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Targeted lipidomics reveals activation of resolution pathways in knee osteoarthritis in humans

H.S. Jónasdóttir † ‡ [a], H. Brouwers ‡ [a], J.C. Kwekkeboom ‡, H.M.J. van der Linden §, T. Huizinga ‡, M. Kloppenburg ‡ ||, R.E.M. Toes ‡, M. Giera †, A. Ioan-Facsinay ‡ [*]

† *Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, The Netherlands*
‡ *Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands*
§ *Department of Orthopedics, Leiden University Medical Center, Leiden, The Netherlands*
|| *Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands*

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SUMMARY

Objective: To investigate the presence of inflammation and resolution pathways in osteoarthritis (OA).
Design: Tissues were obtained from knee OA patients and control rheumatoid arthritis (RA) patients. Cells in synovial fluid (SF) were visualized by flow cytometry. Cytokines and chemokines were measured by multiplex assay. Lipid mediators (LMs) were determined by targeted lipidomics using liquid-chromatography mass spectrometry.
Results: SF of OA patients contained less cells, especially neutrophils, less cytokines and comparable levels of chemokines compared to RA controls.
Thirty-seven lipids were detected in the soluble fraction of SF, including polyunsaturated fatty acids (PUFAs) and their pro-inflammatory and pro-resolving lipoxygenase (LOX) and cyclooxygenase (COX) pathway markers in both OA and RA patients. Among these, pro-inflammatory LM such as prostaglandin E_2 (PGE_2) and thromboxane B_2, as well as precursors and pathway markers of resolution such as 17-HDHA and 18-HEPE were detected. Interestingly, the pro-resolving lipid RvD2 could also be detected, but only in the insoluble fraction (cells and undigested matrix). Ratios of metabolites to their precursors indicated a lower activity of 5-LOX and 15-LOX in OA compared to RA, with no apparent differences in COX-derived products. Interestingly, synovial tissue and SF cells could produce 5-LOX and 15-LOX metabolites, indicating these cells as possible source of LM.
Conclusions: By using a state-of-the-art technique, we show for the first time that resolution pathways are present in OA patients. A better understanding of these pathways could guide us to more effective therapeutic approaches to inhibit inflammation and further structural damage in OA and RA.

Introduction

Osteoarthritis (OA) is the most common form of arthritis, with a prevalence of more than 70% in the elderly population. Characteristic radiographic features of OA are cartilage degradation and the presence of osteophytes (bone spurs). It has recently become evident that synovitis is an accompanying feature of OA in a significant number of patients and that inflammation is an important player in OA (reviewed in 1 and 2) as it is associated with pain, as well as radiographic progression. The association with radiographic progression seems to be even stronger in patients with persistent inflammation[3]. The reason for persistent inflammation in some patients is unclear, but one intriguing possibility is that the essential resolution pathways are incompletely/not activated.

Inflammation is usually a self-resolving process initiated as a response to danger signals. This response is tightly regulated and involves the concerted and timely action of several molecular and

* Address correspondence and reprint requests to: A. Ioan-Facsinay, Department of Rheumatology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands. Fax: 31-71-526-6752.
E-mail addresses: H.S.Jonasdottir@lumc.nl (H.S. Jónasdóttir), H.Brouwers@lumc.nl (H. Brouwers), J.C.Kwekkeboom@lumc.nl (J.C. Kwekkeboom), H.M.J.van_der_linden@lumc.nl (H.M.J. van der Linden), T.W.J.Huizinga@lumc.nl (T. Huizinga), G.Kloppenburg@lumc.nl (M. Kloppenburg), R.E.M.Toes@lumc.nl (R.E.M. Toes), M.A.Giera@lumc.nl (M. Giera), A.Ioan@lumc.nl (A. Ioan-Facsinay).
[a] These authors contributed equally to this work.

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cellular players. Extensive studies of acute inflammation in a model of self-resolving inflammation in mice indicated that the initial phases of inflammation are characterized by neutrophil recruitment, followed by macrophage accumulation during the resolution process[4,5].

At the molecular level, cytokines and lipids are involved in regulating inflammation. Pro-inflammatory mediators, such as cytokines, chemokines and eicosanoids (e.g., prostaglandins and leukotrienes), a class of lipid mediators (LMs) derived from arachidonic acid (AA), are released during the initial phases of inflammation, driving recruitment and activation of immune cells[4]. Resolution of inflammation has been shown to be an active process originating early in inflammation, being driven by anti-inflammatory and pro-resolving mediators[6–8]. Among these, several families of specialized pro-resolving mediators (SPMs) have been identified (lipoxins, resolvins, protectins and maresins) that can induce resolution of inflammation in murine acute inflammatory models[9–11]. Moreover, they appear to be regulated during the disease course in asthma, Alzheimer's disease[12–14], multiple sclerosis[15], cystic fibrosis[16], as well as ulcerative colitis patients[17], indicating a possible role for SPMs in regulating inflammation in human disease.

The SPMs known to date are synthesized enzymatically through lipoxygenase (LOX), cyclooxygenase (COX), or cytochrome P450 (CYP) pathways from polyunsaturated fatty acids (PUFAs) such as AA, docosahexaenoic acid (DHA), or eicosapentaenoic acid (EPA), mostly through transcellular processes involving different types of cells[18,19]. More recently, other mechanisms such as microparticle uptake, phagocytosis and sequential stimulation with different stimuli have been implicated in the generation of SPMs[20,21].

In contrast to cytokines and chemokines, the presence of bioactive (oxy)lipids has only scarcely been investigated in OA. The available studies indicated the presence of the 15-LOX product 15-HETE and the COX product prostaglandin E_2 (PGE_2) in plasma and these appeared to be higher in OA patients than healthy controls[22]. Similarly, the 5-LOX product leukotriene B_4 (LTB_4) was described in synovial fluid (SF) of OA patients[23], as well as $PGF_{2\alpha}$, the non-enzymatically made 8-iso-$PGF_{2\alpha}$, and the deactivation product 15-keto-13,14-dihydro-$PGF_{2\alpha}$ in both SF and plasma[24]. Both LTB_4 and PGE_2 have been shown to be secreted by OA synovial explants[25]. Interestingly, 5-LOX and 15-LOX have been shown to be present in the OA synovium, however most of their LM products were not yet studied in detail[26]. Specifically, the presence of LMs associated with resolution of inflammation, SPMs or their precursors, has not yet been investigated in OA, despite the important role inflammation plays in the progression of structural damage.

The aim of this study was to investigate the activation of resolution in OA by studying the presence of bioactive lipids associated with resolution pathways in SF of OA patients. To this end, we employed a state-of-the-art targeted lipidomics approach to detect SPMs and their precursors in end-stage knee OA patients. Moreover, we extensively characterized inflammatory cells, cytokines and chemokines in SF and compared the results to rheumatoid arthritis (RA), as a control chronic inflammatory disease.

