# The infrapatellar fat pad induces inflammatory and degradative effects in articular cells but not through leptin or adiponectin

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## Abstract

### Objective

*Based on a novel approach suggesting a role of adipose tissue in osteoarthritis (OA), we aimed to determine whether the infrapatellar fat pad (IFP) may affect joint cell functions through adipokines.*

### Methods

*The conditioned media of IFP and subcutaneous adipose tissue from OA patients were used to determine the production of leptin and adiponectin, and to stimulate chondrocytes and fibroblast-like synoviocytes (FLS). Blocking experiments were carried out to evaluate the contribution of adipokines to IFP effects. The gene expression of inflammatory and degradative proteins, growth factors and components of the extracellular matrix, and the production of inflammatory mediators and metalloproteases were determined to evaluate cell response to fat-conditioned media.*

### Results

*IFP releases elevated amounts of leptin and adiponectin independently of the body mass index and the gender. The conditioned media from IFP strongly induce the expression of inflammatory genes in both articular cells and the expression of degradative genes in chondrocytes, but remain ineffective in regulating the expression of aggrecan, type 2 collagen or growth factors. Blocking leptin or adiponectin does not change the cell response to IFP. A great variability between patients is found when compared the inflammatory activity of paired samples of IFP and subcutaneous adipose tissue.*

### Conclusion

*IFP may trigger both cartilage destruction and inflammation of the synovium, but not through leptin or adiponectin. The data suggest also that IFP may have specific inflammatory phenotypic features independent from the general phenotype found in obesity.*

**Key words**

adipokines, infrapatellar fat pad, obesity, osteoarthritis

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*Competing interests: none declared.*

## Introduction

During the last few years, great progress has been made to understand the worldwide increasing prevalence of obesity and this has revealed that white adipose tissue is a major endocrine organ. The intensive researches have also indicated that adipose-derived proteins might also be synthetised at other sites and might participate in various relevant functions unrelated to those with adipose tissue including inflammation. Indeed, a noteworthy feature of obesity is the development of a chronic low-grade inflammation which develops locally in the expanding adipose tissue, but becomes systemic through the release of numerous proinflammatory mediators into the circulation. The adipose tissue-derived inflammatory mediators are secreted by the enlarged adipocytes, but with increasing adipose tissue expansion also by macrophages infiltrating the adipose tissue (1, 2).

During the last decade and until very recently, it has become increasingly evident that non-mechanical factors associated with obesity may induce osteoathritis (OA) (3-6). A very recent study showed more especially that a higher comorbidity count reduced health-related quality of life in OA patients (7). The chronic low-grade inflammation and metabolic disturbances associated with obesity may contribute to this degenerative joint disease. Adipokines, including leptin and adiponectin, can be currently considered as key players of the complex network of soluble factors involved in the pathophysiology of rheumatic diseases. Both adipokines are produced locally in OA-affected joints and up-regulate degradative enzymes and inflammatory mediators (8). Clinical data further support the prominent role of leptin and adiponectin in OA pathophysiology as they have shown association between their levels and OA progression (9-11).

OA has been usually regarded as a disease whose central pathological feature is hyaline cartilage. However, it is now well established that OA is an inflammatory disease which affects also many periarticular tissues. Recently, an adipose intraarticular tissue which was neglected, has emerged as a potential joint structure involved in cartilage changes during OA. The infrapatellar fat pad (IFP) is interposed between the joint capsule externally and the synovium-lined joint cavity internally. It thought to have biomechanical functions by improving the distribution of the synovial fluid and by absorbing forces through the knee joint (12). The most emerging role of IFP is probably related to its endocrine functions. This large adipose deposit may serve as a local source of cytokines, growth factors and adipokines within the joint, and may therefore be an important player in the initiation and the progression of OA (13, 14). Recent studies have shown that immune cells infiltrated the IFP in human OA joints (15). T cells in IFP have a predominant T helper 1 phenotype while macrophages display a mixed pro- and anti-inflammatory phenotype (16). These inflammatory changes may occur early during the disease as suggests the acute inflammatory cell infiltrate found at the earlier stage of an experimental model of OA (17).

As IFP secretes pro-inflammatory cytokines and is infiltrated with immune cells, one can expect that this joint adipose tissue might contribute to the development of OA. However, a longitudinal study has suggested that IFP with large area had a protective role for knee symptoms and cartilage damage in older female adults (18). *In vitro* experiments have also provided contradictory results (19, 20). To further understand the role of IFP in the joint and the contribution of adipokines in IFP effects, we analysed first the production of leptin and adiponectin in IFP according to the gender and the body mass index (BMI) of the patients, and then studied the interactions between IFP and cells in neighbouring tissues known to be involved in the pathogenesis of OA, namely chondrocytes and fibroblast-like synoviocytes (FLS). Blocking experiments have been carried out to evaluate the role of leptin and adiponectin. As adipose tissues have specific features depending on their distribution, we aimed also to determine whether the release of adipokines and the articular cell responsiveness to IFP differ from that induced by the subcutaneous adipose tissue.

