chains categorised by chain length in OA vs non-OA hACs (FDR < 0.05) per lipid class (dot colour). (G) Number of fatty acid chains categorised by chain unsaturation in hACs by lipid class (bar colour). (H) Log_2 fold changes of fatty acid chains categorised by chain unsaturation in OA vs non-OA hACs. Each dot represents 1 fatty acid chain. The dot colour indicates the significance level. (I) Log_2 fold changes of significantly altered fatty acid chains categorised by chain unsaturation in OA vs non-OA hACs (FDR < 0.05) by lipid class (dot colour). BH, Benjamini-Hochberg; CE, cholesteryl esters; DAG, diacylglycerols; DCER, dihydroceramides; DMM, destabilisation of the medial meniscus; FC, fold change; FDR, false discovery rate; FFA, free fatty acids; hAC, human articular chondrocyte; LCER, lactosylceramides; LCFA, long-chain fatty acids; MCFA, medium-chain fatty acids; MUFA, monounsaturated fatty acids; OA, osteoarthritis; PC, phosphatidylcholines; PE, phosphatidylethanolamines; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SM, sphingomyelins; TAG, triacylglycerols; VLCFA, very-long-chain fatty acids.

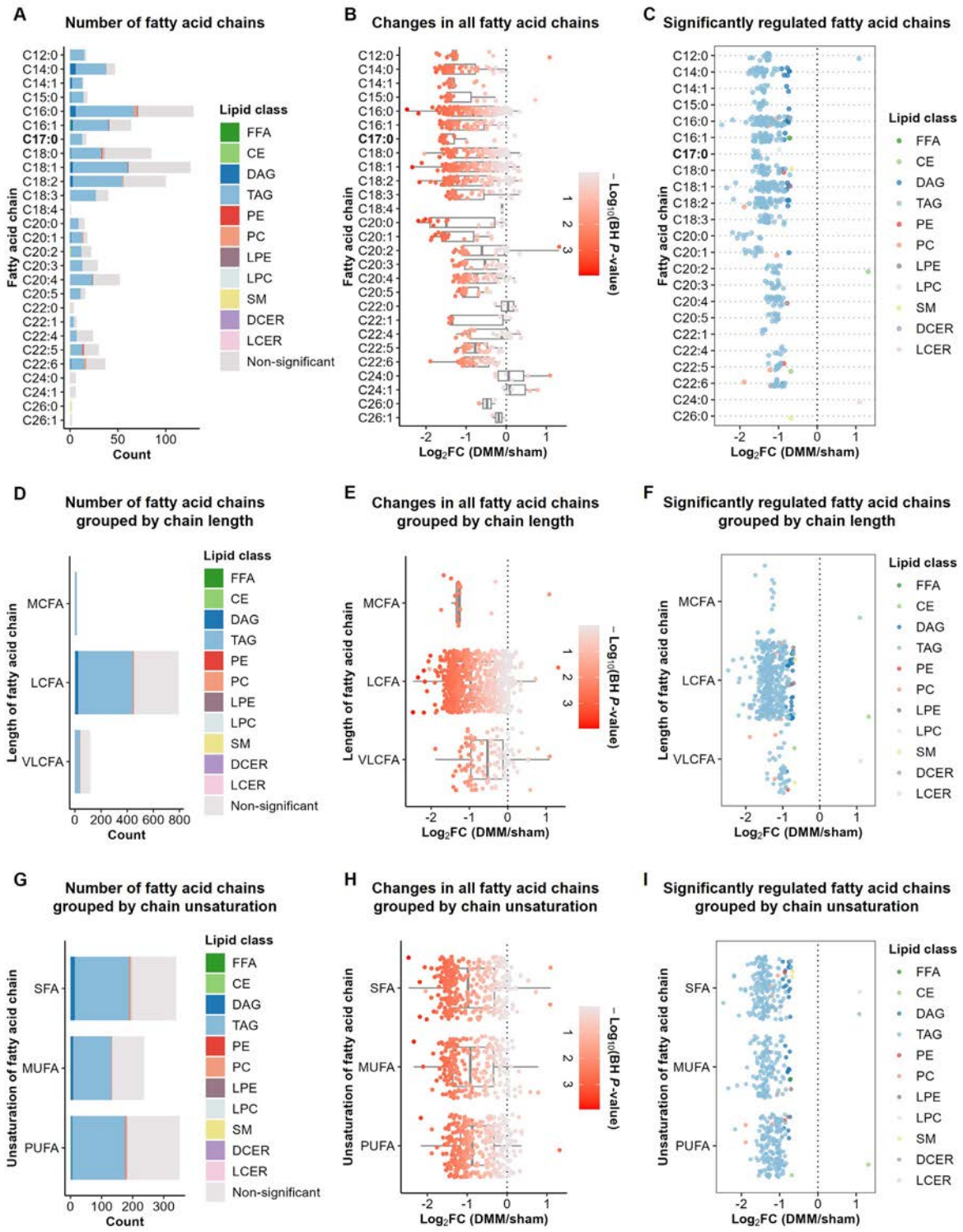

Figure 4. Changes in fatty acid chain composition associated with mouse osteoarthritis. (A) Number of fatty acid chains identified in mouse articular cartilage by lipid class (bar colour). (B) Log_2 fold changes of individual fatty acid chains in articular cartilage of DMM vs sham mice. Each dot represents 1 fatty acid chain. The dot colour indicates the statistical significance level. (C) Log_2 fold changes of significantly altered fatty acid chains in articular cartilage of DMM vs sham mice (FDR < 0.05) by lipid class (dot colour). (D) Number of fatty acid chains categorised by chain length in mouse articular cartilage by lipid class (bar colour). (E) Log_2 fold changes of fatty acid chains categorised by chain length in articular cartilage of DMM vs sham mice. Each dot represents 1 fatty acid chain. The dot colour indicates the significance level. (F) Log_2 fold changes of significantly altered fatty