Materials and methods

Chemicals and materials

Listing of chemicals and other materials can be found in Supplementary Materials and Methods.

Patients and tissue sample collection

SF and synovial tissues from knee OA and RA patients were obtained as anonymized leftover material from patients undergoing knee arthroscopy at the department of Rheumatology or undergoing knee-replacement surgery at the Departments of Orthopaedic Surgery in the LUMC or Alrijne Hospital in Leiden, performed for standard clinical care. Diagnosis in all patients was established by the treating physician. Age, gender, and BMI are reported in Supplementary Table 1. This procedure was approved by the local ethical committee. SF samples were treated as described below and in Supplementary Fig. 1 and were stored at −80 °C until analysis. The average time to analysis was 7 months (range: 1 day−19 months).

Isolation of soluble and insoluble fraction of SF

One mL SF was treated with hyaluronidase, followed by centrifugation at 931 ×*g* for 10 min as described in Supplementary Fig. 1. The supernatant (soluble fraction) was removed and the pellet (insoluble fraction) was resuspended in 1 mL water. Proteins were precipitated from both soluble and insoluble fractions with 3 mL methanol (MeOH) (3184 ×*g* for 15 min at 4 °C). The MeOH supernatant was removed, the protein pellet washed again with 1 mL MeOH and internal standard (IS) was added (LTB_4-d4, 15(*S*)-HETE-d8 and PGE_2-d4, 150 pg each and DHA-d5 1500 pg). Next, the sample was spun again before combining the MeOH supernatants. After drying down the MeOH, diluting it with water and acidifying, the samples were loaded on 3 mL 500 mg Bond Elut C-18 solid-phase extraction (SPE) columns (Agilent Technologies Santa Clara, CA, USA) as described in the legend of Supplementary Fig. 1 and lipids were analyzed as described below.

Lipid analysis

Targeted lipidomics analysis of the SF was carried out after SPE as previously described[27] with some modifications (Supplementary Fig. 1). Liquid-chromatography combined with mass spectrometry (LC-MS/MS) analysis was carried out as previously published[28] with some modifications (Supplementary Materials and Methods).

Synovial tissue cells

Synovial tissue cells (synoviocytes) were isolated from fresh synovial tissue digested for 1.5 h with 1 mg/mL collagenase type 2 (Worthington Biochemical Corporation, Lakewood, NJ, USA) in serum free IMDM medium (Lonza, Basel, Switzerland). Digested tissue was filtered over a 70 μm cell strainer (Falcon, Corning Incorporated, Life Sciences, Durham, NC, USA) to obtain the cells present in synovium. The cells were washed 3 times with serum free IMDM medium before use.

Stimulation of SF cells and synoviocytes

Both SF cells (SFCs) and synoviocytes were first filtered over a 70 μm cell strainer (Falcon, Corning Incorporated, Life Sciences, Durham, NC, USA), then isolated cells suspended in PBS with calcium and magnesium (PBS (+/+), Sigma Aldrich, Steinheim, Germany) were stimulated with 4 μM calcium ionophore A23187 (Sigma, Saint Louis, MO, USA) or vehicle control for 10 min. For LPS stimulation experiments, SFC and synoviocytes were suspended in PBS (+/+) containing 0.1% fatty acid free BSA (Sigma, Saint Louis, MO, USA) and stimulated with 10 ng/mL LPS (Sigma, Saint Louis, MO, USA) for 72 h. Next, proteins were precipitated by adding 3 volumes of MeOH and IS (0.75 ng/mL final concentration). Samples were stored under argon at −80 °C until analysis. Before LC/MS−MS analysis, samples were centrifuged at 16,100 ×*g* for 10 min at 4 °C and supernatants diluted 1:1 with water. Precipitated protein was

quantified using a Micro BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA). The amount of protein per sample varied between 1 μg and 15 μg for SFC and between 5 μg and 34 μg for synoviocytes. Lipid analysis was performed as described in the Supplementary Materials and Methods.

Cytokine and FACS analysis

Cytokine and FACS analysis were performed as described in the Supplementary Materials and Methods.

Data analysis and statistics

LC-MS/MS peaks were integrated with manual supervision and area corrected to corresponding IS with MultiQuant™ 2.1 (Sciex, MA, USA). When possible, lipids were quantified based on a calibration line. Values were normalized to the amount of SF from which they originated (presented as ng lipid/mL SF) or to the amount of protein present in the samples as surrogate for cell numbers (presented as area ratio/mg protein). Paired samples were compared by a 2-tailed Spearman's correlation (SPSS Statistics for Windows, IBM Corp, Armonk, NY USA) and analytes with *P*-values < 0.05 were used in further analyses.

Differences between the two batches of SF, and differences between OA and RA groups for cell numbers, cytokine concentrations, and lipid concentrations were assessed using Mann–Whitney signed rank tests with Bonferroni corrections (GraphPad Prism 6, GraphPad Software, La Jolla, California, USA). All *P*-values indicated in the figure legends are Bonferroni corrected. Uncorrected *P*-values for lipids can be found in Supplementary Table 2.

Results

Inflammatory cells and cytokines in SF

To assess the inflammatory state of the patients, FACS analysis of SFC was performed on samples from 11 OA patients and 12 RA patients. The number of inflammatory cells was low in most OA samples [Fig. 1(A)]. Neutrophils, monocytes and T cells were present in comparable numbers, while the number of B cells was very