## Materials and methods

*Adipose and articular tissue collection and preparation of fat-conditioned medium*

Specimens of subcutaneous adipose tissue and joint tissues (cartilage, synovium and IFP) were obtained from OA patients undergoing total knee replacement surgery (n=22; ages 48-83 years, mean 66.5 years; BMI 27-40 kg/m$^2$, mean 33 kg/m$^2$). All patients were evaluated by an orthopaedic surgeon and diagnosed based on the criteria of the American College of Rheumatology (21). The human study described here was conducted in conformity with the declaration of Helsinki principles and was approved by the local Research Institution (Commission de la Recherche Clinique; agreement UF 9607 – CPRC 2005). Written informed consent was obtained from all participants.

The subcutaneous adipose tissue samples were harvested from the thigh, next to the incision. Beside, the inner part of the IFP was taken to properly separate it from the synovium. Both adipose tissues were washed twice in a phosphate buffer solution (PBS), cut into small pieces and then cultured at a concentration of 100 mg/ml in Dulbecco's Modified Eagles Medium/Ham's F-12 (DMEM/Ham's 12) supplemented with L-glutamine (2 mM), penicillin (0.1 U/ml), streptomycin (100 ng/ml) and Amphotericin B (250 ng/ml) (InVitrogen, Cergy-Pontoise, France). After 3 hrs, the medium was refreshed to remove possible contamination with cell rests and blood-derived soluble factors. After 24 hrs in fresh medium, adipose tissues were discarded and the supernatants were collected and stored at -80°C until use.

*Isolation of human articular cells*

Cartilage samples were obtained from femoral condyles and tibial plateaus while synovium were dissected from the underlying connective tissues. Both articular tissues were washed in PBS and then cut into small pieces. Chondrocytes were isolated after a sequential digestion of the extracellular matrix with pronase (0.15%, w/v) (Roche Applied Science, Germany) for 2 hrs and collagenase (0.2%, w/v) (Roche Applied Science, Germany) overnight at 37°C with constant mechanical stirring. FLS were also obtained after an enzymatic digestion of synovial tissues with a mix of collagenase (0.2%, w/v)/dispase (1 mg/ml) (Roche Applied Science Mannheim, Germany) overnight at 37°C with constant mechanical stirring. After centrifugation of the resulting cells and suspension in Dulbecco's Modified Eagles Medium/Ham's F-12 (DMEM/Ham's 12) supplemented with foetal calf serum (10%, v/v), L-glutamine (2 mM), penicillin (0.1 U/ml), streptomycin (100 ng/ml) and Amphotericin B (250 ng/ml) (InVitrogen, Cergy-Pontoise, France), the chondrocytes were seeded as primary culture in six-well plates (3X10$^4$ cells/cm$^2$) while FLS were cultured in 75 cm$^2$ culture flasks. The cells were expanded at 37°C in monolayer in a humidified atmosphere containing 5% $CO_2$ until reaching confluence. FLS were subcultured until the passage 3 to prevent any contamination by macrophage-like cells (22) and then seeded in six-well plates at high density (3x10$^4$ cells/cm$^2$).

*Study design*

Confluent primary chondrocytes and FLS were stimulated for 6 or 48 hrs with fat-conditioned medium after overnight starvation in serum-free medium. Articular cells incubated in serum deprived medium were used as unstimulated control. After the 6-hour treatment, cells were lysed for gene expression analyses while supernatants were collected at 48 hrs and stored at -80°C until analyses.

For blocking experiments, confluent articular cells were incubated for 6 hrs with IFP-conditioned medium in the presence or not of a goat anti-human adiponectin antibody or a recombinant human leptin R/Fc chimera (100 ng/ml) (R&D Systems, Lille, France). Goat IgG (100 ng/ml) was used as a control. The cells were then harvested for gene expression analyses.

*Gene expression analyses*

Total RNA was extracted from articular cells using the RNeasy Mini Kit (Qiagen, Courtaboeuf, France) according to the manufacturer's instructions. RNA samples were then reverse transcribed for 90 min at 37°C using oligo-dT primers (100 pmol) and M-MLV reverse transcriptase (200 U) (Invitrogen, Cergy-Pontoise, France). Gene expression was analysed by quantitative real-time PCR (Lightcycler, Roche, Mannheim, Germany) using the SYBRgreen master mix system (Qiagen) according to the manufacturer' protocol. The gene-specific primer pairs optimised for this method and the corresponding product's size are summarised in Table I. Quantification was achieved using a specific calibration curve obtained from serial dilutions of a positive PCR standard with a known amount of the corresponding purified PCR products. For standardisation of gene expression levels, mRNA ratios relative to RP29 as housekeeping gene were calculated.

