COMP in the Infrapatellar Fat Pad—Results of a Prospective Histological, Immunohistological, and Biochemical Case-Control Study

David Grevenstein,1,2 Juliane Heilig,2 Jens Dargel,2,3 Johannes Oppermann,1,2 Peer Eysel,1,2 Christoph Brochhausen,4 Anja Niehoff2,5

1Department for Orthopaedic and Trauma Surgery, Faculty of Medicine and University Hospital of Cologne, Cologne, Germany, 2Cologne Center for Musculoskeletal Biomechanics, Faculty of Medicine and University Hospital of Cologne, Cologne, Germany, 3Department of Orthopedic Surgery, St. Josef’s-Hospital, Wiesbaden, Germany, 4Institute for Pathology, University Hospital Regensburg, Regensburg, Germany, 5Institute of Biomechanics and Orthopaedics, German Sport University Cologne, Cologne, Germany

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ABSTRACT: Knee osteoarthritis (OA) involves several structures and molecules in the joint, which interact in a pathophysiological process. One of these molecules is the cartilage oligomeric matrix protein (COMP). Elevated COMP levels in the synovial fluid as well as in the serum have been described in OA patients. However, this has not been described in the infrapatellar fat pad (IPFP) tissue before. In this prospective trial, we collected 14 IPFPs from patients with high-grade OA (mean age 63.8 ± 17.6 years) who underwent total knee replacement (OA group) and from 11 healthy patients (mean age 33.7 ± 14.8 years) who underwent anterior cruciate ligament reconstruction (control group). The presence of macrophages (CD68 and CD206) and proinflammatory cytokines (interleukin 1β [IL-1β] and IL-6) was analyzed. Histological and immunohistological examinations as well as immunoblotting analysis for COMP, leptin, and matrix-metalloproteinase-3 were performed. The IPFPs of both the OA and control group consisted of adipose tissue and fibrous tissue, and the fibrous tissue showed higher score values than the adipose tissue for COMP staining (intensity as well as stained area) in both groups. Although COMP could be detected in most samples, leptin expression was found only in single specimens. COMP could be detected mostly in the fibrous tissue portion of the IPFP. We speculate that it is involved in a remodeling process taking place in the IPFP during OA. Presence of leptin was irregular in immunohistology, and the control group showed higher scores in case of presence. Interestingly, immunoblotting could detect leptin in all analyzed samples. © 2019 The Authors. Journal of Orthopaedic Research® published by Wiley Periodicals, Inc. on behalf of Orthopaedic Research Society. J Orthop Res 38:747–758, 2020

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Osteoarthritis (OA) is a common progressive, degenerative joint disease that affects a large number of the population in old age. However, the pathogenesis of OA is not completely understood until today. In the last few years, pathological changes on several structures of the joint, like the synovial fluid,1,2 the subchondral bone2 or the infrapatellar fat pad (=Hoffa’s fat pad, IPFP)4 have become a target of OA research. All named structures are part of a complex pathological process at the molecular level, which is in the end characterized by an irreversible destruction of the hyaline articular cartilage. Clinical symptoms are joint swelling and inflammatory pain, which are at least partly caused by synovitis, an inflammation of the synovial membrane.5 The synovial fluid contains proinflammatory mediators, which lead to an invasion of mononuclear cells.5

Hereby, the concentration of proinflammatory cytokines like interleukin 1β (IL-1β), interleukin 6 (IL-6), or tumor necrosis factor α (TNF-α) increases6 and causes an excessive synthesis of proteolytic enzymes responsible for cartilage breakdown.2 Due to the mentioned processes, the balance between cartilage extracellular matrix synthesis and degradation is disturbed. Cartilage destruction in turn amplifies synovial inflammation, leading to a vicious circle.