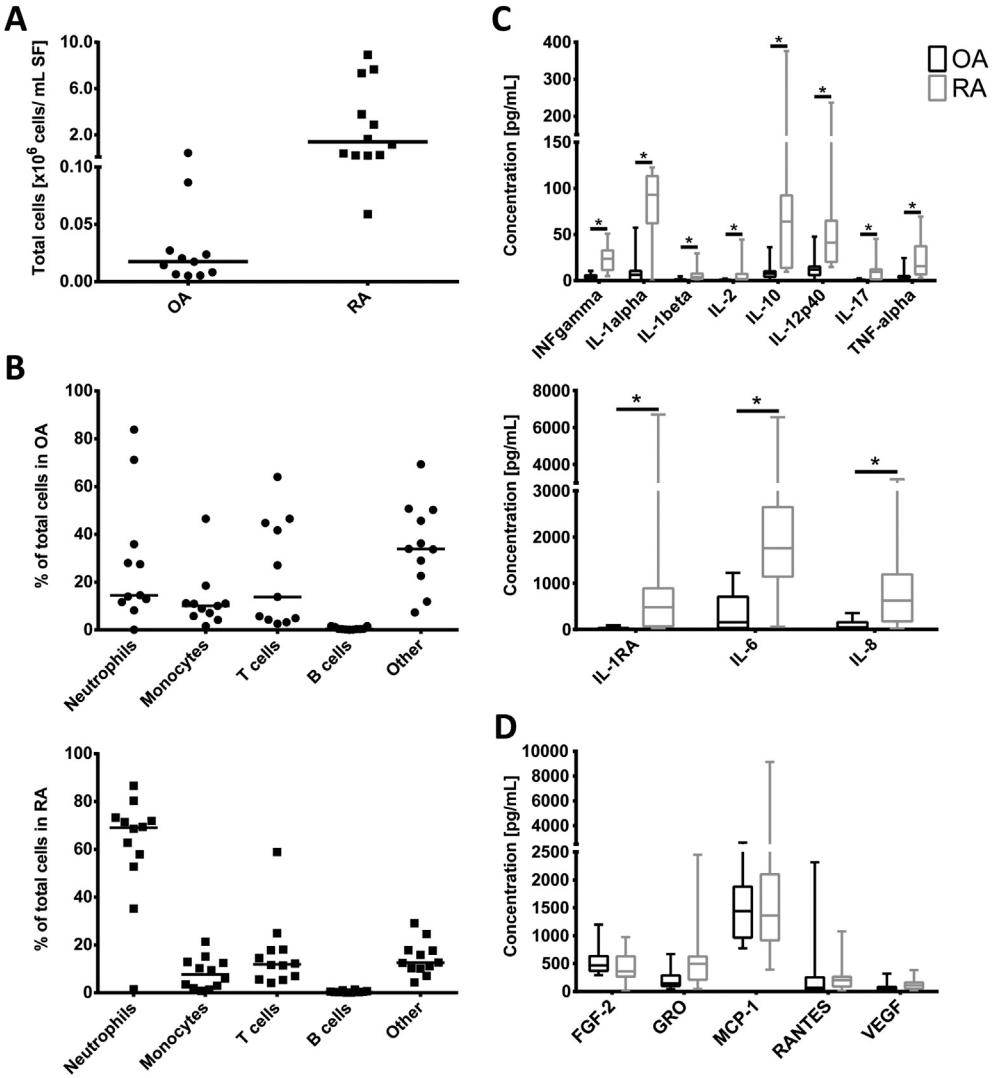

Fig. 1. Inflammatory cells and cytokines in SF. **A**, Quantification of inflammatory cells in OA ($n = 11$, circles) and RA ($n = 12$, squares) samples. **B**, Percentages of different cell populations in SF of OA (top) and RA (bottom) are shown. Each dot represents one patient. Medians are depicted. Quantification of SF cytokines (**C**) and chemokines (**D**) in OA ($n = 30$) and RA ($n = 15$) samples. Medians with interquartile range are indicated. Whiskers indicate the minimum and maximum concentrations. Groups were compared using Mann–Whitney signed rank tests. *: $P < 0.002$.

low. Interestingly, a relatively large percentage of cells could not be attributed to these populations and remains to be determined [Fig. 1(B), top]. The RA samples contained higher cell numbers and neutrophils were predominant, while monocytes, T cells and B cells were present in percentages comparable to those in the OA samples [Fig. 1(B), bottom].

Both pro- and anti-inflammatory cytokines were detectable in the 30 OA patients and 15 RA patients we analyzed, but had lower levels in OA samples [Fig. 1(C)], compared to RA samples. Most cytokines correlated well with total cell numbers (data not shown). All measured chemokines were similar in OA and RA [Fig. 1(D)].

Targeted lipidomics analysis

With the LC-MS/MS platform used in this study, we can detect 60 analytes (Supplementary Table 2), including SPMs, such as for example resolvin E2 (RvE2) in whole blood supplemented with EPA[28] or RvD2 spiked into SF before hyaluronidase treatment (data not shown). Of these analytes, 37 were detected in at least one of the SPE worked-up samples (Supplementary Table 2). Concentrations in OA samples that were hyaluronidase-treated before storage were compared to samples treated after storage for several analytes (data not shown) and as no systematic differences were found, the batches were combined and further analyzed as one. SPE precipitation after storage was compared to MeOH precipitation immediately upon collection in 20 randomly selected samples with rheumatic diseases (OA, RA and others)[29]. For 28 of the 37 detected analytes, the concentrations determined by the two methods correlated well (Supplementary Table 2). Further analysis was restricted to these 28 analytes, which were determined upon SPE treatment in 24 OA and 10 RA samples.

LMs derived from ω-6 and ω-3 PUFA

Levels of PUFAs and oxylipids in both OA and RA samples are depicted in Figs. 2 and 3, and Supplementary Fig. 2. The analyte concentration in each patient group is depicted in Supplementary Table 2. Seven PUFAs were detected in SF of OA patients: the ω-6 FAs, AA, adrenic acid (AdA) and linoleic acid (LA), and the ω-3 FAs, EPA, DHA, docosapentaenoic acid (DPA_{n-3}) and alpha-linolenic acid (ALA)/gamma-linolenic acid (GLA). Moreover, oxidized products (both enzymatic and non-enzymatic) of these PUFAs were detected, including COX-1/2 and 12-LOX products of AA and 5- and 15-LOX products of multiple PUFAs. These included the precursors of SPMs: 15-HETE (precursor of lipoxin A_4), 17-HDHA (precursor of D series resolvins) and 18-HEPE (precursor of E series resolvins). LTB_4, 6-trans-LTB_4, and 20-OH-LTB_4 were low to undetectable in the OA samples. In general, the metabolites detected in OA were present at comparable levels in the RA samples, except the 5- and 15-LOX products of AA: 15-HETE, 6-trans-LTB_4, and 20-OH-LTB_4 (Fig. 2), and the 15-LOX metabolite of AdA, 17-HDoTE (Fig. 3), which were higher in RA than OA samples. None of the SPMs that can be measured with our platform (see Supplementary Table 2) could be detected in any of the samples.