*Determination of adipokines, $PGE_2$, MMP-13 and NO*

The levels of adiponectin, leptin, pro-MMP-13 and $PGE_2$ were determined in duplicate by a sandwich enzyme-linked immunosorbent assay for adipokines and pro-MMP-13, and by a competitive enzyme immunoassay for $PGE_2$ using commercially available kits (R&D Systems, Lille, France).

NO release was determined spectrophotometrically by measuring the accumulation of nitrite in culture supernatants by the Griess reaction (23). Briefly, 100 µl of culture supernatant were mixed with 100 µl of Griess reagent (sulphanilamide [1%] in 2.5% of $H_3PO_4$ and N-Naphtylethylenediamine dihydrochloride [0.1%] in $H_2O$) for 5 min. The absorbance ($OD_{550}$) was measured using a MR5000 (Dynatech) microplate reader.

*Statistical analyses*

The effect of fat-conditioned medium with or without adipokines neutralisation was tested in triplicate for each patient. PCR analyses and determination of $PGE_2$, NO and MMP-13 in supernatants were performed in duplicate on each sample. Data represent means with 95% confidence intervals (lower limit, upper limit), except for the comparisons between the IFP and the subcutaneous adipose tissue for which data showed individual points, each representing the mean of a triplicate found for one patient. Statistical analyses were

**Table I.** Sequences of primers and amplicon size.

| Genes | Sequences | Temperature (°C) | Amplicon size (bp) |
|---|---|---|---|
| RP29 | Fw AAGATGGGTCACCAGCAGCTCTACTG<br>Rv AGACGCGGCAAGAGCGAGAA | 60 | 156 |
| Adiponectin | Fw CCTAAGGGAGACATCGGTGA<br>Rv GTAAAGCGAATGGGCATGTT | 57 | 173 |
| Leptin | RT-PCR primer Set from SuperArray | 55 | 181 |
| Type 2 collagen | Fw ACACTGGGACTGTCCTCTGC<br>Rv GGCAGTCTTTCACGTCTTCA | 62 | 53 |
| Aggrecan | Fw TCGAGGACAGCGAGGCC<br>Rv TCGAGGGTGTAGCGTGTAGAGA | 63 | 84 |
| TGF-β | Fw TGCGGCAGCTGTACATTGA<br>Rv TGGTTGTACAGGGCCAGGA | 59 | 186 |
| IGF-1 | Fw GTATTGCGCACCCCTCAA<br>Rv TTGTTTCCTGCACTCCCTCT | 57 | 126 |
| COX-2 | Fw GCTGGAACATGGAATTACCCA<br>Rv CTTTCTGTACTGCGGGTGGAA | 58 | 98 |
| mPGES | Fw AACGACATGGAGACCATCTAC<br>Rv ACATCAAGTCCCCAGGTATAGCC | 60 | 314 |
| iNOS | Fw ACAAGCCTACCCCTCCAGAT<br>Rv TCCCGTCAGTTGGTAGGTTC | 58 | 157 |
| MMP-13 | Fw TGGTGGTGATGAAGATGATTTG<br>Rv TCTAAGCCGAAGAAAGACTGC | 57 | 161 |
| ADAMTS 4 | Fw TCCTGCAACACTGAGGACTG<br>Rv CGTGGCTCCAGCACATAGTA | 62 | 209 |

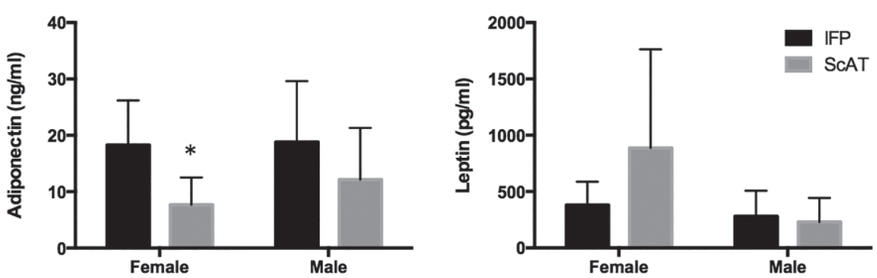


**Fig. 1.** Release of adiponectin and leptin from infrapatellar fat pad (IFP) and subcutaneous adipose tissue (ScAT).
Adipokine levels were determined in duplicate in fat-conditioned media of adipose tissues collected from 22 OA patients (16 female and 6 male) and then cultured for 24 hrs. Bars show the means with 95% confidence intervals. *$p$<0.05 between IFP and ScAT.

performed with the GraphPad Prism 5 software. Differences between women and men in the production of adipokines by fat-conditioned media were analysed using the nonparametric Mann-Whitney U-test. The average gene expression and the average production of $PGE_2$, NO and MMP-13 in articular cells cultured in control medium *versus* IFP-conditioned medium were compared using the Mann-Whitney U-test. The Wilcoxon signed-rank test was used to compare the effects of fat-conditioned medium derived from IFP and paired subcutaneous adipose tissue. Correlations were evaluated using the Spearman ranked correlation test. A $p$-value less than 0.05 was considered significant for differences and correlations.

