LXB4 and RvE1 regulate tendon inflammation

Proresolving mediators LXB4 and RvE1 regulate inflammation in stromal cells from patients with shoulder tendon tears

Stephanie G Dakin¹, Romain A Colas², Kim Wheway¹, Bridget Watkins¹, Louise Appleton¹, Jonathan Rees¹, Stephen Gwilym¹, Christopher Little¹, Jesmond Dalli²,³*, Andrew J Carr¹*

¹ Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Botnar Research Centre, University of Oxford, Nuffield Orthopaedic Centre, Oxford, OX3 7LD, UK

² Lipid Mediator Unit, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London, EC1M 6BQ, UK

³Centre for inflammation and Therapeutic Innovation, Queen Mary University of London, London, London, UK

* equal senior author contribution

Corresponding author Associate Professor Stephanie G Dakin:

stephanie.dakin@ndorms.ox.ac.uk

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ABSTRACT:

Tendon stromal cells isolated from patients with chronic shoulder rotator-cuff tendon tears show dysregulated resolution responses. Current therapies do not address the biological processes concerned with persistent tendon inflammation, therefore new therapeutic approaches targeting tendon stromal cells are required. We determined if two specialised pro-resolving mediators (SPM) LXB₄ and RvE1, modulated the bioactive lipid mediator (LM) profiles of IL-1β stimulated tendon cells derived from patients with shoulder tendon tears and healthy volunteers. We also determined if LXB₄/RvE1 treatments moderated the pro-inflammatory phenotype of tendon tear stromal cells. Incubation of IL-1β treated patient derived tendon cells in LXB₄/RvE1 upregulated concentrations of SPM. RvE1 treatment specifically increased 15-epi-LXB₄ and regulated PGF₂α. LXB₄ or RvE1 also induced expression of the SPM biosynthetic enzymes 12-lipoxygenase (ALOX12), and ALOX15. RvE1 treatment upregulated proresolving receptor ERV1 compared to vehicle treated cells. Incubation in LXB₄ or RvE1 moderated the proinflammatory phenotype of patient derived tendon tear cells, regulating markers of tendon inflammation, including Podoplanin, CD90, STAT-1 and IL-6. These treatments also suppressed JNK1/2/3, Lyn, STAT-3 and STAT-6 and induced p70s6kinase phospho-kinase signalling. LXB₄ and RvE1 counter-regulate inflammatory processes in tendon stromal cells, supporting the role of these molecules as potential therapeutics to resolve tendon inflammation.

KEY WORDS: inflammation, resolution, shoulder, tendon, tendinopathy, tear, Lipoxin, Resolvin
INTRODUCTION

Diseases of the joint are a considerable global economic burden, accounting for 5 of the top 15 causes of years lived with disability in well-resourced health systems. Shoulder rotator cuff tendon tears are a progressive inflammatory and fibrotic condition affecting 15% of 60-year olds and 50% of 80-year olds. Affected patients experience pain and restricted joint motion, severely limiting activities and disrupting life quality. Current treatments include physical therapy, non-steroidal anti-inflammatory drugs, platelet rich plasma, glucocorticoid injections and surgery to repair torn tendons. These therapies are frequently ineffective, glucocorticoids are potentially harmful and tendon tear surgery is associated with high post-operative failure rates. Of importance, COX-2 selective NSAIDs dampen protective responses regulating resolution of inflammation, paradoxically impeding the ability of inflamed tendons to heal. To address this unmet clinical requirement, effective new therapies are required that target the biological mechanisms and cells driving tendon disease.

Growing evidence supports the pivotal role of resident stromal cells including fibroblasts in inflammatory diseases of the joint. Fibroblasts are implicated in the switch from acute to chronic inflammation. Exposure to an inflammatory milieu induces fibroblasts to undergo phenotypic change whereby these cells exhibit characteristics of an activated state and show capacity for inflammation memory. Cross-talk between fibroblasts with tissue resident macrophages, infiltrating immune cells and endothelial cells via cytokine and chemokine gradients in inflamed tissues of the joint further promotes the development of persistent inflammation. We recently identified tendon stromal cells isolated from patients with shoulder tendon...
tears exhibited a pro-inflammatory phenotype, and showed dysregulated resolution responses compared to respective cells isolated from the tendons of healthy volunteers\textsuperscript{15}. Specialised proresolving mediators (SPM) including 15-epi-LXA\textsubscript{4} and MaR1 were found to counter-regulate the dysregulated resolution responses of diseased tendon stromal cells \textsuperscript{15}. This study also identified SPM including Lipoxin B\textsubscript{4} (LXB\textsubscript{4}) and E series resolvins were differentially regulated in cultures of tendon stromal cells isolated from patients with shoulder tendon tears compared to cells from the tendons of healthy volunteers\textsuperscript{15}. The main objective of the current study was to identify new therapeutic approaches to target pathogenic stromal cells and promote resolution of inflammation in cells isolated from patients with shoulder tendon tears. Through experiments with representative SPM including LXB\textsubscript{4} and RvE1, we provide evidence that these SPM regulate the proinflammatory phenotype and promote resolution responses in patient-derived tendon stromal cells.

\textbf{MATERIALS AND METHODS}

\textbf{Study approval}

Tendon tissues were collected from patients under Research Ethics from the Oxford Musculoskeletal Biobank (09/H0606/11). Full informed consent according to the Declaration of Helsinki was obtained from all patients.

\textbf{Collection of patient tendon tissues}

Patients with rotator cuff shoulder tendon tears were recruited from orthopaedic referral clinics. Patients had failed non-operative treatment, including a course of physical therapy, and had experienced pain for a minimum of 3 months. The presence of a supraspinatus tendon tear was identified by ultrasound scan. Patients
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completed the Oxford Shoulder Score (OSS), a validated and widely used clinical outcome measure scoring from 0 (severe pathology) to 48 (normal function).

Supraspinatus tendon tears were collected at the time of surgical debridement of the edges of the torn tendons from 15 male and female patients aged between 46 and 75 (mean 57 ± 16.3 years). All patients were symptomatic and had small to medium tears (≤1 cm to ≤3 cm in anterior-posterior length). Exclusion criteria for all patients in this study included previous shoulder surgery, other shoulder pathology and inflammatory arthritis. Diabetic patients and those receiving systemic anticoagulant therapy were also excluded from the study. Samples of healthy volunteer hamstring tendons were collected from 10 male and female patients undergoing surgical reconstruction of their anterior cruciate ligament. All healthy volunteer patients were aged between 20 and 45 (mean 27.2 ± 10 years).