Enzymatic pathways

The presence of a certain oxylipid is dependent on both the availability of its precursor and the activity of the enzyme involved in its generation. To assess the relative presence of certain enzymatic pathways in OA compared to RA patients, we established the ratios of oxylipids to their respective PUFA precursor. These ratios indicated that five metabolites of AA, one of DHA, and one of AdA,

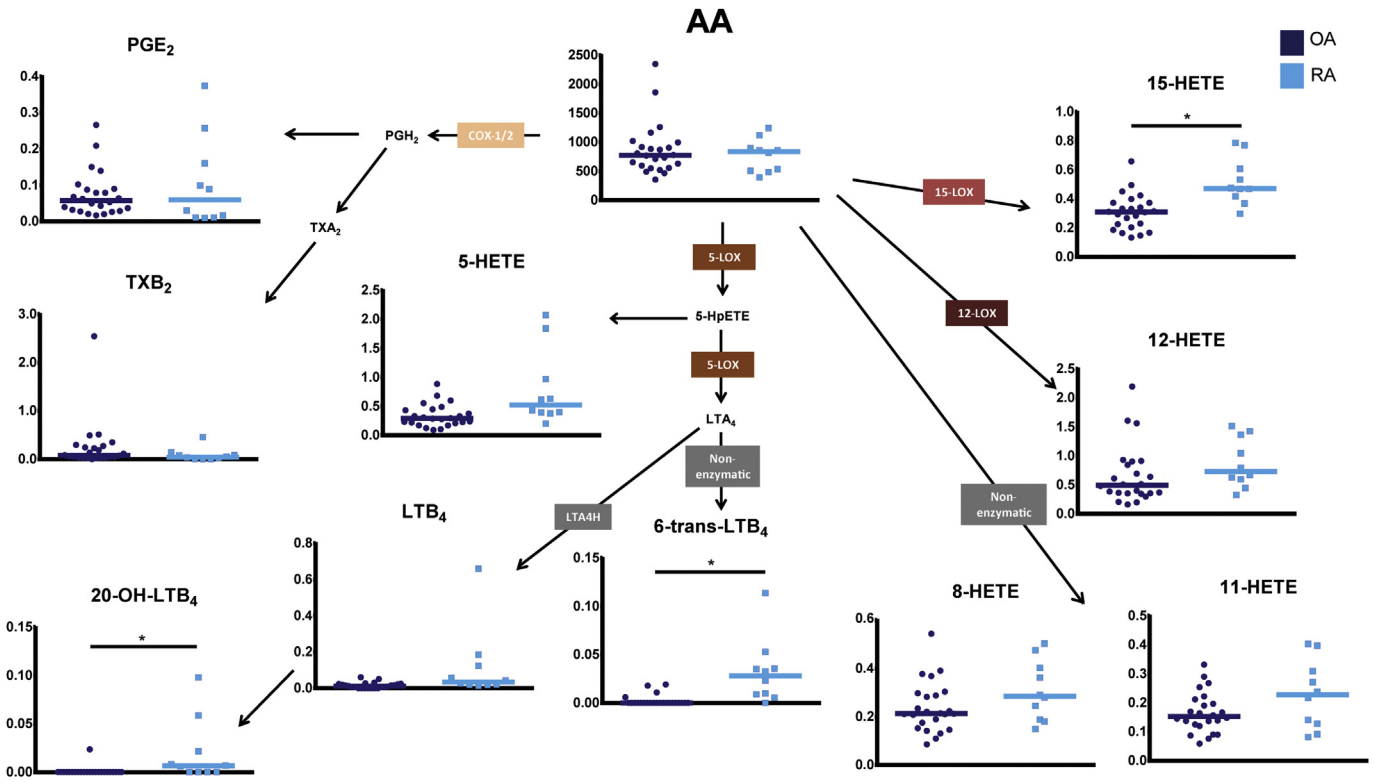

Fig. 2. Concentrations (in ng per mL SF) of AA and its metabolites measured in 24 OA and 10 RA samples. Each dot represents one patient. Medians are depicted. Groups were compared using Mann–Whitney signed ranked tests. *: $P < 0.01$. HETE: hydroxyeicosatetraenoic acid, LTA_4: leukotriene A_4, PGH_2: prostaglandin H_2, TXA_2: thromboxane A_2.

