# Raised levels of $F_2$-isoprostanes and prostaglandin $F_{2\alpha}$ in different rheumatic diseases

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**Abstract**

***Objective*—To evaluate oxidative injury and inflammatory status in various rheumatic diseases by measuring the levels of isoprostanes and prostaglandins in serum and synovial fluid.**

***Methods*—The concentrations of 8-iso-$PGF_{2\alpha}$ ($F_2$-isoprostane indicating oxidative injury) and 15-keto-dihydro-$PGF_{2\alpha}$ (a major metabolite of prostaglandin $F_{2\alpha}$) were measured in both serum and synovial fluid aspirated from 26 patients with various arthritic diseases, including rheumatoid arthritis (RA), reactive arthritis (ReA), psoriatic arthritis (PsA), and osteoarthritis (OA). These prostaglandin derivatives were also measured in serum samples collected from 42 healthy control subjects.**

***Results*—Overall, serum levels of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ were much higher in patients with arthritic diseases than in the healthy control subjects. The levels of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ in synovial fluid aspirated from knee joints were also high and varied among various types of arthritic patients. Although the synovial fluid level of these prostaglandin derivatives was sometimes higher than in the corresponding serum sample, this was not a consistent finding. Overall, there was no correlation between serum and synovial fluid levels of 8-iso-$PGF_{2\alpha}$, or between serum and synovial fluid levels of 15-keto-dihydro-$PGF_{2\alpha}$. However, a strong relation was found between the levels of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$, in both serum ($r_s$=0.53, p<0.001) and synovial fluid ($r_s$=0.62, p<0.001).**

***Conclusions*—These data suggest that both free radical mediated oxidative injury and cyclo-oxygenase dependent inflammatory responses are closely correlated in various types of arthritis.**

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Reactive oxygen species and reactive nitrogen species have been shown to cause tissue injury and pathophysiological consequences in chronic inflammatory conditions such as rheumatoid arthritis and other rheumatic diseases.[1–7] However, the degree of free radical injury and inflammatory response may vary in different types of inflammatory arthritis and also in different people depending on the severity and duration of these diseases.

A major problem associated with the assessment of oxidative stress in inflammatory arthritis has been the limitation in available assay methods for in vivo measurement of free radical generation or end products of free radical catalysed oxidation of lipids.[8] In 1990, Morrow, Roberts, and their colleagues reported the discovery of isoprostanes, a family of prostaglandin derivatives generated in vivo by non-enzymatic free radical catalysed oxidation of arachidonic acid.[9] It has also been noted that one of the major isoprostanes, 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$, fig 1) is increased in several syndromes that are associated with oxidant injury, and the measurement of isoprostanes is widely regarded as a reliable biomarker for in vivo measurement of lipid peroxidation.[9–17]

Cyclo-oxygenase-2 (COX-2), an isoform of cyclo-oxygenase, has been shown to be expressed in macrophages, epithelial cells, and fibroblasts after exposure to several proinflammatory stimuli (for example, cytokines, growth factors), leading to the release of prostaglandins (fig 1).[18–20] 15-Keto-13,14-dihydro-$PGF_{2\alpha}$ (15-keto-dihydro-$PGF_{2\alpha}$), a major metabolite of prostaglandin $F_{2\alpha}$, is increased in the inflammatory response.[21 22]

We recently developed a highly specific and sensitive radioimmunoassay (RIA) by raising antibodies in rabbits against both 8-iso-$PGF_{2\alpha}$ (indicating oxidative injury)[22 23] and 15-keto-dihydro-$PGF_{2\alpha}$ (indicating inflammatory response).[21 22]. The antibodies clearly discriminate between these two closely related substances (fig 1).[21 23] By applying these parameters, we showed that oxidative modification of arachidonic acid, through both non-enzymatic lipid peroxidation and enzymatic (cyclo-oxygenase) pathways, is involved in endotoxin induced inflammation in septic shock,[13 17] hepatotoxin induced oxidative injury,[14] and cerebral oxidative injury after resuscitation from cardiac arrest.[15] These results suggest that oxidative injury and inflammation are closely associated in various syndromes.