acid chains categorised by chain length in articular cartilage of DMM vs sham mice (FDR < 0.05) per lipid class (dot colour). (G) Number of fatty acid chains categorised by chain unsaturation in mouse articular cartilage by lipid class (bar colour). (H) Log_2 fold changes of fatty acid chains categorised by chain unsaturation in articular cartilage of DMM vs sham mice. Each dot represents 1 fatty acid chain. The dot colour indicates the significance level. (I) Log_2 fold changes of significantly altered fatty acid chains categorised by chain unsaturation in articular cartilage of DMM vs sham mice (FDR < 0.05) by lipid class (dot colour). BH, Benjamini-Hochberg; CE, cholesteryl esters; DAG, diacylglycerols; DCER, dihydroceramides; DMM, destabilisation of the medial meniscus; FC, fold change; FDR, false discovery rate; FFA, free fatty acids; LCER, lactosylceramides; LCFA, long-chain fatty acids; LPC, lysophosphatidylcholines; LPE, lysophosphatidylethanolamines; MCFA, medium-chain fatty acids; MUFA, monounsaturated fatty acids; PC, phosphatidylcholines; PE, phosphatidylethanolamines; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SM, sphingomyelins; TAG, triacylglycerols; VLCFA, very-long-chain fatty acids.

C18:1, and C18:2 again showed the highest counts in our analysis (Fig 4A). Among the decreased fatty acids in osteoarthritic mouse cartilage, a striking reduction in odd-chain C17:0 and to a lesser extent C15:0 was again observed (Fig 4B). The analysis of the significantly dysregulated lipids in osteoarthritic mouse cartilage provided more details about their type and distribution among lipid classes (Fig 4C). Most significantly decreased fatty acids side chains in the articular cartilage of mice with OA were again found in TAG species (Fig 4C), thereby also confirming a striking reduction in the concentrations of TAG-associated odd-chain fatty acids (Fig 4C). Therefore, the analysis in osteoarthritic mouse articular cartilage parallels and validates key observations on fatty acid side chain shifts in osteoarthritic human chondrocytes.