## Results

### *Production of adipokines by IFP and subcutaneous adipose tissue*

The conditioned media from cultured specimens of IFP and subcutaneous adipose tissue obtained from OA patients were analysed for secretion of adiponectin and leptin. There was no significant difference between the female (n=16) and male (n=6) patients with respect to age (mean ± SD, 68.5±9.1 years *vs*. 61.3±5.3 years, respectively) or BMI (mean ± SD, 32.2±3.5 kg/m$^2$ *vs*. 33.6±3.4 kg/m$^2$, respectively).

As illustrated in Figure 1, all IFP samples consistently released high amounts of adiponectin. The joint adipose tissue was even more active to synthesise this adipokine than the subcutaneous adipose tissue, but the difference reached statistical significance in the female group only. IFP specimens produced also leptin with similar efficiency compared to the subcutaneous adipose tissue. No significant correlation was found between the BMI of the patients and the release of adipokines from IFP. Interestingly, the levels in IFP-conditioned medium of adiponectin but not those of leptin were associated with the concentrations of $PGE_2$ (r=0.5008 and $p$=0.0245 for adiponectin; r=0.03684 and $p$=0.8810 for leptin).

### *Effect of IFP on articular cells*

Chondrocytes and FLS obtained from patients with end-stage knee OA have been stimulated with autologous IFP-conditioned medium. As the production of adipokines by IFP did not show any sexual dimorphism, data from male and female OA patients were pooled for the next experiments.

All IFP-conditioned media strongly upregulated the expression of inflammatory and degenerative genes in chondrocytes compared to control cells incubated with fresh medium (Fig. 2A). By contrast, no change in the expression of adipokines, type 2 collagen, aggrecan or TGFβ1 was found. The mRNA level of the IGF-1 gene was increased but the difference with unstimulated cells did not reach statistical significance. The data indicated also that the BMI of the patients did not influence chondrocyte response to IFP.

The IFP-conditioned medium collected from OA patients also induced the expression of COX-2 and mPGES in FLS but was ineffective in stimulating iNOS in these cells (Fig. 2B). As found with chondrocytes, the expression of genes encoding adiponectin, leptin or TGFβ1 remained unchanged upon exposure of

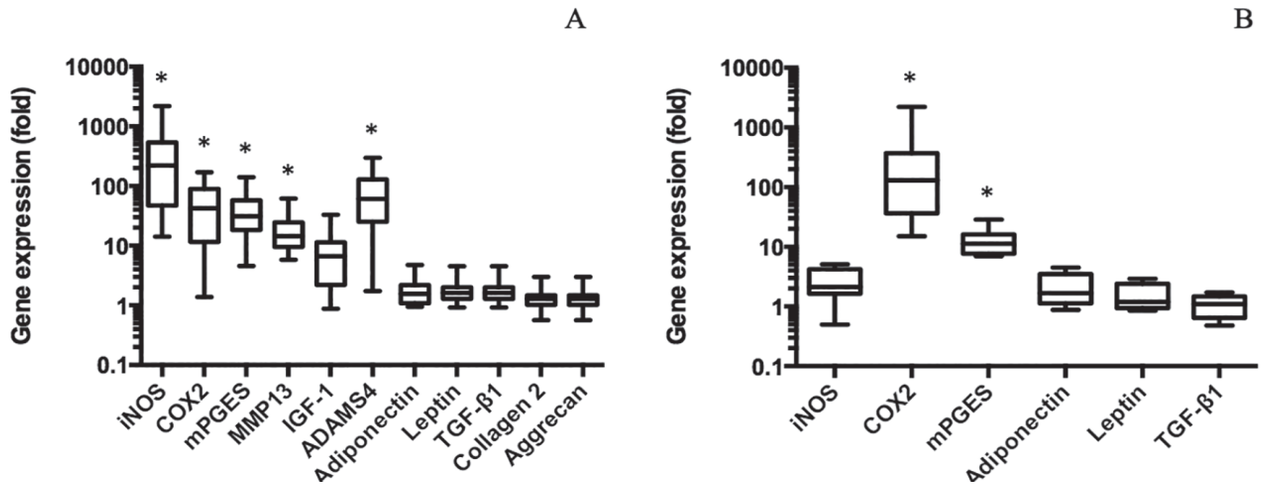


**Fig. 2.** Effect of IFP-conditioned medium on gene expression in chondrocytes (**A**) and fibroblast-like synoviocytes (FLS) (**B**).
Chondrocytes and FLS were collected from 20 OA patients. PCR analyses were performed in duplicate 6 hrs after stimulation of articular cells with autologous IFP-conditioned media. For every patient, experiments were carried out in triplicate. Data are expressed as relative to control without IFP-conditioned medium and are presented as box plots, in which each box represents the 25th to 75th percentiles, line inside the boxes represents the median, and lines outside the boxes represent the minimum and maximum values. *$p$<0.05 between cells stimulated with IFP-conditioned medium and control cells without IFP-conditioned medium. Inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), microsomal prostaglandin E synthase (mPGES), metalloproteinase-13 (MMP-13), insulin growth factor-1 (IGF-1), a disintegrin and metalloproteinase with thrombospondin motifs-4 (ADAMTS-4) and transforming growth factor-β (TGF-β).