On the cellular level, fibroblasts as well as chondrocytes participate in the inflammatory reaction by synthesis of proinflammatory cytokines.7 Most proinflammatory cytokines act through activation of the transcription factor nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), which again leads to synthesis of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), nitric oxide (NO), and pros-taglandin E2 (PGE-2). Subsequently, chondrocytes in osteoarthritic joints reduce synthesis of components of the extracellular matrix, like collagen II or aggregan and increase the expression of matrix-metalloproteinases (MMPs).8,9 In particular, IL-1β stimulates chondrocytes to synthesize MMPs, which seem to play an important role in the pathogenesis of OA.6 MMP-1, -3 and -13 are collagenases, and in particular, MMP-3 is a good marker for proinflammatory processes and cartilage degradation in diseased joints.10,11 Moreover, the hormone leptin stimulates chondrocytes to...
synthesize MMPs and proinflammatory cytokines.12–15 Previous studies have found significantly increased serum leptin levels in OA patients.16 Interestingly, leptin could be found in the IPFP,17 even if only patients with diagnosed OA were investigated in this study. The IPFP is localized infrapatellar, intracapsular and extrasynovial. The exact function is not clear yet. It is richly vascularised and innervated18 and it may work as a shock absorber and protect nearby structures.19 The IPFP consists of white adipose tissue and thin septa.20 Overall, in OA, it seems to play a role in the genesis and the maintenance of the proinflammatory reaction, which is responsible for cartilage degradation.4–21 Several studies have investigated changes in the composition of the IPFP in patients suffering with OA using not only radiological but also histological and biochemical techniques. With magnet resonance imaging (MRI), no difference in the volume could be detected, compared with a control group,22 but inflammatory changes could be found.23 In accordance to this, studies could show a proinflammatory cytokine profile in the IPFP by detection of, for example, TNF-α, VEGF (vascular endothelial growth factor)24,25 and IL-6.26 Favero et al.25 found a different cytokine profile in the fat pad of osteoarthritic patients compared with a control group of an organic-donor program. However, it has to be mentioned, that the donors of the control group had a mean age of 81 years, which makes the exclusion of osteoarthritic changes, even if there were no clinical symptoms known, quite difficult. A molecule which has not been investigated in the extracellular matrix of the IPFP is cartilage oligomeric matrix protein (COMP). COMP or thrombospondin-5 is mainly present in cartilage extracellular matrix. In smaller amounts, the protein could also be detected in highly stressed parts of the tendon,27 in the bone,28 in menisci and ligaments.29 This molecule has been described as an adapter molecule that influences the synthesis and remodeling of the extracellular matrix in physiological and pathophysiological situations.30 COMP is also believed to play an important role in fibrillogenesis of collagen I and II.31 Furthermore, COMP interacts with growth factors, and besides regulating extracellular matrix composition and assembly, it also affects cellular functions such as proliferation, differentiation, and attachment of cells.32 Several studies have shown that the localization and expression of COMP differs between healthy hyaline articular cartilage and osteoarthritic cartilage.33 In addition, elevated serum COMP levels have been reported in OA,34,35 rheumatoid arthritis,36 and injured knees.37 COMP has also been detected in human adipose tissue and there are differences in expression between abdominal fat and gluteal fat.38 However as far as we know, COMP has not been investigated in the extracellular matrix of the IPFP yet. In previous studies, the analysis of different cartilage genes including COMP has been used to determine the chondrogenic differentiation potential of both stromal vascular fraction cells39 and mesenchymal stem cells40 from the IPFP. To our knowledge, there is no study which compares the IPFP extracellular matrix from patients with high-grade OA with a control group of IPFPs from young and non-osteoarthritic patients. There is one recent study41 which analyzed IPFP derived mesenchymal stem cells from old patients with high-grade OA and from young patients with an anterior cruciate ligament (ACL) rupture and no signs of OA. Here, a proteomic study of the mesenchymal stem cells from the IPFP was done. Therefore, the purpose of the present study was (i) to analyze if COMP could be detected in the extracellular matrix of the IPFP and (ii) to study if the localization and the expression of MMP-3, -9, -13, leptin, IL-6, IL-1β, and COMP in the IPFP of OA patients differ from that of young subjects without OA.