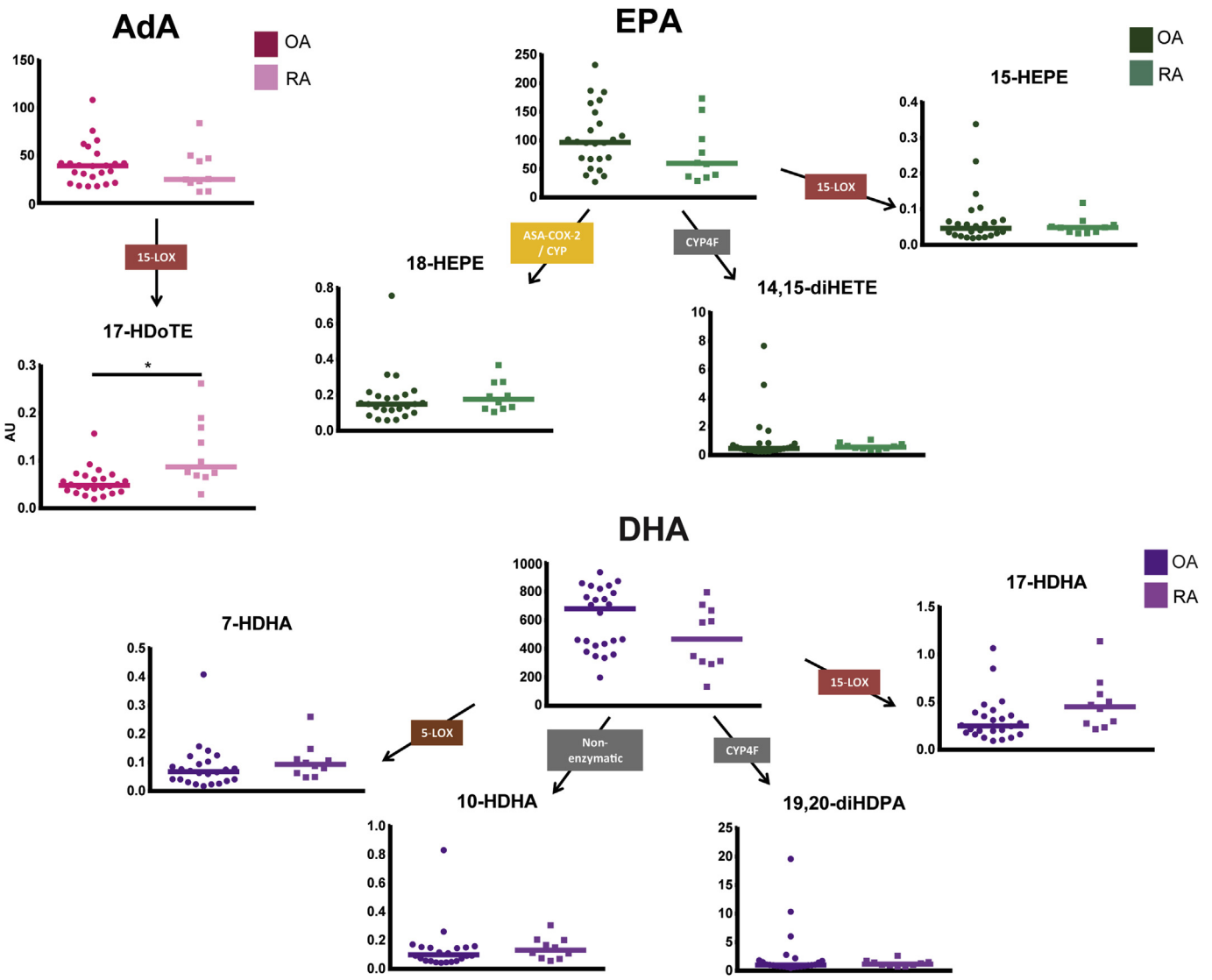

Fig. 3. Concentrations (in ng per mL SF) of EPA, DHA, AdA, and their metabolites measured in 24 OA and 10 RA samples. Levels of 17-HDoTE are in AUs. Each dot represents one patient. Medians are depicted. Groups were compared using Mann–Whitney signed ranked tests. *: $P < 0.05$. HEPE: hydroxyeicosapentaenoic acid, HDHA: hydroxydocosahexaenoic acid, diHETE: dihydroxyeicosatetraenoic acid, diHDPA: dihydroxydocosapentaenoic acid, HDoTE: hydroxydocosatetraenoic acid. ASA-COX: acetylated cyclooxygenase, CYP4F: Cytochrome P450 4F.

are less efficiently generated in OA than in RA [Fig. 4(A) and (B) and data not shown for 20-OH-LTB_4]. Of these, four are generated via the 5-LOX pathway [Fig. 4(A) and 20-OH-LTB_4] and three via the 15-LOX pathway [Fig. 4(B)]. These metabolites included the SPM precursors 15-HETE and 17-HDHA. No differences were found in metabolites generated via the COX-driven pathway [Fig. 4(C)], or the activity of leukotriene A4 hydrolase (LTA4H), assessed indirectly by using the concentration ratio of LTB_4 to 5-HETE [Fig. 4(D)]. These data are interesting, as they indicate that the 5-LOX and 15-LOX pathways are less activated in OA than in RA, while other enzymatic pathways are similar.

Bioactive LMs and their precursors/pathway markers in OA joint cells

To assess which cells present in the knee joint could be responsible for the production of the oxylipids detected in OA SF, we isolated synoviocytes and SFC from OA patients. These were studied either unstimulated, directly *ex vivo* or after 3 days of culture, or after stimulation (Fig. 5 and Supplementary Fig. 3). Calcium ionophore stimulation was used as a potent activator of cPLA2 and subsequent bioactive LM synthesis[20,30,31], while LPS was used as a model TLR4 stimulus, as TLR4 is believed to mediate activation of synovial cells in OA through binding of extracellular matrix breakdown products[32]. The unstimulated synoviocytes contained detectable levels of AA, EPA and DHA, as well as AA 5-LOX derivatives 5-HETE and LTB_4, and 15-LOX derivative 15-HETE indicating presence of activated 5-LOX and 15-LOX in these cells [Fig. 5(A), "−"]. In contrast, these lipids were only detectable in a part of the patients in SFC [Fig. 5(B), "−"]. Remarkably, LTB_4 could not be detected in SFC samples, while it was detectable in synoviocytes of all patients [Fig. 5(A) and (B), "−"]. After 3 days of culture, synoviocytes additionally contained detectable levels of the SPM precursors 17-HDHA and 18-HEPE, while these metabolites were undetectable in all SFC samples [Fig. 5(C) and (D), "−"]. Upon calcium ionophore stimulation, increased levels of AA, EPA, DHA, 5-HETE, 15-HETE, and LTB_4 [Fig. 5(A), "+"] were observed in synoviocytes of all patients. A similar trend was observed for SFC after calcium ionophore stimulation for AA, 5-HETE and LTB_4, although the data is likely underpowered to reach significance [Fig. 5(B),