The proposed role of reactive oxygen species in inflammatory joint disease suggests the need for a marker of oxidative injury, such as 8-iso-$PGF_{2\alpha}$. A previous study has shown that plasma 8-iso-$PGF_{2\alpha}$ levels are raised in systemic lupus erythematosus and other rheumatic diseases with vascular involvement, though no increase was seen in patients with rheumatoid arthritis (RA).[24] However, this particular study examined plasma levels only. We considered it likely that markers of oxidative injury are raised at local sites of inflammation. Patients with joint diseases who present with a synovial effusion provide an opportunity to examine localised oxidative injury as well as systemic responses.

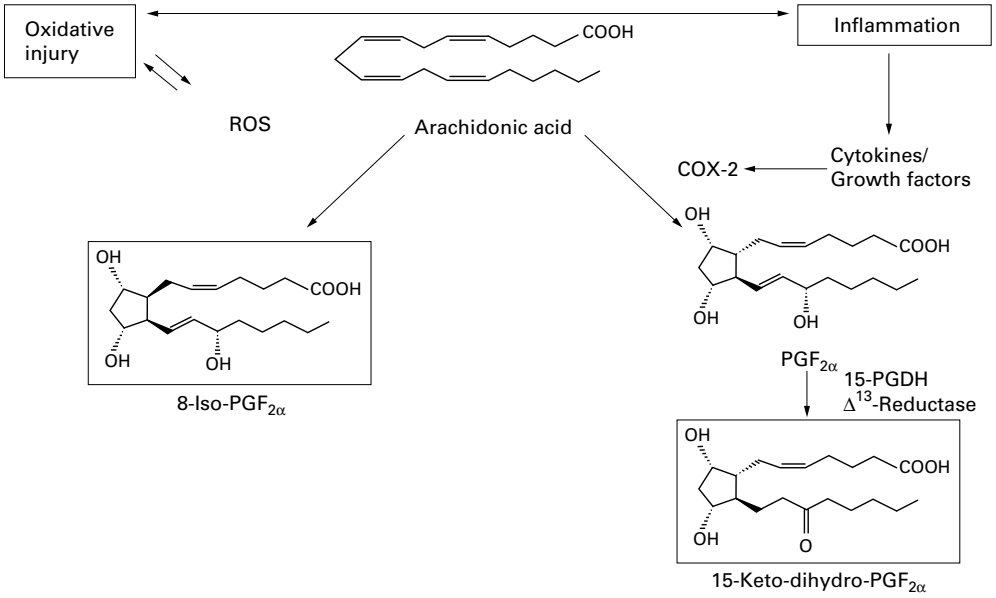


*Figure 1 Schematic diagram of relation between inflammation and oxidative injury, and endogenous formation of 8-iso-PGF$_{2\alpha}$ through free radical and 15-keto-dihydro-PGF$_{2\alpha}$ via cyclo-oxygenase catalysed oxidation of arachidonic acid. ROS = reactive oxygen species; COX = cyclo-oxygenase; 15-PGDH = 15-hydroxyprostaglandin dehydrogenase, $\Delta^{13}$-reductase.*

Thus, in this study, oxidative injury and inflammatory response in patients with various arthritic disorders were investigated by the measurement of 8-iso-PGF$_{2\alpha}$ and 15-keto-dihydro-PGF$_{2\alpha}$ in peripheral blood and synovial fluid aspirated from knee joints. Simultaneous measurement of an $F_2$-isoprostane and of a prostaglandin metabolite in blood samples and synovial fluid provides information about both systemic and local (knee joints) oxidative damage and inflammatory response.

## Materials and methods

### ANIMALS

Unlabelled 8-iso-PGF$_{2\alpha}$, 15-keto-dihydro-PGF$_{2\alpha}$, and other related isoprostanes and prostaglandins were purchased from Cayman Chemicals, Ann Arbor, MI, USA. The tritium labelled 8-iso-PGF$_{2\alpha}$ (specific activity 608 GBq/mmol) was synthesised and purified as described previously.[23] The tritium labelled 15-keto-dihydro-PGF$_{2\alpha}$ (specific activity 6.77 TBq/mmol) was obtained from Amersham (Buckinghamshire, UK). Antibodies against both 8-iso-PGF$_{2\alpha}$ and 15-keto-dihydro-PGF$_{2\alpha}$ were raised at our laboratory and are well characterised.[21 23]