METHODS

Tissue Samples
In this prospective case–control study, we collected 14 IPFPs from patients who underwent total knee replacement surgery due to high-grade OA. A control group of 11 IPFP-samples was collected from young patients, who underwent knee-arthroscopy because of a rupture of the ACL and did not show any radiological or arthroscopical signs of OA. Arthroscopy was performed due to clinical reasons (ACL-reconstruction). Only a small part of the IPFP was removed in cases where it was clinically necessary, for example, when a prominent IPFP disturbed intrarticular sight. The inclusion criteria was that the trauma which leads to the surgery had to be acute (<4 weeks), so that no chronic changes in the joint occurred. Exclusion criteria were autoimmune diseases, especially rheumatoid arthritis, acute joint-infection, and malignoma. A positive vote was obtained from the local ethical commission (Ethical commission of the University hospital of Cologne, study 17–217). Informed written consent was obtained from tissue donors.

Histology
Samples were fixed in 10% neutral buffered 5% formalin (48h), embedded in paraffin, and cut in 7-μm-thick sections. Sections were submitted to a Masson’s trichrome staining (Sigma-Aldrich, Corporation, St. Louis, MO) to visualize collagen in blue, cytoplasm and muscle tissue in red and nuclei in black. The staining procedure was performed according to the manufacturer’s instructions. Shortly, after deparaffinization and rehydration, the sections were incubated in Bouin’s solution for 15 min at 56°C. The sections were washed with water and incubated for 5 min in Weigert’s Iron Hematoxylin solution. Following washing with water, the sections were stained with Biebrich Scarlet-Acid Fuchs in for 5 min. After rinsing in water, the sections were incubated in phosphotungstic/phosphomolybdic acid solution for 5 min and then stained with Aniline Blue solution for 5 min. The sections were then placed in 1% acetic acid for 2 min and subsequently dehydrated in ethanol, isopropanol and xylol and mounted in DPX mounting medium. Masson’s trichrome staining was used to evaluate the portion of fibrous-tissue (ft) in the sections using a semiquantitative score. Score values reached from 0 to 4 (0 = no ft, 1 = <25% ft, 2 = 25–50% ft, 3 = 50–75% ft, 4 = >75% ft). One representative section of each specimen was evaluated.
**Immunohistochemistry and Immunofluorescence**

For immunohistochemical analysis, the following primary antibodies were used: rabbit polyclonal antibodies against IL-6 (21865-1-AP, 1:200 diluted; Proteintech, Europe, Manchester, UK), IL-1β (16806-1-AP, 1:100 diluted; Proteintech), CD206 (ab64693, 1:500 diluted; Abcam plc, Cambridge, UK), COMP (1:1000 diluted), leptin (ab16227, 1:100 diluted; Abcam plc), MMP-3 (ab53015, 1:100 diluted; Abcam plc), MMP-9 (ab38898, 1:500 diluted; Abcam plc), and MMP-13 (ab39012, 1:500 diluted; Abcam plc). For COMP and leptin detection, enzymatic antigen retrieval was performed by incubation with 500 U/ml hyaluronidase in 100 mM NaH₂PO₄ and 100 mM NaCl (pH 5.0) for 30 min at 37°C and with proteinase K (10 µg/ml) in 50 mM Tris-HCl (pH 7.4) and 1 mM EDTA for 10 min at 55°C, for CD206, MMP-3, MMP-9, and MMP-13, unmasking was performed incubating the sections for 1 h in sodium citrate buffer (pH 6) at 60°C. The sections were blocked for 5 min with blocking solution (Zytomed Systems GmbH, Bargteheide, Germany) and incubated with the primary antibody overnight at 4°C. Horseradish peroxidase (HRP)-coupled anti-rabbit IgG (Zytochem Plus HRP polymer; Zytomed Systems GmbH) was used as the secondary antibody.