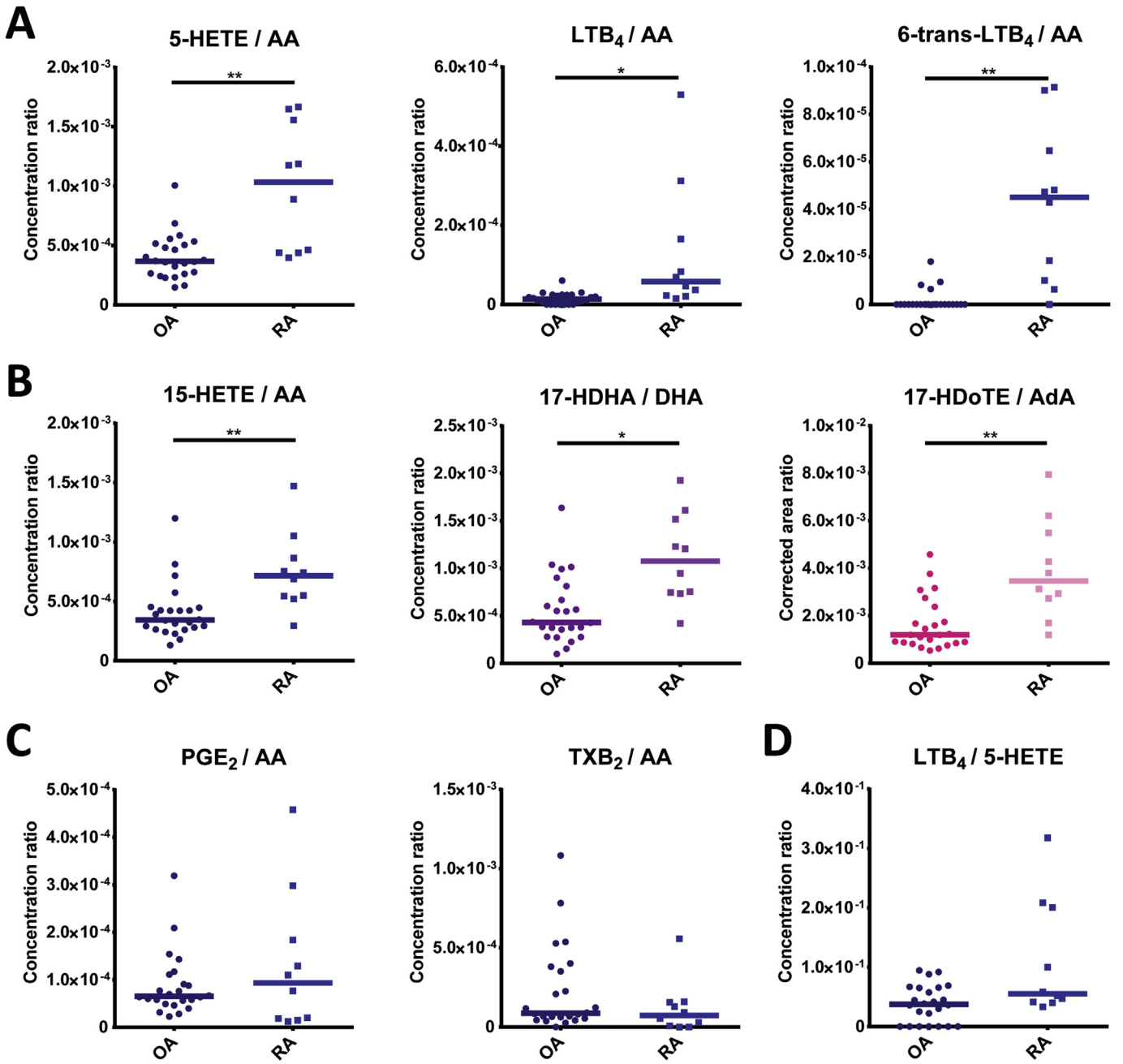

Fig. 4. **A**, Concentrations ratios of 5-LOX products, 5-HETE, LTB_4, and 6-trans-LTB_4 to their precursor AA. **B**, Concentration ratios of 15-LOX products 15-HETE to AA, 17-HDHA to DHA and corrected area ratio of 17-HDoTE to AdA. **C**, Concentration ratios of COX products PGE_2 and TXB_2 to AA. **D**, Concentration ratio of 5-LOX products LTB_4 and 5-HETE. Each dot represents one patient (24 OA and 10 RA). Medians are depicted. Groups were compared using Mann–Whitney signed ranked tests. *: $P < 0.05$, **: $P < 0.01$.

"+"]. LPS stimulation over 3 days had overall low effects and resulted in a significant, albeit small increase in EPA and 15-HETE in synoviocytes [Fig. 5(C), "+"]. Neither RvD2, nor other SPMs could be detected in either stimulated or unstimulated cells. These data indicate that both synoviocytes and SFC could contribute to the LM profile observed in SF of OA patients.

LMs in soluble and insoluble fraction of SF

Our data indicate the activation of resolution pathways in OA and RA. Because we did not detect the final pro-resolving lipids, we questioned whether this could be due to the isolation procedure. To investigate this possibility, we did a crude fractionation of five OA SF samples, in which we treated SF with hyaluronidase and then separated the supernatant (the soluble fraction) from the pellet (the insoluble fraction) (Fig. 6 and Supplementary Fig. 4).

Consistent with the results in Figs. 2 and 3, and Supplementary Fig. 2, we detected PUFAs, the monohydroxylated precursors of the SPMs like 15-HETE and 17-HDHA, as well as pro-inflammatory LMs such as PGE_2 and thromboxane B_2 (TXB_2) in the soluble fraction of all patients (Fig. 6 and data not shown). In the insoluble fraction, these analytes were also detectable in most patients (Fig. 6 and data not shown). Remarkably, although no SPMs could be detected in the soluble fraction, RvD2, a SPM derived from 17-HDHA, could be detected in the insoluble fraction in four out of five samples (Fig. 6 and Supplementary Fig. 4), indicating that the complete resolution pathway is activated in OA and is detectable in the joint.

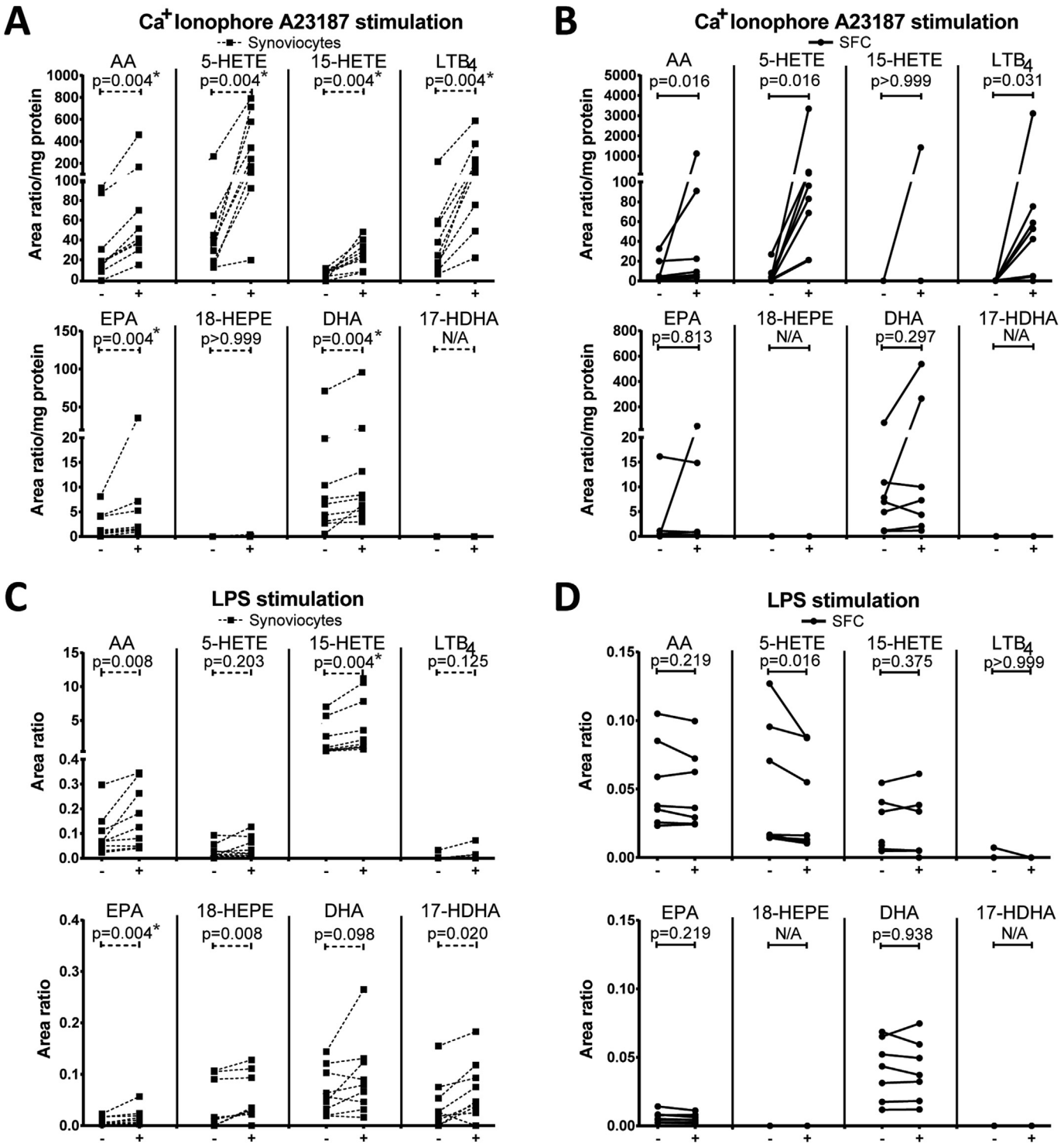

Fig. 5. Protein corrected levels of PUFAs and oxylipids in calcium ionophore stimulated ("+") and unstimulated ("−") synoviocytes from nine patients (**A**) and SFCs from seven patients (**B**). Areas corrected to IS of PUFAs and oxylipids in LPS stimulated ("+") and unstimulated ("−") synoviocytes from nine patients (**C**) and SFCs from seven patients (**D**). Samples were compared using Wilcoxon matched-pairs signed rank test. * indicates significant differences after Bonferroni correction ($P < 0.0042$).