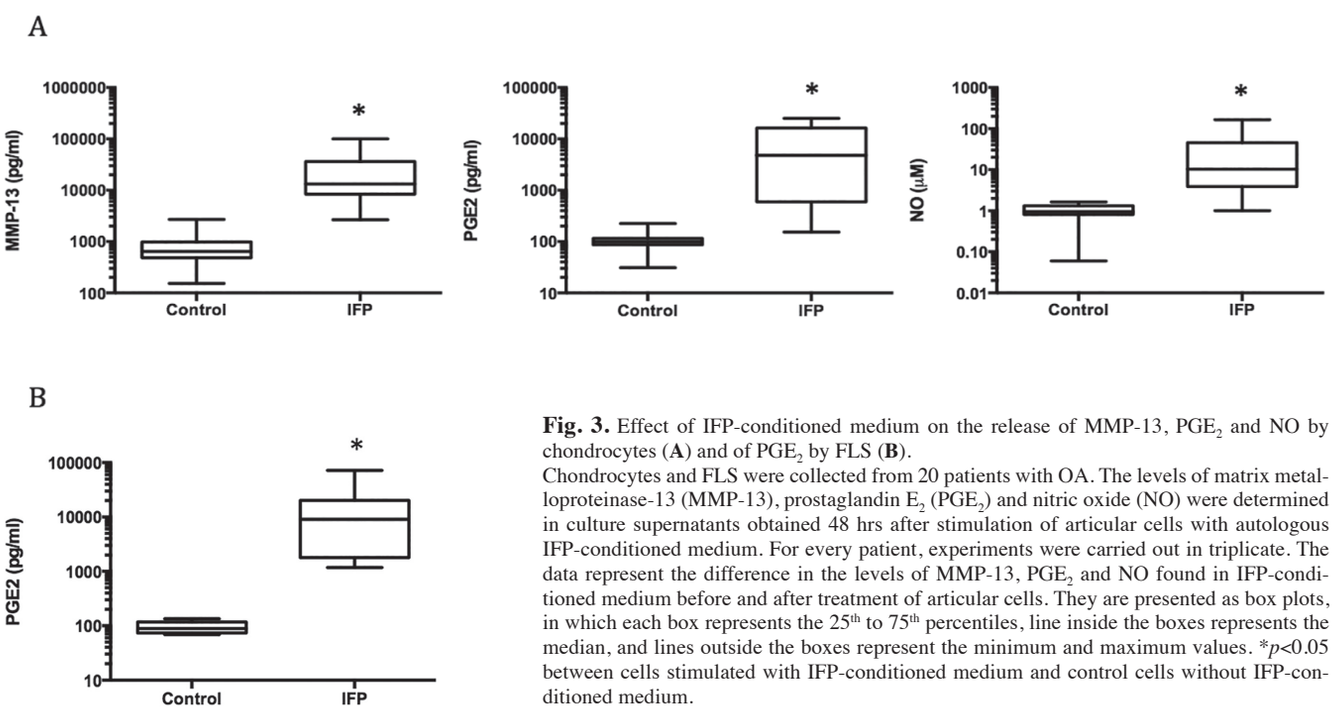


**Fig. 3.** Effect of IFP-conditioned medium on the release of MMP-13, $PGE_2$ and NO by chondrocytes (**A**) and of $PGE_2$ by FLS (**B**).
Chondrocytes and FLS were collected from 20 patients with OA. The levels of matrix metalloproteinase-13 (MMP-13), prostaglandin $E_2$ ($PGE_2$) and nitric oxide (NO) were determined in culture supernatants obtained 48 hrs after stimulation of articular cells with autologous IFP-conditioned medium. For every patient, experiments were carried out in triplicate. The data represent the difference in the levels of MMP-13, $PGE_2$ and NO found in IFP-conditioned medium before and after treatment of articular cells. They are presented as box plots, in which each box represents the 25$^{th}$ to 75$^{th}$ percentiles, line inside the boxes represents the median, and lines outside the boxes represent the minimum and maximum values. *$p$<0.05 between cells stimulated with IFP-conditioned medium and control cells without IFP-conditioned medium.

FLS to IFP-derived products, and no significant relationship was shown between the BMI of the patients and the increase in the mRNA levels of COX-2 and mPGES.

The analyses of cultured supernatants collected 48 hrs after articular cell stimulation with IFP-conditioned media further supported PCR data. IFP strongly increased the release of MMP-13, NO and $PGE_2$ ($p$<0.0001) in chondrocytes and the synthesis of $PGE_2$ in FLS ($p$<0.0001) (Fig. 3). No detectable amount of NO was found in the cultured supernatant of FLS incubated with IFP-conditioned media.