For the detection of bound antibodies, 1% DAB solution was applied to the sections until color development. After counterstaining with hematoxylin for 5 min, the sections were washed with water, dehydrated in ethanol, isopropanol, and xylol and mounted in DPX mounting medium. Immunofluorescence stainings were performed using a mouse monoclonal antibody directed against CD68 (M087629-1:100 diluted; Dako, Agilent Technologies, Santa Clara, CA). After epitope retrieval by incubating the sections for 1 h in sodium citrate buffer (pH 6) at 60°C, the sections were blocked with blocking solution (Zytomed Systems GmbH) for 5 min and incubated with the primary antibody overnight at 4°C. Alexa Fluor 555 coupled anti-mouse IgG (Thermo Fisher Scientific, Waltham, MA) was used as the secondary antibody, and nuclei were stained using 4',6-diamidino-2-phenylindole (DAPI).

Images were acquired using an Axiohot 2 microscope (Carl Zeiss AG, Oberkochen, Germany). Analysis of the immunohistochemical stainings for IL-6, IL-1β, COMP, and leptin was performed by semiquantitatively scoring the staining intensity as well as the extent of the stained area. One to five different sections were analyzed per single sample. The number of sections was dependent of the size and the morphology of the specimen. The extent of the extracellular staining was judged by an individual semiquantitative score ranging from 0 to 3 points with 0 points indicating no staining, 1 point for staining of ≤1/3 of the total area, 2 points for staining of 1/3–2/3 of the total area, and 3 points for staining found in >2/3 of the total area. Anti-IL-6, anti-IL-1β, anti-COMP, and anti-Leptin stainings were additionally analyzed regarding staining intensity using a score from 0–3 (0 = no staining, 1 = weak staining, 2 = average staining, 3 = strong staining) (Fig. 1). Analysis of CD68 and CD206 staining was conducted by counting the number of positive cells in relation to the total number of cells (in %) in three pictures per subject of each adipose and fibrous tissue.

**Immunoblotting**

Human infrapatellar fat samples were minced in small pieces and homogenized in 10 x volume 4 M Guanidine-HCl using a rotor-stator homogenizer. Protein extraction was carried out overnight at 4°C. Total extracts were centrifuged, and the fat layer which was formed on top of the aqueous protein extract was removed. The Guanidine-HCl containing protein extracts were dialyzed against tris-buffered saline (TBS) for 18 h. Protein concentrations were measured with a bicinchoninic acid (BCA) based protein quantitation kit (Uptima Interchim, Montlucon, France). Twenty micrograms of total protein were then separated by SDS-PAGE (non-reducing conditions were used for the detection of COMP, reducing conditions for the

![Intensity score 0](image1)

![Intensity score 1](image2)

![Intensity score 2](image3)

![Intensity score 3](image4)

**Figure 1.** Scoring system from 0 to 3 for immunohistochemical staining intensity. Four different samples with different intensities for the staining grades with a-COMP antibody are shown. Scale bar 200 µm. [Color figure can be viewed at wileyonlinelibrary.com]
The number of CD68-positive adipose and fibrous tissue samples (Fig. 3A and B). Phages with different phenotypes were present in OA and CD206 staining indicated that macrophages showed increased IL-1β and IL-6 staining intensity and a more extended stained area compared with the controls (p = 0.0122) (Fig. 3B). Furthermore, in both groups, there were more positive CD68 cells in the adipose compared with the fibrous tissue (p = 0.0006 [OA] and p = 0.0016 [control]). Analysis of positively stained cells in relation to total number of cells did not reveal a significant difference for CD206-positive cells between OA samples and the control group (Fig. 3D).

**IL-1β and IL-6 Immunohistochemistry**

IL-1β and IL-6 were stained to detect inflammatory cytokines. Staining could be observed in most adipose and fibrous tissue samples (Fig. 4A and C). We could not detect a significant difference in the staining intensity or stained area between samples of the OA and the control group, while in each group, the fibrous tissue showed increased IL-1β and IL-6 staining intensity and a more extended stained area than the respective adipose tissue (Fig. 4B and D). Mean intensity scores were 1.5 ± 0.7 (OA ft), 1.6 ± 0.6 (control ft), 1.0 ± 0.6 (OA at) and 0.9 ± 0.6 (control at) for IL-1β and 1.5 ± 0.5 (OA ct), 1.4 ± 0.5 (control ct), 0.8 ± 0.8 (OA at), and 0.6 ± 0.5 (control at) for IL-6 (Fig. 4B).