**Isolation of tendon-derived stromal cells from healthy and diseased tendons.**

Tendon derived stromal cells were isolated from the tendons of patients and healthy volunteers using previously published protocols. For experiments, cells were incubated in DMEM F12 media (Gibco) containing 1% heat inactivated human serum (Sigma) and 1% Pen-Strep. Passage 1–3 cells were used for all experiments. We previously characterised tendon stromal cells as CD45neg and CD34neg cells exhibiting fibroblast morphology.

**Cytokine treatment of tendon-derived stromal cells**

IL-1β is known to induce expression of NF-κB target genes highly expressed in shoulder tendon disease. We therefore investigated the bioactive LM profiles in tendon-derived stromal cells isolated from the tendons of healthy volunteers and patients in the presence of IL-1β (10 ngmL⁻¹, Sigma) in medium (DMEM F12 phenol...
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red free medium, Gibco), containing 1% heat-inactivated human serum (Sigma) and 1% penicillin-streptomycin. Non-treated (vehicle only) cells served as experimental controls. After cytokine/vehicle treatment, cells were incubated at 37°C and 5% CO2 for 24 hours until experimental harvest of the media and lysate for bioactive LM profiling.

Modulating bioactive lipid mediator profiles of IL-1β stimulated tendon-derived stromal cells with LXB4 and RvE1

Tendon stromal cells were isolated from healthy volunteers or tendon tear patients (n-5 each) and seeded at a density of 60,000 cells per well. Once cells were 80% confluent, they were pre-incubated with 10 nM LXB4 (Cayman Chemical) or 10 nM RvE1 (Cayman Chemical) for 24 h in DMEM F12 phenol red free medium (Gibco) containing 1% heat inactivated human serum (Sigma) and 1% penicillin-streptomycin. Cells were stimulated with IL-1β (10ngml⁻¹) in the presence of media containing either LXB4, RvE1 or vehicle control as previously described 15. Parallel experiments were performed and cell lysates harvested to investigate if incubating cells in these SPM moderated the expression of markers of the pro-inflammatory phenotype of diseased tendon stromal cells and potentiated expression of SPM synthetic enzymes and receptors mediating resolution of inflammation. The concentration and integrity of mediators used for these incubations were validated using UV-spectrophotometry and LC-MS-MS in accordance with published criteria 17. Bioactive LM profiling of media and lysate samples from IL-1β stimulated tendon cells was performed using previously described methodology 15. Calibration curves were obtained for each using authentic compound mixtures and deuterium labelled
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LM at 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50, 100, and 200 pg. Linear calibration curves were obtained for each LM, which gave $r^2$ values of 0.98–0.99.

**Immunocytochemistry for LXB\textsubscript{4} and RvE1 treated tendon stromal cells**

Tendon stromal cells isolated from patients and healthy volunteers were grown in chamber slides and stimulated with IL-1\textsubscript{β} in the presence of LXB\textsubscript{4}, RvE1 or vehicle for 24 hrs as described above. Cells were fixed in ice cold methanol for 5 mins and washed with PBS. Immunofluorescence staining protocols and image acquisition are adapted from a previously published protocol\textsuperscript{15}. Tendon stromal cells isolated from healthy volunteers and patients with tendon tears (n=3 each) were incubated with the following primary antibodies: anti-ALX (Abcam, ab26316), anti-ALOX15 (Abcam ab119774), anti-ALOX12 (Abcam, ab211506), anti-ERV1 (Abcam ab167097), anti-BLT1 (Abcam ab18886), anti-STAT-1 (Abcam ab29045), anti-Podoplanin (Abcam ab10288) and anti-IL-6 (Abcam ab9324) in PBS containing 5% goat serum in Saponin for 3 hrs at room temperature. For negative controls the primary antibody was substituted for universal isotype control antibodies: cocktail of mouse IgG\textsubscript{1}, IgG\textsubscript{2a}, IgG\textsubscript{2b}, IgG\textsubscript{3} and IgM (Dako) and rabbit immunoglobulin fraction of serum from non-immunized rabbits, solid phase absorbed. Isotype control staining is shown in Figure S1. Immunofluorescence images were acquired on a Zeiss LSM 710 confocal microscope using a previously published protocol\textsuperscript{15}.

**Expression of proinflammatory and proresolving genes in tendon stromal cells incubated in LXB\textsubscript{4} or RvE1.** Tendon-derived stromal cells from healthy volunteers or patients with shoulder tendon tears (n=6 each) were seeded at a density of 20,000 cells per well in a 24 well plate. Cells were allowed to reach confluence prior
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to preincubation with LXB₄ or RvE1 and subsequent stimulation with IL-1β (10ngmL⁻¹). Non-treated cells (vehicle only, containing 0.1% endotoxin free BSA, Sigma) served as controls for each experiment. After treatment, cells were then incubated at 37 °C and 5% CO₂ until harvest of the cell lysate for mRNA after 24 h. RNA isolation, cDNA synthesis and quantitative PCR were performed using previously published protocols. Pre-validated Qiagen primer assays (ALOX15, ERV1, IL6, PDPN, CD90, β-actin and GAPDH) were used for qPCR. Results were calculated using the ddCt method using reference genes for human β-actin and GAPDH. Results were consistent using these reference genes and data are shown normalized to β-actin.

Quantification of Interleukin-6 in tissue culture media

IL-6 is an important cytokine implicated in inflammation and is abundantly released by tendon stromal cells isolated from patients with shoulder tendon tears after stimulation with IL-1β. IL-6 in tissue culture supernatants was measured using enzyme-linked immunosorbent assay (ELISA) reagents (BD Biosciences) using incubations isolated from 5 donors. Minimum detectable IL-6 concentration for this assay was 2.2 pgml⁻¹. Optical density was read on a spectrophotometric ELISA plate reader (FLUOstar Omega, BMG Labtech) and analysed using MARS data analysis software.

Phospho-signalling in LXB₄ and RvE1 treated tendon stromal cells

A human phospho-kinase array kit (R&D Systems ARY003B) was used to investigate the effects of incubating IL-1β stimulated patient derived tendon cells in LXB₄ or RvE1 on protein kinase signalling pathways (n=3 donors). Experimental
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protocols were performed according to manufacturer’s instructions on protein lysates harvested after 24h incubation in either LXB₄ or RvE1. Images were captured using a chemiluminescence documentation system (UVITEC), and densitometry analysis of proteins of interest was performed using ImageJ software (NIH).