*Comparison between IFP and subcutaneous tissue*

Chondrocytes and FLS have been stimulated with the conditioned medium obtained from paired samples of subcutaneous tissue. PCR data analyses revealed heterogeneous findings for the expression of COX-2 and mPGES in both chondrocytes and FLS (Fig. 4-5). The IFP from some patients, but not all, exhibited a strong inflammatory pattern compared to the paired subcutaneous tissue. Beside, the conditioned

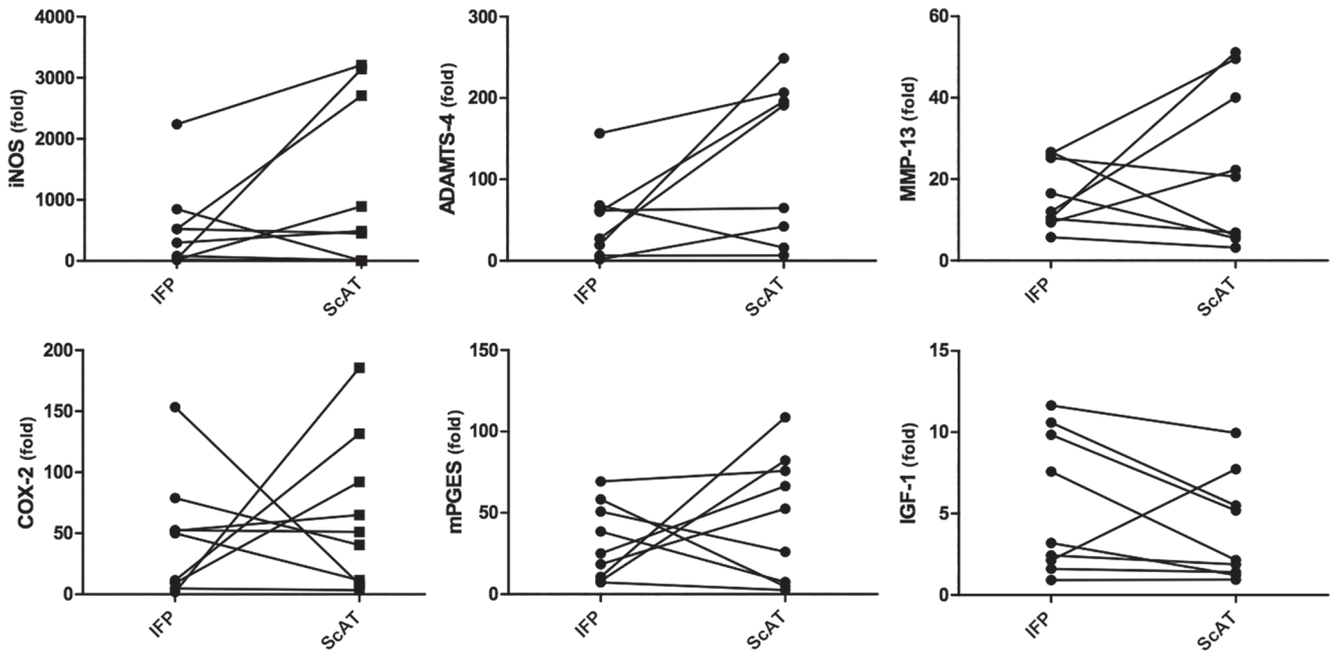


**Fig. 4.** Comparison of the stimulatory effect of IFP and ScAT in chondrocytes.
PCR analyses were performed in duplicate 6 hrs after incubation of articular cells from 9 OA patients with or without the conditioned medium obtained from paired samples of infrapatellar fat pad (IFP) and subcutaneous adipose tissue (ScAT). For every patient, experiments were carried out in triplicate. Data are expressed as relative to control without fat-conditioned medium and each line represents individual patient. Inducible nitric oxide synthase (iNOS), a disintegrin and metalloproteinase with thrombospondin motifs-4 (ADAMTS-4), metalloproteinase-13 (MMP-13), cyclooxygenase-2 (COX-2), microsomal prostaglandin E synthase (mPGES) and insulin growth factor-1 (IGF-1).

media derived from the subcutaneous tissue showed a trend towards a higher stimulatory effect on the expression of iNOS, MMP-13 and ADAMTS-4 while those derived from IFP were the most efficient to increase the mRNA level of the IGF-1 gene.

*Effect of blocking endogenous adiponectin and leptin*

As adipokines are able to modulate the functions of articular cells, we next determined whether leptin or adiponectin derived from IFP might be involved in the up-regulation of the inflammatory and degenerative genes. Surprisingly, the expression of iNOS or MMP-13 in chondrocytes remained unchanged upon neutralization of leptin or adiponectin (Table II). Similarly, the addition of leptin R/Fc chimera or adiponectin neutralising antibody to conditioned media before stimulation of FLS did not change the effects of IFP on the expression of mPGES (Table II) .