We also interrogated differences in fatty acid chain length between osteoarthritic and sham-operated mouse articular cartilage. As for human chondrocytes, LCFA were the most abundant fatty acids found in mouse articular cartilage, with fewer VLCFA and MCFA showing the lowest number (Fig 4D; Supplementary Fig S6A). Most MCFA, LCFA, and VLCFA were downregulated in mouse osteoarthritic cartilage (Fig 4E; Supplementary Fig S6B). Among the significantly regulated fatty acids, most downregulated MCFA, LCFA, or VLCFA were TAG and to a lesser extent DAG (Fig 4F; Supplementary Fig S6C).

Next, we analysed differences in fatty acid chain unsaturation between osteoarthritic and control mouse articular cartilage. As in human samples, higher counts of SFA and PUFA were detected compared to MUFA (Fig 4G; Supplementary Fig S6D). Most SFA, MUFA, and PUFA were decreased in osteoarthritic cartilage (Fig 4H; Supplementary Fig S6E). The analysis of significantly regulated fatty acids grouped by unsaturation degree exhibited similar changes among SFA, MUFA, and PUFA because the reduced fatty acids were mainly species from the TAG class and to a lesser extent from the DAG class (Fig 4I; Supplementary Fig S6F). Taken together, all these data from a controlled animal model validate our findings obtained in human chondrocytes.

We also performed a time course analysis of the fatty acid chains after OA induction. At 4 weeks, the total levels of C22:4 and C24:0 were significantly elevated in the articular cartilage of DMM-operated mice compared with sham controls (Supplementary Fig S4C). At 12 weeks, most fatty acids, including C12:0, C14:0, C14:1, C15:0, C16:0, C16:1, C17:0, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C22:1, C22:5, C22:6, and C26:0, were markedly reduced (Supplementary Fig S4C).

Depletion in 2-hydroxyacyl-CoA lyase 1-mediated odd-chain fatty acid C17:0 synthesis triggers a shift in the molecular signature of human articular chondrocytes towards an OA-like profile

The intriguing reduction in odd-chain fatty acids, especially in C17:0, in osteoarthritic chondrocytes from human and mouse cartilage triggered us to investigate their functional role in the disease. In mammals, odd-chain fatty acids originate from food sources such as dairy products but are also produced endogenously. The C15:0 level appears to be largely influenced by exogenous dietary sources, whereas C17:0 appears to be substantially generated de novo [21]. The observed reduction in odd-chain fatty acids in cartilage of the diet-standardised OA mouse model (Fig 2H; Fig 4A-C) excluded the diet factor as causative of this dysregulation and thus suggested a deficient endogenous synthesis of odd-chain fatty acids in osteoarthritic chondrocytes. Odd-chain fatty acids can be endogenously synthesised by 1-carbon removal of corresponding 2-hydroxy fatty acids via 2-hydroxyacyl-CoA lyase 1 (HACL1)-catalysed alpha-oxidation in the peroxisome or via HACL2-catalysed alpha-oxidation in the endoplasmic reticulum [22,23]. In particular, the synthesis of C17:0, also known as margaric acid, involves 1-carbon removal from 2-hydroxy stearic acid (2-OH C18:0), in which HACL1 and HACL2 act as key enzymes [22,23] (Fig 5A).

To evaluate the effects of reducing the synthesis of odd-chain fatty acid C17:0 in cartilage, we knocked down HACL1 or HACL2 in human articular chondrocytes from non-osteoarthritic individuals using small interfering RNA (siRNA) (Supplementary Fig S7). Silencing of HACL1 (siHACL1) in chondrocytes led to a decrease in C17:0, whereas silencing of HACL2 (siHACL2) had no consistent effect (Fig 5B). The amount of C15:0 was not affected by siHACL1 or siHACL2 (Fig 5B). These data indicate that HACL1 contributes to the synthesis of odd-chain C17:0 in human articular chondrocytes. Then, to investigate the role of C17:0 in OA, we triggered its depletion by knocking down HACL1 (and HACL2 as negative control) and examined effects on articular chondrocyte markers. Silencing of HACL1 and subsequent reduction in odd-chain C17:0 resulted in a downregulation of chondrocyte anabolic markers type 2 collagen (COL2A1) and aggrecan (ACAN), essential constituents of the healthy articular cartilage ECM (Fig 5C). Conversely, silencing of HACL1 led to an up-regulation in disease-associated markers including alpha-1 type 1 collagen (COL1A1) and tissue-destructive matrix metalloproteinases 1 and 3 (MMP1 and MMP3) (Fig 5C). Silencing of HACL2 had no effect on the expression of these chondrocyte markers (Fig 5C). These data indicate that a reduction in the synthesis of odd-chain C17:0 by HACL1 leads to a shift in the molecular signature of healthy articular chondrocytes towards an OA-like profile.