Statistical analysis

Statistical analyses were performed using GraphPad Prism 7 (GraphPad Software). Normality was tested using the Shapiro-Wilk normality test. Analysis of bioactive LM profiles from tendon cells derived from patients and healthy volunteers was performed using multivariate statistical analysis, orthogonal-partial least squares-discriminant analysis (o-PLS-DA) using SIMCA 14.1 software (Umetrics, Umea, Sweden) following unit variance scaling of LM amounts. PLS-DA is based on a linear multivariate model that identifies variables that contribute to class separation of observations (cell incubations) on the basis of their variables (LM levels). During LM classification, observations were projected onto their respective class model. The score plot illustrates the systematic clusters among the observations (closer plots presenting higher similarity in the data matrix). Loading plot interpretation identified the variables with the best discriminatory power (Variable Importance in Projection greater then 1) that were associated with tight clusters for LM profiles obtained from incubations with cells from healthy volunteers or patients with tendinopathy. For levels of proresolving mediators and inflammation initiating eicosanoids, data are shown as summed with SEM, where n is the biological replicate. Unpaired t-tests were used to test for differences in LM levels between tendon cells derived from
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healthy volunteers and patients with shoulder tendon tears. Pairwise Mann Whitney U tests were used to determine differences in expression of proinflammatory and proresolving genes and IL-6 protein in IL-1β treated tendon stromal cells in the presence or absence of LXB₄, RvE₁ or respective vehicle. *P* < 0.05 was considered statistically significant.

RESULTS

**LXB₄ and RvE₁ treatments induce SPM release from tendon-derived stromal cells**

We previously identified that tendon stromal cells isolated from patients with shoulder tendon tears show dysregulated resolution responses compared to cells isolated from healthy volunteer tendons.¹⁵ This study identified SPM including LXB₄ and E series resolvins were differentially regulated in these incubations. To gain further insights into whether these SPM counter-regulate tendon inflammation, we therefore investigated whether LXB₄ and RvE₁ modulated the bioactive LM profiles of IL-1β stimulated tendon stromal cells isolated from the tendons of patients with shoulder tendon tears and healthy volunteers. Multivariate analysis identified differences in bioactive LM profiles between IL-1β stimulated tendon cells isolated from either healthy volunteer donors or patients with tendon tears in the presence of 10nM LXB₄ compared to vehicle only incubations, demonstrated by the distinct clustering of the LM profiles (Figure 1A-D). The molecules profiled, together with the concentrations of the individual lipid mediators identified are listed in Table S1. In these incubations, LXB₄ upregulated concentrations of SPM in IL-1β stimulated
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tendon cells from healthy volunteers (p=0.008) and tendon tear patients (p=0.008) (Figure 1E). Multivariate analysis also identified distinct clustering of the LM profiles between IL-1β stimulated tendon cells isolated from healthy volunteer donors and tendon tear patients incubated in the presence of 10nM RvE₁ compared to vehicle only (Figure 2A-D). The molecules profiled, together with the concentrations of the individual lipid mediators identified are listed in Table S1. In these incubations, RvE₁ upregulated concentrations of SPM in healthy volunteer tendon cells (p=0.008) and tendon tear patients (p=0.008) (Figure 2E). In tendon tear incubations, RvE₁ upregulated the concentrations of specific SPM including 15-epi-LXB₄ (p=0.04) and decreased levels of the pro-inflammatory eicosanoid PGF₂α (p=0.02) (Figure 2E).

LXB₄ and RvE₁ upregulate the expression of SPM biosynthetic enzymes and proresolving receptors in tendon-derived stromal cells

We next investigated the mechanisms by which LXB₄ and RvE₁ potentiated the further release of SPM. Incubation of IL-1β stimulated tendon cells isolated from tendon tear patients in LXB₄ or RvE₁ induced ALOX₁₅ mRNA expression relative to respective vehicle controls (p=0.03 respectively, Figure 3A). The same treatment of healthy volunteer tendon cells also upregulated ALOX₁₅ mRNA expression relative to respective vehicle controls (p=0.03 and p=0.01 respectively, Figure 3A). Immunostaining demonstrated these treatments also increased ALOX₁₂ and ALOX₁₅ proteins expression implicated in the biosynthesis of SPM (Figure 3B and 3C). Induction of these biosynthetic enzymes was profound in tendon cells isolated from tendon tear patients compared to healthy volunteer donors (Figures 3B&C). We also investigated if incubation of tendon tear cells in RvE₁ moderated expression of receptors to which RvE₁ is known to bind. In the presence of vehicle only, IL-1β
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...stimulated tendon tear cells showed increased CHEMR23/ERV1 mRNA compared to respective healthy tendon cells (Figure 3D). Indeed, RvE1 treatment upregulated the expression of ChemR23/ERV1 and BLT1 receptors in IL-1β stimulated tendon tear cells compared to respective vehicle controls (Figure 3E).

LXB₄ and RvE1 moderate the pro-inflammatory phenotype of tendon stromal cells, dampening pro-inflammatory signalling pathways

We next assessed whether LXB₄ and RvE1 also regulated known markers of tendon inflammation in tendon cells isolated from tendon tear patients and healthy volunteers. Incubation of IL-1β stimulated diseased cells in LXB₄ or RvE1 for 24 hrs reduced fibroblast activation marker Podoplanin (PDPN), STAT-1 and IL-6 compared to respective vehicle controls (Figure 4A). The same treatment of IL-1β stimulated healthy tendon cells also reduced PDPN, STAT-1 and IL-6 compared to respective vehicle controls (Figure 4B). Measurement of IL-6 levels in supernatant from IL-1β stimulated diseased cells demonstrated incubation in LXB₄ or RvE1 reduced IL-6 levels compared to vehicle only (p=0.02 and 0.006 respectively, Figure 4C). In HV incubations, LXB₄ or RvE1 treatment also reduced IL-6 levels (p=0.03 and p=0.01 respectively, Figure 4D). We next determined if LXB₄ or RvE1 treatment moderated expression of pro-inflammatory genes and signalling pathways in IL-1β stimulated tendon tear cells. Incubation of these cells in LXB₄ reduced IL6, PDPN and CD90 mRNA expression compared to vehicle controls (p=0.004, p=0.002 and p=0.015 respectively, Figure 4E). RvE1 treatment of IL-1β stimulated tendon tear cells also reduced IL6, PDPN and CD90 mRNA compared to vehicle controls (p=0.02, p=0.02 and p=0.015 respectively, Figure 4E). In these incubations, LXB₄ or RvE1 treatments regulated phospho-kinase signalling pathways identified in inflamed tendons,
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including JNK1/2/3 (phosphorylation sites T183/Y185, T221/Y223), Lyn (Y397), STAT-3 (Y705) and STAT-6 (Y641) compared to respective vehicle control treated cells (Figure 5). Incubation in RvE₁ also induced p70S6 (T389) kinase compared to respective vehicle controls (Figure 5).