**RESULTS**

We included 14 specimens in the OA group from seven females and seven males with a mean age of 63.8 ± 17.6 years. Eleven IPFPs could be included in the control group (three females and eight males) with a mean age of 33.7 ± 14.8 years.

**Histology**

The Masson trichrome staining was performed to give an overview of the specimen and to quantify the portion of fibrous tissue in the samples. Most samples showed a considerable amount of fibrous tissue (Fig. 2A). Semiquantitative scoring of the fibrous tissue portion resulted in a mean score of 1.6 ± 0.8 for the OA and of 1.2 ± 1 for the control group, indicating a slightly higher content of fibrous tissue in the OA specimens, although we were not able to detect statistical significant difference (Fig. 2B).

**CD68 and CD206 Immunohistochemistry and Immunofluorescence**

CD68 and CD206 staining indicated that macrophages with different phenotypes were present in most adipose and fibrous tissue samples (Fig. 3A and C). The number of CD68-positive cells was significantly higher in the fibrous tissue of the OA group compared with the controls (p = 0.0122) (Fig. 3B). Furthermore, in both groups, there were more positive CD68 cells in the adipose compared with the fibrous tissue (p = 0.0006 [OA] and p = 0.0016 [control]). Analysis of positively stained cells in relation to total number of cells did not reveal a significant difference for CD206-positive cells between OA samples and the control group (Fig. 3D).

![Figure 2. Tissue morphology of the infrapatellar fat pads (IPFP).](wileyonlinelibrary.com)
COMP Immunohistochemistry

COMP could be detected in most specimens of the OA group as well as in most samples of the control group (Fig. 5A). However, in both groups, single samples were found to completely lack COMP staining. In both groups, staining was predominantly found in the fibrous tissue portion of the IPFPs. Stainings were quantified using a scoring system for the intensity of the staining as well as for the extent of the stained area (Fig. 1). Application of this scoring system resulted in mean intensity scores of 1.7 ± 0.8 (OA ft), 1.4 ± 1.3 (control ft), 0.2 ± 0.8 (OA at), and 0.5 ± 0.9 (control at) demonstrating a significantly higher score for OA fibrous tissue compared with OA adipose tissue ($p = 0.0001$), the respective comparison for the control tissue lacked statistical significance but showed a strong tendency ($p = 0.054$) (Fig. 5B). The comparison between OA and control samples showed no statistical significant differences in COMP staining in intensity, neither for fibrous tissue nor for adipose tissue. Additionally, scoring the extent of the stained area also

Figure 3. Detection of CD68-positive and CD206-positive macrophages using immunofluorescence and immunohistochemical stainings. (A) CD68-positive macrophages in the fibrous tissue and adipose tissue of infrapatellar fat pads of the osteoarthritis (OA) group and the control group are shown in red, nuclei are stained in blue using 4',6-diamidino-2-phenylindole. Scale bar 50 µm. (B) Quantification of the number of CD68-positive cells. Three different regions of interest (ROIs) within the fibrous tissue and the adipose tissue of each sample were analyzed for the number of CD68-positive and CD68-negative cells. $n = 14$ (OA), $n = 11$ (control). ***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$. (C) CD206-positive macrophages were immunohistochemically stained using DAB (brown) and nuclei were counterstained using hematoxylin. Scale bar 50 µm. (D) CD206-positive cells were counted in three different ROIs of both fibrous tissue and adipose tissue of each sample. $n = 14$ (OA), $n = 11$ (control). [Color figure can be viewed at wileyonlinelibrary.com]
resulted in statistical significant differences between the fibrous tissue and the adipose tissue with mean area scores of $1.7 \pm 0.9$ (OA ft), $1.2 \pm 1.0$ (control ft), $0.4 \pm 0.9$ (OA at), and $0.4 \pm 0.5$ (control at) ($p = 0.0011$ for OA ft vs. OA at and $p = 0.0476$ for control ft vs. control at) (Fig. 5B).