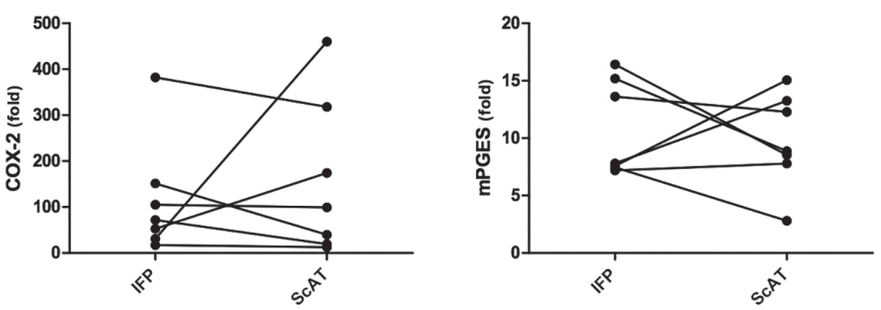


**Fig. 5.** Comparison of the stimulatory effect of IFP and ScAT in fibroblast-like synoviocytes (FLS).
PCR analyses were performed in duplicate 6 hrs after incubation of articular cells from 9 OA patients with or without the conditioned medium obtained from paired samples of infrapatellar fat pad (IFP) and subcutaneous adipose tissue (ScAT). For every patient, experiments were carried out in triplicate. Data are expressed as relative to control without fat-conditioned medium and each line represents individual patient. Cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase (mPGES).

## Discussion

Recently, it has become more evident that IFP tissue in the joint may contribute to the inflammatory and degenerative process during OA. The current study aimed therefore to determine whether adipokines known to induce destructive responses in cartilage may mediate the effects of IFP. The data indicate that the IFP tissue is a major source of adiponectin compared with the subcutaneous adipose tissue from the same patient independently of the gender. Conversely, a trend toward a lower production of leptin by the IFP was found for female patients. In fact, the subcutaneous adipose tissue has been identified as a strong active organ for the secretion of leptin (24, 25). More especially, subcutaneous adipocytes from females have been shown to release significantly more leptin than did omental cells from the same subject (5). The lack of sexual dimorphism for the release of leptin by IFP and the elevated production of adiponectin suggest that even if this joint adipose deposit is structurally similar to the subcutaneous adipose tissue, it has its own pattern of secretion of adipokines. In addition, no correlation between BMI and IFP adipokines production was demonstrated, suggesting that

**Table II.** Effect of blocking adiponectin or leptin on the IFP-induced expression of iNOS, MMP-13 and mPGES.

| | Chondrocyte | | FLS |
|---|---|---|---|
| | iNOS | MMP-13 | mPGES |
| IFP | 197.4 (-9.59 to 404.4) | 31.23 (-34 to 96.46) | 13.95 (4.24 to 23.61) |
| Leptin R/Fc | 4.19 (1.38 to 7) | 1.15 (0.49 to 1.81) | 1.69 (0.63 to 2.75) |
| IFP + Leptin R/Fc | 354.0 (1.56 to 706.4) | 40.06 (-59.95 to 140.1) | 14.88 (0.86 to 28.89) |
| Goat IgG | 4.47 (-2.23 to 11.17) | 2.62 (-3.07 to 8.31) | 2.47 (-0.92 to 5.86) |
| IFP + Adiponectin Ab | 316.0 (-221.6 to 853.6) | 23.89 (-26.54 to 74.32) | 10.80 (4.68 to 16.91) |

PCR analyses were performed in duplicate 6 hrs after stimulation of chondrocytes and fibroblast-like synoviocytes (FLS) with autologous IFP-conditioned media in the presence or not of recombinant human leptin R/Fc Chimera (leptin R/Fc) or a goat anti-human adiponectin antibody (adiponectin Ab) (100 ng/ml). A goat IgG (100 ng/ml) was used as control. The data are expressed as relative to control value without IFP-conditioned medium and represent mean (95% confidence interval) of nine OA patients with three samples per patient and per condition.
Inducible nitric oxide synthase (iNOS), metalloproteinase-13 (MMP-13), and microsomal prostaglandin E synthase (mPGES).

systemic metabolic changes associated with obesity do not affect IFP.

Although excessive adiposity triggers the secretion of inflammatory mediators in adipose tissue, we failed to show any association between the levels of $PGE_2$ in IFP-conditioned media and BMI, further supporting that IFP may have specific inflammatory phenotypic features independent from the general phenotype found in obesity. Interestingly, we found a positive association between the productions of adiponectin and $PGE_2$ by IFP suggesting that the release of adiponectin by IFP is up-regulated under inflammatory conditions. By contrast and despite the well-known inflammatory role of leptin, no correlation between the levels of leptin and $PGE_2$ in IFP-conditioned medium was demonstrated. These unexpected data are, however, in agreement with those found by Distel *et al*. who have shown a negative association between the productions of IL-6 and leptin by IFP (14).