### EXPERIMENTAL PROTOCOL

*Patients*

All patients were resident in north Staffordshire, UK, and had various rheumatic conditions (table 1). These included rheumatoid arthritis (RA), psoriatic arthritis (PsA), reactive arthritis (ReA), and osteoarthritis (OA). Patients presenting at the Staffordshire Rheumatology Centre with a knee joint effusion were recruited consecutively into this study. Treatment was given as clinically indicated. All patients were being treated with at least one non-steroidal anti-inflammatory drug (NSAID), and in the case of patients with RA, with one or more disease modifying antirheumatic drug (DMARD), including hydroxychloroquine, sulfasalazine, gold, or methotrexate. None of the patients was being treated with oral corticosteroids and none had been injected with steroids in the three months preceding their presentation at the clinic. All patients with RA satisfied the American College of Rheumatology (formerly the American Rheumatism Association) 1987 ARA criteria for RA.[25] The patients with OA all had localised knee OA.

*Table 1 Characteristics of various types of rheumatic patients*

| Disease | Patients (n) | Age (mean (SD)) | Duration (mean (SD)) |
|---|---|---|---|
| Rheumatoid arthritis | 7 | 58.1 (10.1) | 10.6 (9.6) |
| Psoriatic arthritis | 4 | 38.5 (9.1) | 8.5 (5.3) |
| Reactive arthritis | 5 | 31.4 (4.4) | 3.6 (2.4) |
| Osteoarthritis | 10 | 63.6 (7.0) | 6.0 (4.5) |

SD = standard deviation.

### BLOOD AND SYNOVIAL FLUID COLLECTION

Blood was collected by venepuncture from all patients after aspiration of synovial fluid from the affected joint. Synovial fluid was always collected from the joint before any intra-articular injection with corticosteroids. Blood was allowed to clot and was centrifuged before collection of serum. Control blood samples were collected from healthy men and women (mean (SD) age (45 (2)). Synovial fluid was also centrifuged to pellet any cells and clotted material before removal of the fluid. All serum and synovial fluid samples were stored at −70°C.

### RADIOIMMUNOASSAY OF 8-ISO-PGF$_{2\alpha}$ (OXIDATIVE INJURY INDICATOR)

Unextracted serum and synovial fluid were analysed for 8-iso-PGF$_{2\alpha}$ by RIA at our laboratory as described elsewhere.[23] The cross reactivity of the 8-iso-PGF$_{2\alpha}$ antibody with

15-keto-13,14-dihydro-8-iso-$PGF_{2\alpha}$, 8-iso-$PGF_{2\beta}$, $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}$, $TXB_2$, 11β-$PGF_{2\alpha}$, 9β-$PGF_{2\alpha}$, and 8-iso-$PGF_{3\alpha}$, respectively, was 1.7, 9.8, 1.1, 0.01, 0.01, 0.1, 0.03, 1.8, and 0.6%. The detection limit of the assay was about 23 pmol/l.

### RADIOIMMUNOASSAY OF 15-KETO-DIHYDRO-$PGF_{2\alpha}$ (INFLAMMATORY RESPONSE INDICATOR)

Unextracted serum and synovial fluid were analysed for 15-keto-dihydro-$PGF_{2\alpha}$ by an RIA at our laboratory, as described elsewhere.[21] The cross reactivity of the antibody with $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, $PGE_2$, 15-keto-13,14-dihydro-$PGE_2$, 8-iso-15-keto-13,14-dihydro-$PGF_{2\alpha}$, 11β-$PGF_{2\alpha}$, 9β-$PGF_{2\alpha}$, $TXB_2$ , and 8-iso-$PGF_{3\alpha}$ was 0.02, 0.43, <0.001, 0.5, 1.7, <0.001, <0.001, <0.001, 0.01%, respectively. The detection limit was about 45 pmol/l.

### STATISTICAL ANALYSES

The results of this investigation were analysed with the Statistical Analysis System (SAS) and STATA (Stata Corporation, TX, USA) software systems. Variables with a skewed distribution were logarithmically transformed before the statistical analysis. Comparison between patient groups was made using one way analysis of variance (ANOVA) with correction by the Tukey Kramer procedure for multiple comparisons between samples of unequal sizes. Multiple comparisons between patient groups and the control group were corrected by the Bonferroni (versus control) procedure. Comparisons between paired serum and synovial fluid variables were carried out using paired *t* tests or the Wilcoxon signed rank test. Association between variables was determined by Spearman's rank correlation.