**Leptin Immunohistochemistry**
Leptin expression could only be demonstrated in 8 samples using immunohistochemistry, of which four belonged to the OA group and four to the control group (Fig. 5C). Although COMP was predominantly found in the fibrous tissue within the IPFP, leptin was present both in the adipose tissue and in the fibrous tissue of the IPFPs, depending on the respective sample. Scoring for the leptin staining intensity resulted in mean intensity scores of $0.3 \pm 0.6$ (OA ft), $0.7 \pm 1.1$ (control ft), $0.1 \pm 0.3$ (OA at), and $0.6 \pm 0.9$ (control at) (Fig. 5D). No statistical significant differences could be found but the control IPFPs tend to show a higher leptin staining intensity in the adipose portion than the OA fat pads ($p = 0.066$). The
results for the staining extent were comparable with the area score yielding mean values of $0.4 \pm 0.8$ (OA ft), $1.0 \pm 1.4$ (control ft), $0.1 \pm 0.3$ (OA at), and $1.0 \pm 1.4$ (control at) (Fig. 5B). Also here, a trend to an increased area stained positively for leptin could be observed for control adipose tissue compared with OA adipose portion ($p = 0.0601$).

**MMP-3, MMP-9, and MMP-13 Immunohistochemistry**

MMP-9 and MMP-13 could not be detected by immunohistochemical stainings, neither in the adipose tissue nor in the fibrous tissue of the IPFPs of the OA or control group (data not shown). Analysis of MMP-3 expression using immunohistochemistry showed weak staining in a minor number of the samples (5/14 samples could be stained in the OA group and 2/11 samples in the control group) (Fig. 6A). Both stained area and staining intensity were scored with 1 in every of these cases (Fig. 6B). In the specimens which showed positive staining for MMP-3, MMP-3 could only be detected within the fibrous tissue portion of the IPFP, not in the adipose tissue itself.
Immunoblotting

Immunoblotting was performed on four exemplary different specimens (all belonging to the OA group) to detect COMP and leptin (Fig. 7A). Actin was used as loading control. In all analyzed samples, COMP was detected in its intact pentameric form (full length size 524 kDa), fragmentation and bands of smaller oligomers or monomers could not be observed. COMP was found in varying amounts in the different samples, correlating the intensities of the COMP bands to the respective actin band intensities showed that mostly differences in loading amounts accounted for in the COMP immunoblot showed strong variations (Fig. 7B). Interestingly, leptin could be detected in the immunoblot even in samples in which immunohistochemical signals were missing. When correlated to the amounts

Figure 6. Immunohistochemical analysis of matrix metalloproteinase-3 (MMP-3) expression. (A) A minor number of specimens in the osteoarthritis (OA) group as well as in the control group showed positive staining for MMP-3 in the fibrous tissue (left panel), while the adipose tissue portion of the single specimens could not be stained with the MMP-3 antibody. For most samples of both groups, MMP-3 expression was missing both in the fibrous tissue and the adipose tissue (right panel). Scale bar: 200 µm. (B) Scoring of MMP-3 staining intensity as well as the size of the positively stained area. $n = 14$ (OA), $n = 11$ (control). [Color figure can be viewed at wileyonlinelibrary.com]

Figure 7. Immunoblot analysis of cartilage oligomeric matrix protein (COMP), leptin and actin expression. (A) Proteins were extracted from four different specimens of the osteoarthritis (OA) group. COMP could be detected as the pentamer in all samples. Also, leptin was present in all specimens (even in samples in which leptin could not be detected with immunohistochemical staining). Actin was used as a loading control. (B) Quantification of the relative signal intensities shown in (A). Signal intensities of COMP or leptin referred to the corresponding actin signals of the respective sample.
of detected actin, leptin showed variations in expression level, too. Surprisingly, opposing expression levels of COMP and leptin in the single samples could be seen, in samples with higher COMP levels the leptin levels were found to be rather low, while the sample with the highest leptin expression also showed the lowest COMP levels.