Discussion

In this study, we have characterized the inflammation present in the OA knee joint, by identifying inflammatory cells, cytokines, chemokines, PUFAs and oxylipids present in SF and comparing them to SF from RA patients (control). Our data indicate that inflammation is qualitatively and quantitatively different in OA compared to RA, being characterized by a lower inflammatory load (cells and cytokines), in agreement with previously published data[33]. Moreover, by investigating the presence of SPMs and their precursors, as biomarkers of resolution, we found that resolution pathways are activated in OA, as well as RA. Remarkably, the SPM precursors are present in the fluid phase of SF, while SPMs are detectable only in the insoluble fraction. Additionally, the

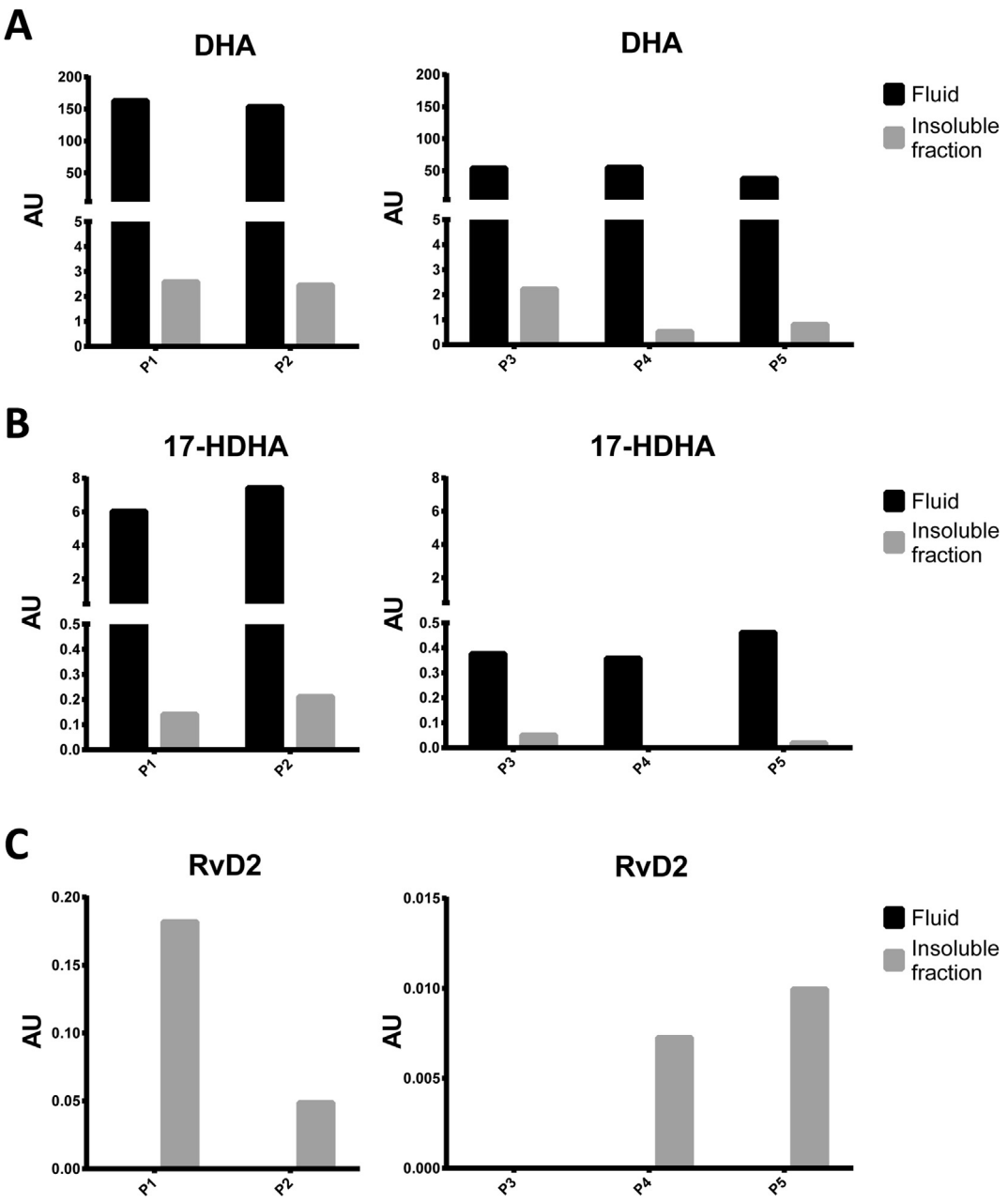

Fig. 6. Both supernatant and insoluble fraction of OA SF samples were worked-up with SPE as described in Materials and Methods. Areas corrected to IS area (AU) are shown for DHA (**A**), its metabolites 17-HDHA (**B**) and RvD2 (**C**) in both the soluble (black) and insoluble (gray) fractions of the SF for five patients (P1–5). Samples were measured in two batches (presented left and right).

enzymatic pathways involved in inflammation and its resolution seem to be less activated in OA than in RA. Finally, our data suggest that metabolites generated by these enzymatic pathways can be produced by OA synoviocytes and SFC.

Our data are in line with previous reports, indicating that SF of OA patients contains less inflammatory cells than of RA patients. Moreover, the composition of the cellular infiltrate was also different in these diseases, with OA SF containing relatively less infiltrating neutrophils than RA SF, but comparable percentages of monocytes, T cells and B cells. While Growth-Regulated Oncogene (GRO) levels are similar in OA and RA, other neutrophil chemoattractants, such as IL-8 and LTB_4, which are lower in OA than RA, could account for the observed differences in the neutrophil population.

We have detected several pro- and anti-inflammatory cytokines, as well as pro- and anti-inflammatory LMs in SF of OA and RA. The inflammatory cell infiltrate correlated with the detected cytokine levels, suggesting that inflammatory cells in SF have a significant contribution to the production of these cytokines. In contrast, chemokine and most lipid levels were not different between OA and RA, despite differences in infiltrating cells numbers.