We then interrogated whether supplementation with exogenous C17:0 in human articular chondrocytes with deficient C17:0 synthesis could restore a healthy molecular profile. We silenced HACL1 in chondrocytes from non-osteoarthritic patients and treated these cells with increasing concentrations of C17:0, but this supplementation did not lead to beneficial effects in healthy or disease chondrocyte markers (Fig 5D; Supplementary Figs S8A, S9A). In line with this observation, supplementation with C17:0 in articular chondrocytes from osteoarthritic patients, which have reduced C17:0 levels, did not ameliorate their disease-associated phenotype (Fig 5E; Supplementary Fig S9B). Together, these data indicate that odd-chain C17:0 itself may not exert beneficial effects in articular chondrocytes, at least under our experimental conditions. This suggests that a reduction of C17:0 is likely not causative of joint damage in OA but is indicative of deficient synthesis by HACL1.

HACL1 expression is downregulated in human and mouse osteoarthritic articular cartilage

We then investigated potential differences in the expression of HACL1 in OA and control articular cartilage. First, looking at transcriptome datasets from literature, we found that HACL1 was significantly downregulated in human OA articular cartilage compared with control cartilage (Table 1) [24–26]. We then specifically investigated available single-cell transcriptome datasets of human osteoarthritic and control cartilage (GSE220244 [27] and GSE104782 [28]) and found a decreased number of chondrocytes with detectable HACL1 in OA samples compared to the non-OA controls and in late stages compared to early stages of OA (Fig 5F). We additionally found that protein amounts of HACL1 in articular cartilage from mice with DMM-induced OA were substantially decreased compared with