DISCUSSION

Resident stromal cells including fibroblasts are increasingly recognised as important cell types driving chronic inflammatory joint disease ¹⁰, ¹⁸, ¹⁹. After exposure to an inflammatory milieu, tendon and synovial fibroblasts adopt a pro-inflammatory phenotype exhibiting activation and inflammation memory ¹¹, ¹², ²⁰. Distinct fibroblast subtypes that mediate joint inflammation and tissue damage have been characterised in rheumatoid synovium ²¹. Recent advances in the knowledge of how resident stromal cells behave under inflammatory conditions of the joint has prompted further investigation of the resolution responses of these cells. Given that stromal fibroblasts comprise the majority cell type of musculoskeletal soft tissues, improved understanding of how these cells respond to an inflammatory milieu is required to inform the development of therapeutic strategies targeting these cells.

We recently identified that tendon stromal cells isolated from patients with tendon tears showed increased levels of SPM and inflammation initiating eicosanoids compared to cells isolated from healthy volunteer tendons, reminiscent of a dysregulated resolution response characteristic of chronic inflammation ¹⁵. We also identified that incubation of IL-1β stimulated tendon stromal cells in either 15-epi-LXA₄ or MaR₁ regulated pro-inflammatory eicosanoids and potentiated the further
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These treatments moderated the pro-inflammatory phenotype of IL-1β stimulated diseased tendon stromal cells, dampening expression of PDPN, STAT-1 and IL-6. We previously identified that SPM including LXB₄ and E series resolvins were differentially regulated between IL-1β stimulated tendon cells isolated from patients with shoulder tendon tears compared to cells from healthy volunteer tendons. In the current study, we therefore investigated if LXB₄ or RvE₁ modulated the bioactive lipid mediator (LM) profiles of IL-1β stimulated tendon cells derived from these patient cohorts. Tendon stromal cells were stimulated with IL-1β, as this cytokine is known to induce expression of NF-κB target genes highly expressed in human tendon disease, simulating an inflammatory milieu. In these incubations, treatment with LXB₄ or RvE₁ upregulated SPM concentrations. RvE₁ treatment specifically increased 15-epi-LXB₄ and regulated PGF₂α in incubations of IL-1β stimulated diseased tendon cells. We next investigated the mechanism of action underpinning these observations. Incubating in LXB₄ or RvE₁ induced expression of SPM biosynthetic enzymes ALOX₁₂, and ALOX₁₅ in healthy and diseased tendon stromal cells. Notably, expression of these SPM biosynthetic enzymes was increased in diseased compared to healthy tendon stromal cells. We previously identified that incubation of tendon stromal cells in 15-epi-LXA₄ or MaR₁ induced ALOX₁₅ expression. The findings from the current study support these observations, suggesting a common mechanism whereby proresolving mediator activate feed forward cascades leading to the upregulation of other SPM via induction of ALOX biosynthetic enzymes. We next investigated if incubating tendon cells in LXB₄ or RvE₁ influenced expression of proresolving receptors. The receptor to which LXB₄ binds have not yet been identified, while RvE₁ is known to activate ERV₁ and a competitive inhibitor of BLT₁. In the absence of SPM treatment,
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**ERV1** mRNA expression was increased in diseased compared to healthy IL-1β stimulated tendon stromal cells, suggesting a pro-inflammatory phenotype favours increased expression of this proresolving receptor. Incubation of LXB₄ or RvE₁ did not induce ALX expression, although RvE₁ treatment further upregulated ERV1 and BLT1 expression on tendon stromal cells compared to vehicle treated cells. Collectively these findings suggest a positive feedback loop, whereby RvE₁ treatment upregulates ERV1 and BLT1 receptor expression. This may occur as a direct consequence of RvE₁ treatment, or via RvE₁ induced upregulation SPM.

We identified that incubation in LXB₄ or RvE₁ moderated the proinflammatory phenotype of patient derived tendon tear cells, regulating known markers of tendon inflammation, including PDPN, CD90, STAT-1 and IL-6. CD90 is expressed by pathogenic synovial fibroblasts from rheumatoid arthritis patients with an inflammatory and invasive phenotype. We previously identified persistent fibroblast activation may be implicated in the development of chronic tendon inflammation and increased likelihood of recurrent injury. Proresolving mediators may therefore possess therapeutic utility to moderate the pro-inflammatory phenotype of tendon stromal cells via attenuating expression of pathogenic fibroblast activation markers. In support of this, other SPM including 15-epi-LXA₄ and MaR₁ also moderated PDPN expression in IL-1β stimulated diseased tendon cells, suggesting this property is common to different families of SPM including lipoxins, resolvins and maresins.

In addition to moderating the pro-inflammatory phenotype of diseased tendon cells, we also identified LXB₄ and RvE₁ treatments regulated phosphokinases including pJNK₁/₂/₃, pLyn, pSTAT-3 and pSTAT-6. The findings from our study suggest LXB₄...
and RvE1 regulate IL-6 via suppressing STAT-3 signalling in patient derived tendon stromal cells. Suppression of STAT-6 signalling may modulate IL-4 and IL-13 responsive genes known to drive fibrosis in the advanced stages of tendon disease\(^ {16, 24}\). IL-6 is an important cytokine implicated in the cross talk between resident stromal cells including activated endothelial cells, tissue resident macrophages, fibroblasts and infiltrating immune cells\(^ {25-27}\). Given the ability of LXB\(_4\) and RvE1 to regulate IL-6 release from IL-1\(\beta\) stimulated tendon cells, these SPM may play an important role in dampening cytokine mediated cross-talk between stromal cells which actively promotes the retention of immune cells. RvE1 treatment also induced p70s6kinase signalling in IL-1\(\beta\) stimulated diseased tendon cells. RvE1 is also known to activate ERV-1 signalling via rs6 phosphorylation in peripheral blood neutrophils isolated from patients with type 2 diabetes\(^ {28}\). These findings suggest that circulating immune cells and resident stromal fibroblasts share common signalling pathways downstream of the ERV-1 receptor.