## Results

### OXIDATIVE INJURY AS MEASURED BY SERUM AND SYNOVIAL FLUID 8-ISO-$PGF_{2\alpha}$

All rheumatic patients had significantly higher basal levels of serum 8-iso-$PGF_{2\alpha}$ than the healthy control subjects (by ANOVA and correction for multiple comparisons). Figure 2A shows the levels of 8-iso-$PGF_{2\alpha}$ in different classes of patients. The mean (SEM) levels of serum 8-iso-$PGF_{2\alpha}$ were 33 (3.3) pg/ml in healthy control subjects of different ages (n=42). The mean levels of 8-iso-$PGF_{2\alpha}$ were 451 (160) pg/ml in the patients with ReA, 325 (143) pg/ml in the patients with RA, and 92 (226) pg/ml in the patients with PsA. It was also notable, however, that levels of 8-iso-$PGF_{2\alpha}$ were raised in the patients with OA, with a mean value of 187 (53.3) pg/ml.

OA is often considered to be a non-inflammatory arthritis, whereas RA, PsA, and ReA are considered to be inflammatory arthropathies. We therefore divided the patients into "non-inflammatory" and "inflammatory" groups. When stratified in this way the level of serum 8-iso-$PGF_{2\alpha}$ tended to be higher in the inflammatory group than in the non-inflammatory group (329 *v* 187 pg/ml), though this did not achieve significance (p=0.4).

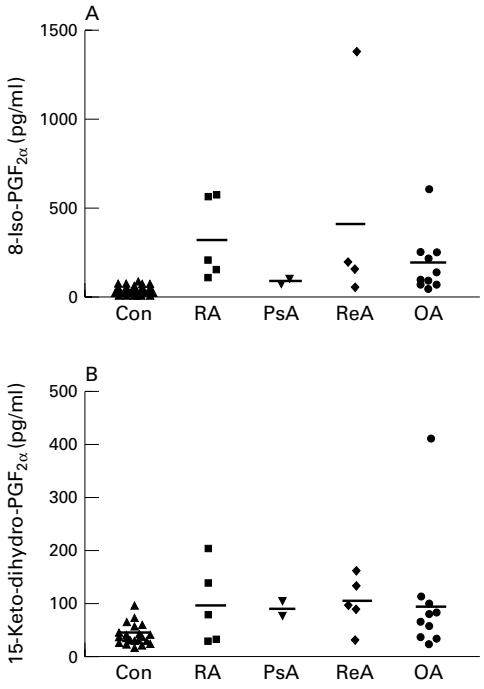


*Figure 2 Serum levels of (A) 8-iso-$PGF_{2\alpha}$ and (B) 15-keto-dihydro-$PGF_{2\alpha}$ in individual patients with various types of rheumatic disease, and controls. The mean level is represented by a bar in each group.*

When the levels of 8-iso-$PGF_{2\alpha}$ were measured in synovial fluid, they were found to be high and varied in different types of rheumatic patients (fig 3A). The synovial fluid levels of 8-iso-$PGF_{2\alpha}$ were 494 (100) pg/ml in patients with OA, 192 (141) pg/ml in patients with ReA, 177 (119) pg/ml in patients with RA, and 522 (158) pg/ml in patients with PsA. Comparison between patient groups by ANOVA showed no significant differences between them after correcting for multiple comparisons. Overall, there was no correlation between serum and synovial fluid levels of 8-iso-$PGF_{2\alpha}$.

Division into non-inflammatory and inflammatory groups disclosed a significantly higher level of synovial fluid 8-iso-$PGF_{2\alpha}$ in the non-inflammatory (OA) group than in the inflammatory group (494 *v* 268 pg/ml, p=0.04).