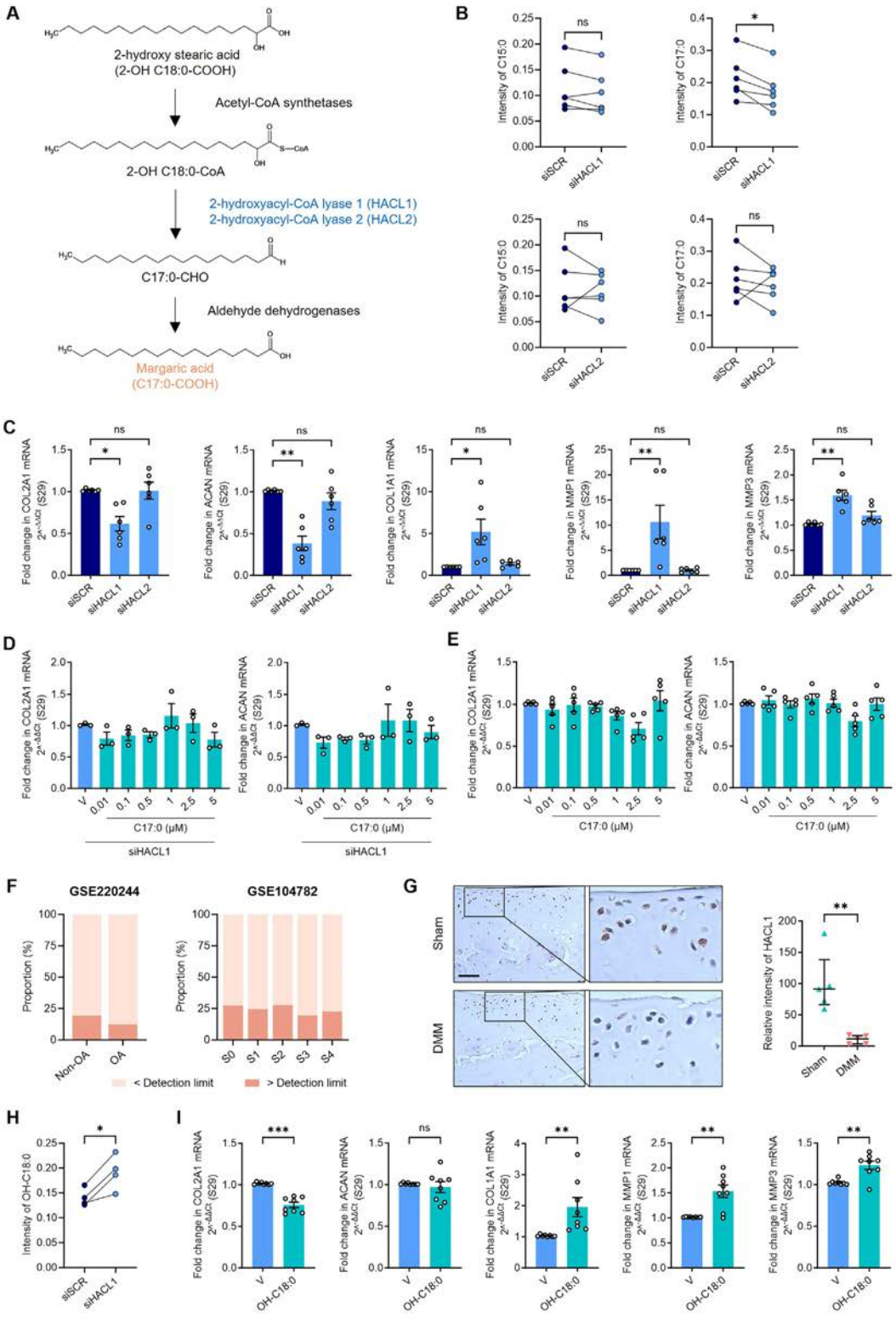

Figure 5. Reduction of odd-chain fatty acid lipid synthesis by HACL1 shifts the molecular profile of articular chondrocytes towards osteoarthritis. (A) Scheme of endogenous biosynthesis of odd-chain fatty acids via alpha-oxidation. (B) Measurement of C17:0 and C15:0 levels in primary hACs of non-OA patients after treatment with siRNA-mediated silencing of *HACL1* (siHACL1), *HACL2* (siHACL2), or scrambled control (siSCR) (n = 6, $^{*}P < .05$ by paired two-tailed Student *t*-test). (C) Real-time PCR analysis of *COL2A1, ACAN, COL1A1, MMP1*, and *MMP3* in primary hACs of non-OA patients after treatment with siHACL1, siHACL2, or siSCR (n = 6, $^{*}P < .05$, $^{**}P < .01$ by RM 1-way ANOVA with Dunnett's test for multiple comparisons to siSCR). (D) Real-time PCR analysis of *COL2A1* and *ACAN* in primary hACs of non-OA patients treated with C17:0 upon siHACL1 (n = 3, analysed by linear mixed model). (E) Real-time PCR analysis of *COL2A1* and *ACAN* in primary hACs of OA patients treated with C17:0 (n = 5, analysed by linear mixed model). (F) Proportions of primary hACs with detectable HACL1 from public single-cell transcriptome datasets GSE220244 and GSE104782. (G) Immunohistochemical detection of HACL1 in the articular cartilage of OA mouse model triggered by DMM surgery compared with sham-operated mice. Images are representative of 5 mice per group. Scale bar: 50 μm (n = 5, $^{**}P < .05$ by 2-tailed Mann–Whitney test, data are shown as individual points and medium with IQR). (H) Measurement of 2-OH C18:0 level in primary hACs of non-OA patients upon siHACL1 (n = 4, $^{*}P < .05$ by paired 2-tailed Student *t*-test). (I) Real-time PCR analysis of *COL2A1, ACAN, COL1A1, MMP1*, and *MMP3* in primary hACs of non-OA patients treated with OH-C18:0 at 40 μM (n = 8, $^{**}P < .01$, $^{***}P < .001$ by paired 2-tailed Student *t*-test). (B-E,H-I) Data are shown as individual points with mean ± SEM where indicated. ACAN, aggrecan; ANOVA, analysis of variance; CoA, coenzyme A; COL1A1, alpha-1 type 1 collagen; COL2A1, type 2 collagen; DMM, destabilisation of the medial meniscus; hAC, human articular chondrocyte; HACL, 2-hydroxyacyl-CoA lyase; MMP, matrix metalloproteinase; ns, not significant; OA, osteoarthritis; PCR, polymerase chain reaction; RM, repeated measures; SCR, scrambled.