Therapies that promote resolution of inflammation are an important future therapeutic strategy to address pathogenic stroma in chronic inflammatory disease of the joint. The pro-resolving mediator resolvin D3 (RvD3) has been shown to regulate leukocyte infiltration pro-inflammatory eicosanoids in murine inflammatory arthritis\(^ {29}\). 17R-RvD1 attenuated arthritis severity, paw oedema and leukocyte infiltration, in acute murine inflammatory arthritis\(^ {30}\). The current study suggests that LXB\(_4\) and RvE1 regulate expression of tendon pro-inflammatory molecules including Podoplanin, CD90, STAT-1, IL-6, and dampen phosphokinases including JNK1/2/3, Lyn, STAT-3 and STAT-6. These SPM also potentiated further release of proresolving mediators in IL-1\(\beta\) stimulated healthy and diseased tendon cells. We
therefore propose that SPM including LXB₄ and RvE1 are potential new therapeutics to target pathogenic stromal cells and potentiate resolution of chronic tendon inflammation.

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AUTHOR CONTRIBUTIONS

Designed research: SGD, JD, AC
Performed research: SGD, RC
Contributed reagents / analytical tools: KW, BW, LA, JR, SG, CL
Analysed data: SGD, RC, JD
Wrote the paper: SGD, RC, JD, AJC
Reviewed submitted manuscript: All authors
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FIGURE LEGENDS

FIGURE 1. LXB4 upregulates SPM concentrations in IL-1β stimulated tendon stromal cells. Tendon stromal cells were derived from healthy volunteers (Healthy, H, n=5 donors) and patients with shoulder tendon tears (Diseased, D n=5 donors). Cells were incubated with LXB4 (10nM) or vehicle for 24 h at 37 °C then with IL-1β (10ngml⁻¹) for 24 h. LM were identified and quantified using LM profiling. (A) 2-dimensional score plot and (B) corresponding 2-dimensional loading plot of LM-SPM from human tendon derived-stromal cell incubations isolated from healthy volunteers incubated with IL-1β and 10nM LXB4 or vehicle only. (C) 2-dimensional score plot and (D) corresponding 2-dimensional loading plot of LM-SPM from human tendon derived-stromal cell incubations isolated from patients with shoulder tendon tears incubated with IL-1β and 10nM LXB4 or vehicle only. (E) Cumulative concentrations of proresolving mediators (DHA-derived RvD, PD, MaR, n-3 DPA-derived RvDₙ₋₃ DPA, PDₙ₋₃ DPA, MaRₙ₋₃ DPA, EPA-derived RvE and AA-derived LX) in IL-1β stimulated tendon stromal cell incubations in the presence of LXB4 (10nM) or vehicle for 24 h. Results are shown as means and SEM and representative of n=5 donors per group.

FIGURE 2. RvE1 increases SPM levels in IL-1β stimulated tendon stromal cells. Tendon stromal cells were derived from healthy volunteers (Healthy, H, n=5 donors) and patients with shoulder tendon tears (Diseased, D n=5 donors). Cells were incubated with RvE1 (10nM) or vehicle for 24 h at 37 °C then with IL-1β (10ngml⁻¹) for 24 h. LM were identified and quantified using LM profiling. (A) 2-dimensional score plot and (B) corresponding 2-dimensional loading plot of LM-SPM from human
tendon derived-stromal cell incubations isolated from healthy volunteers incubated with IL-1β and 10nM RvE1 or vehicle only. (C) 2-dimensional score plot and (D) corresponding 2-dimensional loading plot of LM-SPM from human tendon derived-stromal cell incubations isolated from patients with shoulder tendon tears incubated with IL-1β and 10nM RvE1 or vehicle only. (E) Cumulative levels of proresolving mediators and differentially regulated lipid mediators in IL-1β stimulated tendon stromal cell incubations in the presence of RvE1(10nM) or vehicle for 24 h. Results are shown as means and SEM and representative of n=5 donors per group.

FIGURE 3. LXB₄ and RvE1 induce SPM biosynthetic enzymes and regulate the pro-resolving receptor ChemR23/ERV1 in tendon stromal cells. Tendon stromal cells were derived from patients with shoulder tendon tears (TD, n=6) or healthy volunteers (HV, n=6). Cells were incubated with LXB₄ (10nM), RvE1 (10nM) or vehicle for 24 h at 37 °C then with IL-1β (10ngml⁻¹) for 24 h. (A) Incubation in LXB₄ significantly induced ALOX15 mRNA in both TD and HV cells (p=0.03 respectively) compared to respective vehicle controls. Incubation in RvE1 significantly induced ALOX15 mRNA in both TD (p=0.03) and HV cells (p=0.01) compared to respective vehicle controls. Gene expression is normalized to β-actin; bars show median values. Representative images of immunocytochemistry for SPM synthetic enzymes ALOX15 (green) ALOX12 (violet) in IL-1β stimulated HV (B) and TD tendon stromal cells (C) incubated in 10nM LXB₄, 10nM RvE1 or vehicle control for 24 hrs. Cyan represents POPO-1 nuclear counterstain. All images are representative of n=3 donors. Scale bar, 20 μm. (D) ERV1 mRNA expression in IL-1β stimulated HV and TD cells. Gene expression is normalized to β-actin; bars show median values. (E)
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Representative images of immunocytochemistry for proresolving receptors ALX (green), ERV1 (red) and BLT1 (violet) in IL-1β stimulated diseased tendon stromal cells incubated in 10nM LXB₄, 10nM RvE1 or vehicle control for 24 hrs. Cyan represents POPO-1 nuclear counterstain. All images are representative of n=3 donors. Scale bar, 20μm.

**FIGURE 4.** LXB₄ and RvE1 moderate the pro-inflammatory phenotype of tendon stromal cells. Tendon stromal cells were derived from patients with shoulder tendon tears (TD) or healthy volunteers (HV). Representative images of immunocytochemistry for established markers of tendon inflammation including Podoplanin (PDPN) green, STAT-1 (green) and Interleukin-6 (IL-6, red) in IL-1β stimulated (A) TD and (B) HV tendon stromal cells incubated in 10nM LXB₄, 10nM RvE1 or vehicle control for 24 hrs. Cyan represents POPO-1 nuclear counterstain. All images are representative of n=3 donors. Scale bar, 20μm. ELISA assay of IL-6 protein secretion from IL-1β stimulated TD (C) and HV (D) tendon cells incubated in the presence and absence of 10 nM LXB₄ or 10nM RvE1. Data are shown as means and SEM, n=5 separate donors. (E) mRNA expression of markers of tendon inflammation including IL-6, and fibroblast activation markers PDPN and CD90, in IL-1β stimulated TD cells (n=6 donors) incubated in either 10nM LXB₄, 10nM RvE1 or vehicle control for 24 hrs. Gene expression is normalized to β-actin, bars show median values.