Earlier studies have reported contradictory data on the effect of IFP on cartilage metabolism with a protective role for Bastiaansen-Jenniskens *et al*. (20) and conversely, a degenerative activity for Hui *et al*. (19). Here, we showed that all conditioned media collected from cultured IFP have induced the expression of the genes encoding degenerative enzymes in chondrocytes and inflammatory proteins in both chondrocytes and FLS. These effects of IFP were accompanied with a release of MMP-13, NO and $PGE_2$ in the culture supernatants. No change in the expression of aggrecan, type 2 collagen or growth factors was observed, excluding any protective effect of IFP on cartilage. The use of human OA chondrocytes instead of healthy bovine cartilage may explain the discrepancies with the study of Bastiaansen-Jenniskens *et al*. (20). The response to a stimulus of chondrocytes isolated from osteoarthritic cartilage may differ from that found in cells maintained in an intact extracellular matrix. The discrepancy might also be related to the species. Species-specific regulations have been described in bovine and human chondrocytes, especially for the activation of the NO pathway (27). Even if investigations with human samples raise the question of heterogeneity in tissue and cell metabolic status, the current study provides homogeneous data about the effects of IFP. All specimens tested exhibited an inflammatory activity, only the level of stimulation differs between patients. Alternatively, these findings suggest that IFP may release both protective and deleterious factors which may prevent tissue destruction in healthy cartilage, but may further contribute to the degenerative process in OA cartilage.

In addition to the articular cartilage, the synovium is another joint structure in close contact with IFP. The inflammatory effect of IFP-conditioned medium on FLS indicated that IFP may also contibute to the changes found in the synovium during OA. Surprisingly, IFP was not able to induce the expression of iNOS in FLS whereas it strongly increases the mRNA levels of iNOS and the release of NO by chondrocytes. Previous studies demonstrated that NO production from isolated synoviocytes was up-regulated by *in vitro* exposure to inflammatory cytokines, especially when they were used in association (28,29). The lack of essential cytokines in the conditioned medium may explain the inability of IFP to stimulate NO production in FLS.

Because leptin or adiponectin are known to induce an inflammatory response in articular cells (8), we performed blocking experiments to determine whether these adipokines found in IFP-conditioned medium may contribute to the stimulatory effect of IFP. Blocking leptin or adiponectin did not change the level of expression of IFP-induced genes, suggesting that they did not mediate IFP effects.

In order to gain insight into the inflammatory potency of IFP, we compared articular cell response to conditioned media from paired samples of IFP and subcutaneous adipose tissue. Although previous studies have reported an increased level of inflammatory factors in IFP-conditioned medium compared with subcutaneous adipose tissue, we did not show a clear difference in the response of articular cells between both adipose tissues. In fact, we did not use mean data to compare IFP and subcutaneous adipose tissue because of a great variability between patients. For some of them, IFP appeared to be more inflammatory while the opposite was found for the other ones. These findings suggest that IFP exhibits a specific inflammatory phenotype which is regulated by pathways distinct from those involved in the expression of an inflammatory phenotype in subcutaneous adipose tissue. As shown in the current study as well as in others, this specific inflammatory phenotype develops in IFP independently of adiposity. Neither the levels of most of the inflammatory mediators released by IFP nor the articular cell response to IFP-conditioned medium were associated with BMI (15, 20, 30). Additional experiments would be helpful to identify the factors responsible for a high level of inflammation in IFP.

These findings provide evidence for a key role of IFP in the pathophysiology of OA and are further support by experimental data found in mice. They indicated actually that high fat diet promoted both OA changes and hypertrophy of the IFP (31). These changes in IFP were associated with the expression of inflammatory cytokines, growth factors and adipokines as well as macrophages infiltration. However, clinical data suggest rather that IFP may have a protective role in OA. Pan *et al*. showed in a longitudinal study that higher IFP maximal area at baseline was associated with reduced development and progression of knee pain, cartilage volume loss and cartilage defects over 2.6 years (18). In fact, IFP with maximal area may reduce the mechanical stress in the knee joint with an improved distribution of synovial fluid, a better absorption of the loading and an increased stability of the patellar ligament. However, our study indicated that IFP in an inflammatory environment may also contribute to the progression of OA.

In summary, our findings provide further evidence for a deleterious role of IFP in OA. This articular adipose tissue may trigger both cartilage destruction and inflammation of the synovium. Although it produces adipokines with a pattern of secretion distinct from that of subcutaneous adipose tissue, neither leptin nor adiponectin mediate the inflammatory effect of IFP. In addition, unlike most of the adipose deposits, the secretory activity and the inflammatory effect of IFP do not depend on BMI. As we used IFP from patients with end-stage OA, it remains to determine whether IFP may have protective effect in healthy joints or during the initiation of the disease, but may contribute to degenerative changes under inflammatory conditions.

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