Table 1
HACL1 expression in publications

Publication (OA vs non-OA patients)	HACL1 expression
Karlsson et al [24]. Osteoarthritis Cartilage. 2010	Fold change = −2.908 (P = 3.606E-16)
Fisch et al [25]. Osteoarthritis Cartilage. 2018	Log_2 fold change = −0.277 (P = .066)
Soul et al [26]. Ann Rheum Dis. 2018	Log_2 fold change = −0.408 (P = .002, in 1 subgroup)

HACL1, 2-hydroxyacyl-CoA lyase 1; OA, osteoarthritis.

cartilage from control mice (Fig 5G). Hence, these data indicate that HACL1 expression is downregulated in osteoarthritic articular cartilage. We also investigated whether HACL1 expression is affected by some key signalling pathways involved in cartilage homeostasis, including IL-1β [29], Wnt [30], and hypoxia [31]. Wnt activation reduced HACL1 expression, hypoxic conditions led to a slight increase, and IL-1β stimulation did not affect HACL1 expression (Supplementary Fig S10).

Treatment with 2-hydroxy C18:0, precursor of C17:0, shifts the molecular signature of human articular chondrocytes towards OA

Based on our observations, we hypothesised that deficient HACL1 activity in osteoarthritic cartilage would lead to a subsequent accumulation of 2-hydroxy stearic acid (2-OH C18:0), precursor of C17:0 (Fig 5A), with potential detrimental effects. As expected, we found that 2-OH C18:0 is increased in articular chondrocytes from non-osteoarthritic patients upon silencing of HACL1 (Fig 5H). Then, we investigated the potential effects of 2-OH C18:0 on cartilage health. Treatment of human articular chondrocytes from non-osteoarthritic patients with 2-OH C18:0 led to a decrease in anabolic marker COL2A1 and an increase in catabolic markers COL1A1, MMP1, and MMP3 (Fig 5I; Supplementary Fig S8B), mirroring detrimental changes in chondrocyte markers that were triggered by silencing of HACL1 (Fig 5C). Altogether, our data show that deficient HACL1-mediated synthesis of odd-chain fatty acid C17:0 in osteoarthritic cartilage results in the accumulation of its precursor 2-OH C18:0, which leads to detrimental changes in the articular chondrocytes.

DISCUSSION

Our study unravels the lipidome of human articular cartilage and its shifts in OA. Analysis of patient samples compared to non-OA controls identified notable changes in different lipid classes and a clear separation of the control vs diseased profiles. These findings were validated in a standardised mouse model of OA, with largely analogous lipid alterations observed in articular cartilage from the diseased animals. Detailed fatty acid analysis highlighted a marked reduction in odd-chain fatty acid C17:0 associated with OA. Subsequent functional studies indicated that reduced HACL1-mediated C17:0 synthesis in chondrocytes led to molecular shifts resembling OA pathology. Our mechanistic investigations showed that, instead of the reduction of C17:0, the accumulation of its precursor, 2-hydroxy C18:0, due to HACL1 deficiency, triggers pathological changes in articular chondrocytes. Together, our results provide novel insights into lipid changes in OA and unravel a lipid-related mechanism underlying disease pathogenesis.