**Figure 5.** LXB₄ and RvE1 moderate protein kinase expression in diseased tendon stromal cells. Densitometric analysis was acquired using Image J software to identify the effects of incubating IL-1β treated TD cells in LXB₄ or RvE1 on protein...
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phospho-kinase signalling pathways JNK1/2/3, Lyn, STAT3, STAT6 and p70s6 kinase. Results are shown as means and SEM and representative of n=3 donors per group relative to respective vehicle control treated cells.

Figure S1. Isotype control staining of patient derived tendon stromal cells.

Representative confocal immunofluorescence images showing merged images of stromal cells isolated from patients with shoulder tendon tears, stained with isotype control antibodies for mouse IgG₁, IgG₂a, IgG₂b and rabbit IgG fractions. Cyan represents POPO-1 nuclear counterstain. Scale bar, 20µm.
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Supplemental Table 1. LM-SPM profiles of IL-1β stimulated patient tendon stromal cells in the presence of LXB₄ or RvE1.

| Tendon stromal cells lipid mediator levels pg/incubation | Healthy IL1β | Healthy IL1β+LXB₄ | Healthy IL1β+RvE1 | Disease IL1β | Disease IL1β+LXB₄ | Disease IL1β+RvE1 |
|--------------------------------------------------------|-------------|-----------------|-----------------|-------------|-----------------|-----------------|
| DHA bioactive metabolome                                |             |                 |                 |             |                 |                 |
| RvD1                                                   | Mean ± SEM  | 3.69 ± 0.27     | 3.72 ± 0.36     | 4.01 ± 0.61 | 3.27 ± 0.11     | 3.41 ± 0.29     | 3.97 ± 0.63    |
| RvD2                                                   | Mean ± SEM  | 3.60 ± 0.60     | 3.37 ± 0.71     | 3.65 ± 0.68 | 3.15 ± 0.43     | 3.02 ± 0.57     | 3.30 ± 0.62    |
| RvD3                                                   | Mean ± SEM  | 5.72 ± 0.67     | 5.15 ± 0.92     | 5.08 ± 0.83 | 4.98 ± 0.89     | 5.21 ± 0.60     | 4.34 ± 0.76    |
| RvD4                                                   | Mean ± SEM  | 9.61 ± 0.89     | 8.05 ± 0.77     | 8.63 ± 1.25 | 8.04 ± 0.61     | 7.77 ± 1.00     | 6.18 ± 0.73    |
| RvD5                                                   | Mean ± SEM  | 49.22 ± 3.45    | 45.75 ± 3.51*   | 46.03 ± 4.77 | 39.56 ± 6.76   | 40.38 ± 8.11    | 46.65 ± 11.32  |
| RvD6                                                   | Mean ± SEM  | 17.05 ± 2.21    | 15.82 ± 2.17    | 17.42 ± 2.56 | 12.66 ± 3.33   | 12.38 ± 3.53    | 14.09 ± 4.54   |
| 17R-RvD1                                               | Mean ± SEM  | 1.80 ± 0.09     | 1.75 ± 0.35     | 1.58 ± 0.35 | 2.02 ± 0.30     | 1.46 ± 0.30     | 1.23 ± 0.20*   |
| 17R-RvD3                                               | Mean ± SEM  | 7.77 ± 0.40     | 6.79 ± 0.54     | 6.29 ± 0.63* | 6.56 ± 1.07     | 6.03 ± 0.92     | 5.14 ± 0.58    |
| PD1                                                    | Mean ± SEM  | 0.00 ± 0.00     | 0.00 ± 0.00     | 0.00 ± 0.00 | 0.00 ± 0.00     | 0.00 ± 0.00     | 0.00 ± 0.00    |
| 10S,17SdiHDHA                                          | Mean ± SEM  | 194.7 ± 52.68   | 186.0 ± 51.43   | 208.1 ± 64.86 | 155.7 ± 64.89 | 171.5 ± 71.24  | 209.1 ± 89.95  |
| 22-OHPD1                                               | Mean ± SEM  | 18.49 ± 6.26    | 14.68 ± 5.01*   | 16.65 ± 5.91* | 16.55 ± 5.83   | 15.16 ± 5.38   | 14.91 ± 5.23   |
| 17R-PD1                                                | Mean ± SEM  | 3.64 ± 1.31     | 3.17 ± 1.16     | 3.45 ± 1.54 | 2.86 ± 1.14     | 3.60 ± 1.45     | 3.93 ± 0.58    |
| Maresin1                                               | Mean ± SEM  | 4.36 ± 1.44     | 4.61 ± 2.17     | 4.44 ± 1.73 | 4.18 ± 2.07     | 2.80 ± 1.87     | 4.31 ± 2.06    |
| Maresin2                                               | Mean ± SEM  | 7.31 ± 1.23     | 7.24 ± 1.05     | 7.67 ± 1.49 | 6.46 ± 1.36     | 6.28 ± 1.33     | 7.42 ± 2.20    |
| 22-OH-MaR1                                             | Mean ± SEM  | 14.93 ± 1.14    | 12.91 ± 1.94    | 12.55 ± 1.83* | 14.51 ± 2.19   | 13.74 ± 2.79   | 15.83 ± 1.53   |
| 14-oxo-MaR1                                            | Mean ± SEM  | 0.12 ± 0.05     | 0.22 ± 0.12     | 0.26 ± 0.12* | 0.15 ± 0.08     | 0.08 ± 0.04     | 0.13 ± 0.07    |
| 7S,14S diHDHA                                          | Mean ± SEM  | 57.82 ± 12.55   | 58.35 ± 16.73   | 63.33 ± 14.94 | 45.00 ± 13.70  | 54.77 ± 20.03  | 65.74 ± 23.07* |
| 4,14-diHDHA                                            | Mean ± SEM  | 55.78 ± 14.89   | 50.98 ± 13.88*  | 51.92 ± 13.59 | 37.43 ± 13.38  | 35.82 ± 12.94  | 40.80 ± 15.85  |
| n-3 DPA bioactive metabolome                           |             |                 |                 |             |                 |                 |
| RvT1                                                   | Mean ± SEM  | 0.34 ± 0.16     | 0.25 ± 0.14     | 0.46 ± 0.22 | 0.24 ± 0.12     | 0.40 ± 0.20     | 0.52 ± 0.25    |
| RvT2                                                   | Mean ± SEM  | 0.00 ± 0.00     | 0.07 ± 0.08     | 0.06 ± 0.06 | 0.07 ± 0.08     | 0.26 ± 0.12     | 0.04 ± 0.05    |