**Correlations**

In the OA group, significant correlations were found between the COMP staining intensity in the fibrous tissue and the IL-6 staining intensity in the adipose tissue ($r = -0.534, p = 0.049$), and the COMP staining intensity in the adipose tissue and the leptin staining intensity in the fibrous tissue ($r = 0.624, p = 0.017$) (Table 1). No further correlations could be detected for the OA group. In the control group, there is a significant correlation between the COMP staining intensity in the fibrous and the adipose tissue ($r = 0.517, p = 0.004$).

### DISCUSSION

In the present study, we performed histological, immunohistological and immunoblot analysis of IPFPs from patients who suffered from symptomatic OA and compared this group with a group of IPFPs from young subjects without any signs of OA. As far as we know, this is the first time COMP could be detected in the IPFP extracellular matrix in patients suffering from OA. But also in younger subjects who underwent ACL-reconstruction, COMP could be found. However, due to the number of subjects, we were unable to detect significant differences.
In earlier studies, COMP could be detected in subcutaneous human abdominal adipose tissue as well as in gluteal adipose tissue, with higher expression in the gluteal region. Moreover, authors could show, that there is a positive correlation between COMP expression in adipose tissue, circulating COMP and BMI. In this study, COMP could be identified as a regulator of adipogenesis. Furthermore, chondrogenic differentiation of both stromal vascular fraction cells and mesenchymal stem cells from the IPFP was confirmed by upregulation of different cartilage genes including COMP. In addition, adipose-derived mesenchymal stem cells are able to upregulate chondrogenic gene markers including COMP under cyclic hydrostatic load. However, as far as we know, COMP has not been described in the IPFP extracellular matrix yet. In our study, nearly every specimen showed the presence of fibrous tissue within the IPFP and COMP could be detected mostly in this part of the tissue. This matches previously published research, where the presence of fibrous structures in the IPFP could be described. The staining in the adipose part of the tissue was overall weaker. COMP is well known for playing an important role as an adapter protein in the hyaline articular cartilage, and it could be detected in the developing mouse embryo already at embryonic day 10 in mesenchyme tissue. COMP has been described to interact with many molecules of the extracellular matrix, beside several types of collagen, for example with aggrecan, fibronectin, and proteoglycans. Because of its interaction with different extracellular matrix proteins, which not only occur in the articular cartilage, we speculate that COMP is involved in the remodeling processes of the IPFP to fibrotic tissue. The remodeling with hypertrophy and fibrosis of the IPFP in context with high-grade OA has been described by a few authors, and it is supposed to be a source of pain in these patients. Moreover, it seems, that the IPFP could be involved in the inflammatory reaction pathognomonic for OA, as in an animal model, the injection of arthritogenic factor led to synovitis of Hoffa’s synovia, infiltration with neutrophils and mononuclear cells and fat necrosis. Recent studies have shown that also the IPFP secretes proinflammatory cytokines and may modulate the inflammatory processes contributing to the pathomechanism of knee OA. A higher gene expression of IL-6 was found in mesenchymal stem cells from the fat pad of OA patients compared with a control group of patients with an ACL rupture. Favero et al. detected increased IL-6 protein levels in OA IPFPs compared with control IPFPs from cadavers with no signs of knee OA. We found no differences in the staining intensity scores of IL-1β and IL-6 between OA and control samples from the young with a rupture of the ACL. Previous studies indicate that the rupture of the ACL leads to an increase of intra-articular inflammatory cytokines like IL-1 and IL-6. We speculate that also in our control group, the rupture of the ACL results in an increase of IL-1β and IL-6 in the IPFP. Furthermore, the number of CD206-positive cells did not differ between the groups. Interestingly, the OA group had more CD68-positive cells in the fibrous tissue compared with the control group. Although CD206 is associated with an anti-inflammatory phenotype, CD68 is a general macrophage marker. Even if we saw no differences for the proinflammatory cytokines IL-1β and IL-6 based on the semiquantitative staining intensity scoring, a higher number of CD68-positive macrophages in the OA samples might indicate increased inflammation involving other proinflammatory cytokines than the ones analyzed compared with the control IPFPs. An increased number of CD68-positive cells accompanied by increased levels of TNF-α, VEGF, and TGF-β within the IPFP could also be observed in a high-fat diet-induced OA model. Surprisingly, we could detect more CD206 positive cells than CD68-positive cells. The question arises, whether or not CD206 is expressed by cells other than macrophages or that possibly our staining was weak. However, Kristensen et al. reported similar results in hepatic and adipose tissue.