### INFLAMMATORY RESPONSE AS MEASURED BY SERUM AND SYNOVIAL FLUID 15-KETO-DIHYDRO-$PGF_{2\alpha}$

All rheumatic patients had higher basal levels of serum 15-keto-dihydro-$PGF_{2\alpha}$ than the healthy control subjects, though after correction for multiple comparisons only the levels in patients with OA and ReA were significantly different. Figure 2B shows the levels of 15-keto-dihydro-$PGF_{2\alpha}$ in various subjects and classes of rheumatic patients. The levels of 15-keto-dihydro-$PGF_{2\alpha}$ were 41 (4.4) pg/ml (n=21) in healthy control subjects. The mean serum levels of 15-keto-dihydro-$PGF_{2\alpha}$ were 102 (31) pg/ml in patients with OA, 104 (44) pg/ml in the patients with ReA, 97 (44) pg/ml in the patients with RA, and 94 (70) pg/ml in the patients with PsA. Comparison of non-inflammatory and inflammatory groups

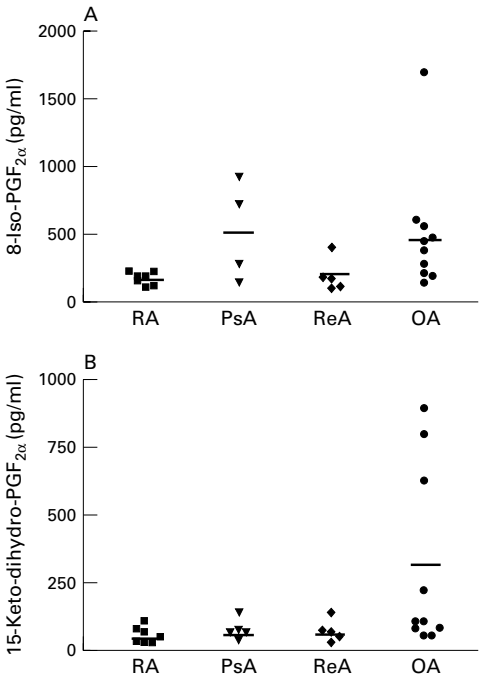


*Figure 3 Synovial fluid levels of (A) 8-iso-$PGF_{2\alpha}$ and (B) 15-keto-dihydro-$PGF_{2\alpha}$ in individual patients with various types of rheumatic disease, and controls. The mean level is represented by a bar in each group.*

showed no significant difference in serum levels of 15-keto-dihydro-$PGF_{2\alpha}$ (102 *v* 99 pg/ml, p=0.5).

When the levels of 15-keto-dihydro-$PGF_{2\alpha}$ were measured in synovial fluid, the levels were found to be high, though there were considerable variations between different types of rheumatic patients (fig 3B). The synovial levels of 15-keto-dihydro-$PGF_{2\alpha}$ were 299 (67) pg/ml in patients with OA, 71 (94) pg/ml in patients with ReA, 56 (80) pg/ml in patients with RA, and 64 (105) pg/ml in patients with PsA. There were no significant differences between patient groups after correction for multiple comparisons. No overall correlation was found between serum and synovial fluid levels of 15-keto-dihydro $PGF_{2\alpha}$.

When divided into non-inflammatory and inflammatory groups a significantly higher level of synovial fluid 15-keto-dihydro-$PGF_{2\alpha}$ was found in the non-inflammatory (OA) group than in the inflammatory group (299 *v* 63 pg/ml, p=0.01).

### COMPARISON OF PAIRED SERUM AND SYNOVIAL FLUID LEVELS OF PROSTAGLANDIN DERIVATIVES

We examined whether there was any difference between serum and synovial fluid paired samples in the levels of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro $PGF_{2\alpha}$. Although the synovial fluid level of these prostaglandin derivatives was sometimes higher than in the corresponding serum, this was not a consistent finding, and overall there were no significant differences between the levels in serum and synovial fluid.

### RELATION BETWEEN LEVELS OF 8-ISO-$PGF_{2\alpha}$ AND 15-KETO-DIHYDRO-$PGF_{2\alpha}$

When all the patients were considered together a strong correlation was found between the levels of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ in both serum ($r_s$=0.53, p<0.001) and synovial fluid ($r_s$=0.62, p<0.001).