[^1]: Data certified by peer review; bioRxiv preprint posted April 11, 2019. doi: 10.1101/606152
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|            |       |       |       |       |       |       |       |       |       |
|------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| RvT3       | 0.00  | ±     | 0.00  | ±     | 0.00  | ±     | 0.00  | ±     | 0.00  |
| RvT4       | 14.32 | ±     | 3.50  | 13.78 | ±     | 3.61  | 14.83 | ±     | 3.83  |
| RvD₁₃ DPA  | 7.54  | ±     | 1.26  | 7.01  | ±     | 0.91  | 7.26  | ±     | 0.80  |
| RvD₂₃ DPA  | 1.54  | ±     | 0.47  | 1.28  | ±     | 0.42  | 1.87  | ±     | 0.35  |
| RvD₅₁₃ DPA| 30.26 | ±     | 3.19  | 27.79 | ±     | 2.97  | 32.86 | ±     | 5.54  |
| DPA        |       | ±     |       |       |       |       |       |       |       |
| PD₁₃ DPA   | 0.00  | ±     | 0.00  | 0.00  | ±     | 0.00  | 0.00  | ±     | 0.62  |
| 10S,17S-diHDPA | 19.08 | ±     | 5.20  | 20.59 | ±     | 4.75  | 21.85 | ±     | 6.21  |
| 15-epi-LXB₄| 3.12  | ±     | 0.67  | 3.46  | ±     | 0.41  | 3.13  | ±     | 0.67  |
| EPA bioactive metabolome |           | | | | | | | | |
| RvE1       | 0.00  | ±     | 0.00  | 0.00  | ±     | 0.00  | 1397. 39| ± | 315.5 4 |
| RvE2       | 33.49 | ±     | 1.82  | 32.39 | ±     | 1.80  | 31.46 | ±     | 2.60  |
| RvE3       | 3.12  | ±     | 0.67  | 3.46  | ±     | 0.41  | 3.13  | ±     | 0.67  |
| AA bioactive metabolome |           | | | | | | | | |
| LXA₄       | 2.02  | ±     | 0.16  | 2.39  | ±     | 0.30  | 2.15  | ±     | 0.22  |
| LXB₄       | 3.84  | ±     | 1.85  | 396.9 | ±     | 90.14 | 3.43  | ±     | 1.88  |
| 5,15-diHETE| 2332. 88±      | 550.6 7 | 2496.44| ±      | 629.0 7 | 2509.74| ±      | 681.7 6 | 1860.42| ±      | 589.7 4 | 1967.32| ±      | 667.7 7 | 2461.83| ±      | 908.88 |
| 15-epi-LXA₄| 56.73 | ±     | 7.92  | 50.34 | ±     | 6.70  | 49.89 | ±     | 9.07  |
| 15-epi-LXB₄| 12.52 | ±     | 2.10  | 13.37 | ±     | 1.08  | 12.82 | ±     | 1.80  |
| 13,14-dihydro-15-oxo-LXA₄| 28.45 | ±     | 4.08  | 23.87 | ±     | 4.04  | 22.27 | ±     | 3.65  |
| 15-oxo-LXA₄| 22.42 | ±     | 15.50 | 18.62 | ±     | 12.75 | 22.04 | ±     | 15.10 |
| LTB₄       | 0.00  | ±     | 0.00  | 0.00  | ±     | 0.00  | 0.00  | ±     | 0.00  |
| 5,12 diHETE| 333.8 7±      | 61.15 9 | 324.7 4| ±      | 57.51 9 | 332.2 4| ±      | 58.63 8 | 261.7 4| ±      | 76.72 5 | 260.6 5| ±      | 77.52 8 | 277.9 8| ±      | 90.72 |
| 6-trans-LTB₄| 133.2 1±      | 12.31 2 | 124.7 2| ±     | 14.20 5 | 126.8 5| ±     | 12.30 4 | 134.8 4| ±     | 28.06 4 | 139.9 4| ±     | 32.82 4| 32.82 | ±     | 32.82 |
| 12-epi-6-trans-LTB₄| 164.9 2±      | 28.04 5 | 146.9 5| ±     | 25.43 3 | 149.3 3| ±     | 23.89 3 | 152.8 3| ±     | 42.66 0 | 150.4 0| ±     | 40.20 6 | 164.8 6| ±     | 49.33 |
Tendon stromal cells (60,000 cells per well) were derived from patients with shoulder tendon tears (Diseased n=5 donors) or healthy volunteer hamstring tendons (Healthy n=5 donors) and incubated in the presence of 10nM LXB₄, 10nM RvE1 or vehicle only in the presence of 10ngml⁻¹ IL1β. Cell incubations were terminated using ice-cold methanol containing internal standards and lipid mediators (LM) were identified and quantified using LM-profiling (see methods for details). Results are expressed as pg/incubation. Mean ± SEM of n = 5 per incubation. * p ≤ 0.05 comparison between respective vehicle incubated tendon stromal cells. The detection limit was ~ 0.1 pg. 0.0, Below limits of detection.
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REFERENCES