The complexity of OA and the existing challenges to find effective strategies for the treatment of this disease underscore the enormous need for comprehensive investigations into its pathogenesis. Although the transcriptome and proteome of articular cartilage have been systematically studied, lipids have received little attention, despite their critical roles in cellular function. Hence, our research fills an important knowledge gap by documenting the broad lipidomic profile of articular chondrocytes and its shifts in OA. Early morphologic studies by histochemical approaches and quantitative studies by chromatography suggested some lipid classes, such as neutral lipids and phospholipids, accumulate in human articular cartilage with age [32–34]. However, lipids can also accumulate without any sign of cartilage degeneration [32]. How this relates to OA remains unclear. Later quantitative studies looking at the level of total fatty acid composition reported that the predominant fatty acids in human articular cartilage are palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) [35,36], in agreement with the observations in our detailed and comprehensive lipidome study. Limited functional studies suggested that lipids might exert diverse effects in OA development. For instance, inhibition of glycosphingolipid synthesis was reported to exacerbate structural damage in a mouse model of OA [37], while blocking cholesterol biosynthesis in mice was shown to protect against the disease [8]. In contrast to the limited existing insights into lipid profiles in articular cartilage health and OA, our global lipidomic analysis and functional experiments provide not only a comprehensive overview of lipids but also a more detailed view on the disease-associated and disease-contributing lipid changes.

Our lipid profiling of osteoarthritic articular cartilage prompted a functional investigation into the impact of the dysregulation of one specific lipid identified by our comprehensive approach. We found that deficient HACL1-mediated synthesis of odd-chain C17:0 in osteoarthritic cartilage results in the accumulation of its precursor, which leads to the loss of chondrocytic molecular identity. The specific deficiency of HACL1-mediated lipid synthesis we identify here may represent an attractive opportunity for the development of novel therapeutic strategies for OA. To date, the factors that regulate HACL1 expression and activity in articular cartilage remain unknown and are an important area for further research. Efforts to modulate HACL1 expression or activity could prevent or reduce the accumulation of 2-OH C18:0, which leads to detrimental effects in articular chondrocytes. This latter finding aligns with a previous report in which 2-OH C18:0 was found to be elevated in synovial fluid after intra-articular ankle injury [38]. There is evidence that 2-OH fatty acids can be further metabolised to 2-keto derivatives by peroxisomal 2-OH acid oxidases, leading to the production of hydrogen peroxide [39,40]. In addition, although in our study we demonstrate detrimental effects of 2-OH C18:0 on articular chondrocytes, whether 2-OH C18:0 is the only HACL1-related molecule responsible for the detrimental effects of HACL1 deficiency in articular cartilage remains to be determined.

As a key component of the lipidomic signature associated with OA pathology, C17:0 levels or other OA-associated lipid changes may serve as a potential biomarker reflecting disease severity or progression. However, to establish its utility as a diagnostic biomarker, further investigation is warranted, including the analysis of such markers in synovial fluid, blood samples,

or urine from OA patients, as direct access to the articular cartilage for biomarker testing is not within regular clinical reach. Furthermore, lipidomic changes in cartilage may also reflect systemic metabolic alterations that can be detected in peripheral fluids, offering a link between local joint pathology and broader physiological mechanisms.

The rapidly changing therapeutic landscape and new targets in the treatment of obesity and diabetes, combined with our data presented here, raise the question whether interventions targeting systemic metabolic pathways could influence lipid metabolism in cartilage and thus help preserve chondrocyte phenotype and cartilage integrity. Semaglutide, a glucagon-like peptide-1 receptor agonist, not only reduces body weight but also alleviates pain in OA patients, suggesting a clear link between metabolic regulation, weight loss, and joint health. An effect on systemic lipid metabolism, which might translate to favourable changes in the lipidome of cartilage, could be part of this. However, while semaglutide may hold promise, the evidence surrounding other metabolic interventions, such as statins, has been less conclusive. Preclinical models and human studies have not consistently shown that statin use leads to significant improvements in cartilage integrity or chondrocyte function, highlighting the complexity of translating metabolic effects to cartilage-specific outcomes. Thus, while treatments targeting systemic metabolic dysfunction may offer therapeutic potential, further research is needed to establish their effectiveness in modulating the lipid landscape of cartilage and preserving joint health.