## Discussion

This study deals with the important phenomenon of oxidative stress induced by the chronic inflammatory state in rheumatic patients of various categories and stages of disease. It investigates the local (knee joint) and systemic inflammatory response index and oxidative injury by measuring a major $PGF_{2\alpha}$ metabolite, 15-keto-dihydro-$PGF_{2\alpha}$, and $F_2$-isoprostane in the synovial fluid and blood, respectively. To our knowledge this study is the first to report a high concentration of 8-iso-$PGF_{2\alpha}$ and moderately high levels of 15-keto-dihydro-$PGF_{2\alpha}$ in various types of rheumatic patients in both synovial fluid and peripheral blood collected from the same patients. These results indicate that both non-enzymatic free radical and enzymatic cyclo-oxygenase catalysed oxidation of arachidonic acid occur in various types of rheumatic diseases, such as RA, OA, ReA, and PsA. In addition to the findings of this study, we have seen high levels of $F_2$-isoprostanes and $PGF_{2\alpha}$ metabolite in both synovial fluid and blood from one patient with scleroderma and one with undifferentiated oligoarthritis (data not shown). Raised levels of urinary and plasma $F_2$-isoprostanes have been shown in scleroderma.[24 26] However, in a recent study no significant increase of isoprostane levels was reported in patients with RA,[24] though the relevance of free radical generation has previously been shown for RA.[2] Ames *et al* showed a wide variation in 8-iso-$PGF_{2\alpha}$ levels in patients and controls,[24] as did our study. Possibly, the difference between our study and that of Ames can be explained by differences in the methods used to measure 8-iso-$PGF_{2\alpha}$. However, there is probably also a difference in the type of patient examined. All the patients in our study presented with an effusion, and were considered to be undergoing a disease flare. This may explain why we found an increase in $F_2$-isoprostanes in these patients.

Our data suggested that the mean serum levels of isoprostanes were higher in patients with inflammatory arthritis than OA, though the mean synovial fluid levels were higher in patients with OA. However, the wide variations in levels indicate that overall differences between the inflammatory and OA groups may not be significant. It has been suggested that highly reactive radical species contribute to injury at sites of chronic inflammation and may also contribute to systemic damage. This study suggests that chronic inflammation, both locally at the knee joints and in the systemic circulation, leads to higher basal levels of both $F_2$-isoprostane and prostaglandin metabolite in body fluids of patients with various arthritides. Interestingly, overall, higher levels of 8-iso-$PGF_{2\alpha}$ than 15-keto-dihydro-$PGF_{2\alpha}$ were found in both serum and synovial fluid. As isoprostanes have significant pharmacological effects, they might thus be major mediators of tissue injury.

The variability of both $F_2$-isoprostane and $PGF_{2\alpha}$ formation in various types of rheumatic patients and among the individual patients within groups could be due to various reasons. Most of the patients in this particular study were receiving NSAIDs, which are likely to affect prostaglandin levels, and the patients with RA were also receiving various DMARDs. These may have a variety of effects on inflammatory and immune mediated responses, as well as on the generation of free radicals.[27 28] Other reasons might be the variability in the endogenous defence mechanism in these patients against oxidative injury and inflammation, and the occurrence of hydroxyl radical generation in synovial fluid, which also varied widely between patients.[29] Furthermore, the number of patients in each disease group was small, so the differences in levels of the $F_2$-isoprostane and prostaglandin metabolite between groups should be treated with caution. However, clearly, the levels of these prostaglandin derivatives are high in the serum and synovial fluid of both inflammatory and non-inflammatory arthritis. Surprisingly, some of the highest synovial fluid levels of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ were found in the patients with OA. The significance of this is unclear because the differences between OA and the other rheumatic conditions were not significant when correction was made for multiple comparisons between patient groups.

It was difficult to estimate the relation between the local and systemic damage in different rheumatic diseases from this study because only a few patients were included in each group. However, the local oxidative injury and inflammatory response were high among patients with OA. These data suggest that significant oxidative injury may occur in OA, and that this condition may also have a significant local inflammatory component. The characterisation of OA as a non-inflammatory arthritis may thus need to be re-evaluated, though further studies with these markers on much larger groups of patients are needed. Nevertheless, our data suggest that oxidative injury and inflammatory response are closely associated in OA as well as in other types of rheumatic disease.

In conclusion, this study has shown that blood and synovial fluid from patients with various rheumatic diseases have high levels of both free radical mediated $F_2$-isoprostanes and the cyclo-oxygenase derived $PGF_{2\alpha}$ metabolite. This suggests that both oxidative injury and inflammation play a part to various degrees in these chronic inflammatory diseases. The possibility of measuring these arachidonic acid metabolites in body fluids opens unique opportunities for studying the role of lipid peroxidation, and its control by drugs.

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