[1] Global Burden of Disease Study C: Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015, 386:743-800.
[2] Thangarajah T, Pendegrass CJ, Shahbazi S, Lambert S, Alexander S, Blunn GW: Augmentation of Rotator Cuff Repair With Soft Tissue Scaffolds. Orthop J Sports Med 2015, 3:2325967115587495.
[3] Minagawa H, Yamamoto N, Abe H, Fukuda M, Seki N, Kikuchi K, Kijima H, Itoi E: Prevalence of symptomatic and asymptomatic rotator cuff tears in the general population: From mass-screening in one village. J Orthop 2013, 10:8-12.
[4] Carr AJ, Cooper CD, Campbell MK, Rees JL, Moser J, Beard DJ, Fitzpatrick R, Gray A, Dawson J, Murphy J, Bruhn H, Cooper D, Ramsay CR: Clinical effectiveness and cost-effectiveness of open and arthroscopic rotator cuff repair [the UK Rotator Cuff Surgery (UKUFF) randomised trial]. Health technology assessment 2015, 19:1-218.
[5] Dean BJ, Franklin SL, Murphy RJ, Javaid MK, Carr AJ: Glucocorticoids induce specific ion-channel-mediated toxicity in human rotator cuff tendon: a mechanism underpinning the ultimately deleterious effect of steroid injection in tendinopathy? Br J Sports Med 2014, 48:1620-6.
[6] Poulsen RC, Watts AC, Murphy RJ, Snelling SJ, Carr AJ, Hulley PA: Glucocorticoids induce senescence in primary human tenocytes by inhibition of sirtuin 1 and activation of the p53/p21 pathway: in vivo and in vitro evidence. Ann Rheum Dis 2014, 73:1405-13.
[7] Carr AJ, Rees JL, Ramsay CR, Fitzpatrick R, Gray A, Moser J, Dawson J, Bruhn H, Cooper CD, Beard DJ, Campbell MK: Protocol for the United Kingdom Rotator Cuff Study (UKUFF): a randomised controlled trial of open and arthroscopic rotator cuff repair. Bone & joint research 2014, 3:155-60.
[8] Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA: Inducible cyclooxygenase may have anti-inflammatory properties. Nat Med 1999, 5:698-701.
[9] Gilroy DW, Lawrence T, Perretti M, Rossi AG: Inflammatory resolution: new opportunities for drug discovery. Nat Rev Drug Discov 2004, 3:401-16.
[10] Buckley CD, Pilling D, Lord JM, Akbar AN, Scheel-Toellner D, Salmon M: Fibroblasts regulate the switch from acute resolving to chronic persistent inflammation. Trends Immunol 2001, 22:199-204.
[11] Crowley T, O'Neil JD, Adams H, Thomas AM, Filer A, Buckley CD, Clark AR: Priming in response to pro-inflammatory cytokines is a feature of adult synovial but not dermal fibroblasts. Arthritis Res Ther 2017, 19:35.
[12] Dakin SG, Buckley CD, Al-Mossawi MH, Hedley R, Martinez FO, Wheway K, Watkins B, Carr AJ: Persistent stromal fibroblast activation is present in chronic tendinopathy. Arthritis Res Ther 2017, Jan 25.
[13] Dakin SG CM, Sherlock J, Powrie F, Carr AJ, Buckley CD: Pathogenic stromal cells as therapeutic targets in joint inflammation. Nature reviews Rheumatology 2018.
[14] Crowley T, Buckley CD, Clark AR: Stroma: the forgotten cells of innate immune memory. Clin Exp Immunol 2018.
LXB₄ and RvE1 regulate tendon inflammation

[15] Dakin SG, Ly L, Colas RA, Oppermann U, Wheway K, Watkins B, Dalli J, Carr AJ: Increased 15-PGDH expression leads to dysregulated resolution responses in stromal cells from patients with chronic tendinopathy. Sci Rep 2017, 7:11009.

[16] Dakin SG, Martinez FO, Yapp C, Wells G, Oppermann U, Dean BJ, Smith RD, Wheway K, Watkins B, Roche L, Carr AJ: Inflammation activation and resolution in human tendon disease. Science translational medicine 2015, 7:311ra173.

[17] Hsiao HM, Thatcher TH, Colas RA, Serhan CN, Phipps RP, Sime PJ: Resolvins D1 Reduces Emphysema and Chronic Inflammation. Am J Pathol 2015, 185:3189-201.

[18] Buckley CD, Filer A, Haworth O, Parsonage G, Salmon M: Defining a role for fibroblasts in the persistence of chronic inflammatory joint disease. Ann Rheum Dis 2004, 63 Suppl 2:ii92-ii5.

[19] Buckley CD: Why does chronic inflammation persist: An unexpected role for fibroblasts. Immunol Lett 2011, 138:12-4.

[20] Dakin SG, Newton J, Martinez FO, Hedley R, Gwilym S, Jones N, Reid HAB, Wood S, Wells G, Appleton L, Wheway K, Watkins B, Carr AJ: Chronic inflammation is a feature of Achilles tendinopathy and rupture. Br J Sports Med 2018, 52:359-67.

[21] Mizoguchi F, Slowikowski K, Wei K, Marshall JL, Rao DA, Chang SK, Nguyen HN, Nos E, Turner JD, Epp BE, Blazar PE, Wright J, Simmons BP, Donlin LT, Kalliolias GD, Goodman SM, Bykerk VP, Iwashvili LB, Lederer JA, Hoehoen N, Ngoivic PA, Filer A, Buckley CD, Raychaudhuri S, Brenner MB: Functionally distinct disease-associated fibroblast subsets in rheumatoid arthritis. Nature communications 2018, 9:789.

[22] Arita M, Ohira T, Sun YP, Elangovan S, Chiang N, Serhan CN: Resolvin E1 selectively interacts with leukotriene B4 receptor BLT1 and ChemR23 to regulate inflammation. J Immunol 2007, 178:3912-7.

[23] Chiang N, Serhan CN: Structural elucidation and physiologic functions of specialized pro-resolving mediators and their receptors. Mol Aspects Med 2017, 58:114-29.

[24] Morita W, Dakin SG, Snelling SJB, Carr AJ: Cytokines in tendon disease: A Systematic Review. Bone & joint research 2017, 6:656-64.

[25] Filer A, Ward LSC, Kemble S, Davies CS, Munir H, Rogers R, Raza K, Buckley CD, Nash GB, McGettrick HM: Identification of a transitional fibroblast function in very early rheumatoid arthritis. Ann Rheum Dis 2017, 76:2105-12.

[26] Freire MO, Dalli J, Serhan CN, Van Dyke TE: Neutrophil Resolvin E1 Receptor Expression and Function in Type 2 Diabetes. J Immunol 2017, 198:718-28.

[27] Arnardottir HH, Dalli J, Norling LV, Colas RA, Perretti M, Serhan CN: Resolvin D3 Is Dysregulated in Arthritis and Reduces Arthritic Inflammation. J Immunol 2016, 197:2362-8.

[28] Norling LV, Headland SE, Dalli J, Arnardottir HH, Haworth O, Jones HR, Irimia D, Serhan CN, Perretti M: Proresolving and cartilage-protective actions of resolvin D1 in inflammatory arthritis. JCI Insight 2016, 1:e85922.