Interestingly, synovial cells (synoviocytes) and, to a lesser extent, SFC contained detectable levels of free fatty acids and derivatives even in the absence of extra stimulation, indicating them as a possible source of pro-inflammatory oxylipids, as well as SPM precursors in the joint. Moreover, synoviocytes and SFC were able to produce AA-derived oxylipids upon activation with calcium ionophore.

Although the stimulus driving inflammation in OA is still unclear, there are indications that TLR4 could play a role in the disease by binding extracellular matrix breakdown products. Our data suggest a dual function for this receptor, both in the induction of inflammation and its resolution since TLR4 stimulation enhanced LXA_4 precursor 15-HETE production in synoviocytes. The temporal relationship between these functions remains to be elucidated. Moreover, in contrast to a previously published study[34], we could not detect LXA_4 upon stimulation of synoviocytes. The discrepancy could be explained by differences in experimental set-up, as the previous study has used synovial tissue explants, while we used isolated cells in order to minimize variations in cell number and composition inherent to explants. Moreover, the previous study has detected LXA_4 by Enzyme-Linked Immunosorbent Assay (ELISA), while we used a more specific targeted lipidomics approach based on LC-MS/MS.

As SPMs and their precursors are biomarkers of resolution pathways, our data indicate that the resolution pathways are activated in OA. Intriguingly, however, although the SPM precursors were readily detectable in the soluble fraction of SF, only one SPM could be found, and only in the insoluble fraction containing cells and undigested tissue matrix. While the mechanisms underlying this dichotomy are still unclear, it is possible that other SPMs are either present only in low amounts in the SF volume that we tested, falling below our detection limit, or that they are short-lived. In either case, their association with the insoluble fraction could imply a short range of action around the cells that secrete them. Future studies are needed to address these possibilities. Likewise, the possible role for RvD2 in OA needs to be further studied. Previous data have indicated that RvD2 could attenuate pain in a fibromyalgia model[35] and in a model of inflammatory pain[36] in mice. Therefore, it is conceivable that the presence of RvD2 could be associated with less joint pain, which is a predominant feature of OA. However, due to lack of data regarding pain, we could not investigate this in our cohort. Likewise, 17-HDHA has been previously shown to reduce pain and tissue damage in a rat arthritis model[37], while 18-HEPE could reduce IL-6 production in cardiac fibroblasts[38]. Whether these lipids also have an effect on cells/tissues involved in OA is yet unknown, but one could imagine that their described functions could be beneficial for OA.

However, despite the presence of RvD2, 17-HDHA and 18-HETE in SF, there is substantial inflammation still present in these patients, indicating that although resolution pathways are activated, they are probably incomplete or suboptimal in OA and RA. Moreover, they fail to counteract the pain and tissue destruction characteristics for these diseases. Likely explanations for this are that the LMs detected in SF might not be able to outcompete the inflammatory signals present in the joint of these patients or might not interfere with all pathways involved in the disease.

In contrast to our previous study[27] and a more recent study[39], we did not detect any SPMs in SF of RA patients. This could be due to differences in SF fluid volume and handling, as our previous samples were not treated with hyaluronidase and could therefore still have contained cells or other insoluble parts before storage and measurement. Additionally, different therapeutic treatments could influence the lipid profiles of the patients and could explain differences between cohorts.

A limited number of studies have previously shown the presence of 5- and 15-LOX in OA synovium, and have indicated in line with our findings, that the expression of these enzymes is lower in OA compared to RA synovium. However, our study now shows that lipids generated by these enzymes are present in SF of OA patients and detectable in synovial cells and to a lesser extent in SF cells, indicating that these enzymes are active in the OA synovium.

The (oxy)lipid profiles detected in RA SF were similar to the ones found in OA. Despite the higher inflammatory load present in the RA samples, the efficiency of the generation of (pro-) inflammatory LMs via the COX seemed similar in OA and RA. Together, these data suggest that SF inflammatory cells do not significantly add to the levels of these COX-derived LMs in SF in either disease.

In contrast, oxylipids that were different between OA and RA are generated via 5-LOX or 15-LOX pathways, suggesting a lower activity of these enzymes in OA compared to RA. This is also supported by differences in ratio between 5- or 15-LOX products and their PUFA precursors. Lower levels of LTB_4/AA in OA samples than RA could, in addition to decreased 5-LOX activity, also indicate a lower expression/activity of LTA4H. However, the ratio of LTB_4 to its pathway marker 5-HETE is not different between the two groups, indicating that the LTA4H activity is likely not different in the two patient groups. This is additionally supported by higher levels of the non-enzymatic LTA_4-derived 6-trans-LTB_4 in RA.

A limitation of our study is the lack of information on the therapeutic background of the patients. As Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are known to affect directly or indirectly enzymes involved in the generation of oxylipids, we cannot exclude that medication influenced oxylipid levels.

In conclusion, we have shown that knee SF of OA patients contains several inflammatory cells, as well as pro- and anti-inflammatory cytokines and oxylipids. In comparison to OA patients, the inflammatory load is higher in RA, with predominantly neutrophil infiltrate, which is accompanied by higher concentrations of cytokines and a higher activity of 5- and 15-LOX enzymes. By using a state-of-the-art technique, we now show for the first time that resolution pathways are present in OA patients. A better understanding of these pathways could guide us to a novel and effective therapeutic approach to inhibit inflammation and further structural damage in inflammatory joint disease as OA and RA.

Author contributions

HS Jónasdóttir participated in acquiring the data, analysis and interpretation of the data, drafting of the article and critical revision of the article for important intellectual content, and final approval of the article.

H Brouwers participated in acquiring the data, analysis and interpretation of the data, drafting of the article and critical revision of the article for important intellectual content, and final approval of the article.

JC Kwekkeboom participated in acquiring and analyzing the data, revising the article critically for important intellectual content, and final approval of the article.

EMJ van der Linden participated in acquisition of data, revising the article critically for important intellectual content, and final approval of the article.

T Huizinga participated in interpretation of data, revising the article critically for important intellectual content, and final approval of the article.

M Kloppenburg participated in interpretation of data, revising the article critically for important intellectual content, and final approval of the article.

REM Toes participated in interpretation of data, revising the article critically for important intellectual content, and final approval of the article.

M Giera participated in the conception and design of the study, interpretation of data, revising the article critically for important intellectual content, and final approval of the article.

A Ioan-Facsinay participated in the conception and design of the study, interpretation of data, drafting and critical revision of the article for important intellectual content, and final approval of the article.

Conflict of interest

None.

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Supplementary data

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