Some study limitations should be acknowledged. Sample size and characteristics of the end-stage OA samples may limit generalizability of the findings. Clinical data about postsurgical OA subtypes are missing, and these samples cannot provide information over the evolution of the articular cartilage lipidome over time. The non-OA samples came mostly from patients with fractures, and underlying osteoporosis or malignancy may impact the lipidome of the cartilage. Therefore, the validation of our findings in a controlled mouse model was essential. Mouse tissues were used for the validation experiment and compared to human cells. The clear overlap between lipid shifts in human and mouse samples in OA vs non-OA controls showed good robustness and validation of our data. Additionally, the pipeline for the analysis of the lipidome data includes essential steps such as batch corrections, data imputation, and a set of criteria applied. This procedure could theoretically exclude certain lipids of interest or under- or overestimate differences between OA and non-OA samples. Despite batch correction steps, residual variation between batches may also impact the analysis. The cross-sectional design of the study in the human subjects from which biological material was obtained at the time of joint replacement surgery may limit the ability to establish true causal relationships between lipid alterations and OA development. However, our functional investigations support a causative role for HACL1-related lipid alterations in OA. Of note, our data cannot claim specificity of HACL1-mediated effects because the direction of changes seen with silencing of HACL2 was similar although the silencing efficacy was smaller. Nevertheless, further *in vitro* and *in vivo* experiments investigating the effects of modulating HACL1 expression on OA development and progression are needed to assess its therapeutic relevance.

While our study provides valuable insights into the dysregulation of odd-chain fatty acid C17:0 synthesis in OA chondrocytes and its impact on the disease, this represents just one facet of the complex changes in the lipidomic landscape associated with OA. Our comprehensive dataset highlights numerous dysregulated lipids across various classes, emphasising the need to further investigate to understand their functional impact on modulating chondrocyte metabolism, cartilage integrity, and disease progression. Understanding how these lipid alterations influence cellular pathways offers a promising avenue for identifying novel therapeutic targets in OA. Future studies should aim to elucidate the functional roles of key dysregulated lipids and their interactions with metabolic and inflammatory processes within the cartilage, other joint tissues, and at the systemic level. By defining these mechanistic links, it may become possible to develop lipid-targeted interventions aimed at restoring homeostasis and slowing or reversing disease progression. Our dataset serves as a valuable resource for the wider OA research community. Moreover, integrating lipidomics with other -omics approaches, such as transcriptomics and proteomics, could provide a better understanding of the pathophysiology of OA and facilitate the discovery of biomarkers for disease diagnosis, prognosis, and treatment response. This comprehensive approach will be critical for translating mechanistic insights into clinically relevant strategies, ultimately driving the development of precision therapies that target lipid dysregulation and improve patient outcomes in OA.

Competing interests

Leuven Research and Development, the technology transfer office of KU Leuven, has received consultancy and speaker fees and research grants on behalf of RJL from AbbVie, Amgen (formerly Celgene), Biocon, Biosplice Therapeutics (formerly Samumed), Eli Lilly, Galapagos, Fresenius Kabi, Janssen, Novartis, Pfizer, and UCB. The other authors declare that they have no competing interests.

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Contributors

RJL, SM, and QZ planned the study and designed all experiments. QZ processed human samples. Mohan Ghorasaini (MG) performed lipidomics sample preparation and measurement. FMFC performed animal experiments. QZ performed data analysis and visualisation of lipidomics and single-cell transcriptome data. QZ, RA, and ADR performed *in vitro* experiments. Martin Giera (MG) was responsible for quality oversight of lipidomics experiments and made suggestions. RJL and QZ were responsible for statistical analyses. RJL, SM, and QZ wrote the manuscript. RJL is the manuscript guarantor.

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Patient consent for publication

Primary human articular chondrocytes were isolated from cartilage from patients with informed consent.

Ethics approval

Ethical approval for experiments with human tissue was granted by the University Hospitals Leuven Ethics Committee and Biobank Committee (Leuven, Belgium) (S56271). Experiments in mice were performed with approval from the Ethics Committee for Animal Research (P159-2016 and P013-2022; KU Leuven) (licence no. LA1210189).

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability

The datasets generated in this study are included within the article and supplementary files. Public data used in this work are accessible in the Gene Expression Omnibus database. Other data supporting the findings of this study are available from the corresponding authors upon request.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.01.009.

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