There are a few further proteins described in patients with high-grade OA, of which elevated levels in the synovial fluid and serum are connected to the proinflammatory reaction characteristic for OA. Here, members of the MMP-family, especially MMP-3, have to be listed. In our specimens, the immunohistochemical staining for MMP-3 failed to detect MMP-3 in most of the cases. Only in a few samples, weak staining could be observed. However, we did not perform an examination of the synovia. It is possible that MMP-3 is not part of the ongoing remodeling of the IPFP and that the trespassing of MMP-3 from the synovial fluid into Hoffa’s fat pad is rare. Analysis of the Masson’s trichrome staining showed a tendency to a higher presence of fibrous tissue in the OA group, which is associated with higher mean COMP levels. This matches the observation that COMP could be detected mainly in the fibrous tissue which is present in the IPFPs. The presence of COMP in the control group may be explained by potential acute inflammatory reactions the trauma patients possibly had due to the recent trauma or the surgery. This consideration would match that of published research. Until now, it is unclear if the present COMP really is synthesized in the examined tissue, or if it reaches the IPFP via for example the synovial fluid.

We could detect leptin in a few cases in both groups but were unable to detect leptin immunohistochemically in most samples. A reason, therefore, could be a weak anchorage of the protein in the tissue and a consecutive wash-out during the processing. Still, a trend to higher leptin amount could be observed for the control group compared with the OA group, both in the fibrous tissue and in the adipose tissue. Leptin has been investigated in the context of OA in studies before and it is known that the disease leads to increased serum leptin levels. Moreover, leptin could be
detected immunohistologically in the IPFP of patients in early as well as in end-stage OA. In connection with trauma, researchers found that leptin has a positive effect on fracture-healing in mice and wound-healing of the skin. As far as we know, leptin has not been described in context with arthritic trauma yet, but the fact that in our subjects leptin expression was higher in the control group could be interpreted as an acute reaction to the trauma in this context and that leptin may support intra-arthicular processes, which are a response to the trauma. Leptin also is connected with pain. Lübbeke et al. described that elevated leptin levels in the synovia of patients with high-grade OA correlate with higher pain-scores. We observed a positive correlation between the COMP staining intensity in the adipose tissue and the leptin staining intensity in the fibrous tissue, and a negative correlation between the COMP staining intensity in the fibrous tissue and the IL-6 staining intensity in the adipose tissue. Future studies are necessary, in which RNA of IPFPs should be analyzed for COMP expression to answer the question of whether the present COMP is synthesized by the tissue itself and if these correlations are direct or indirect. Moreover, we are interested in the role of leptin in articular trauma and aim to analyze IPFPs as well as synovial fluids from patients with an acute knee injury.

In summary, this is the first study which could detect COMP in the IPFP. The meaning of this finding is quite unclear yet, but as we observe a tendentially stronger expression of COMP in OA samples, we speculate that COMP might be involved in a remodeling process taking place in the IPFP during OA. Further investigations including a higher number of samples as well as RNA analysis could help to understand the ongoing processes better. In our study, leptin could be detected in a subset of IPFPs of both groups. With respect to the fact that subjects in the control group received surgery because of trauma, future investigations could aim at answering the question of whether a connection between trauma and the upregulation of leptin persists.

AUTHORS’ CONTRIBUTION
DG, JH, and AN. conceived the study and wrote the manuscript. DG, JH, JD, JO, PE, and CB. collected and analyzed the data. AN. supervised the study. All authors read and approved the final manuscript.

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