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Synovial fluid concentrations of the C-propeptide of type II collagen correlate with body mass index in primary knee osteoarthritis

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Abstract

Objective—To explore in a cross sectional study in patients with primary knee osteoarthritis (OA) the relations between body mass index (BMI), disease stage, and the concentrations of a putative joint fluid marker of type II collagen synthesis, procollagen II C-propeptide.

Patients and Methods—The study included 142 patients with knee OA (median age 68, median BMI 24.1). OA was staged radiologically. The concentrations in synovial fluid of procollagen II C-propeptide were measured by a sandwich enzyme immunoassay.

Results—Joint fluid concentrations of procollagen II C-propeptide were increased in knees with OA (median 3.7 ng/ml), compared with published reference values for knees in healthy adult volunteers (median 1.3 ng/ml). The concentrations of procollagen II C-propeptide were independently related to both OA stage and BMI ($r_s = 0.343$, $p < 0.0001$ and $r_s = 0.253$, $p = 0.002$, respectively).

Conclusions—Joint fluid concentrations of this putative marker of collagen II synthesis are high in early and mid-stage OA, but decrease in end stage disease. In addition and for the first time it was shown that the concentrations in synovial fluid of procollagen II C-propeptide increase with increasing BMI in primary knee OA. The increased joint fluid values of this marker in patients with primary knee OA and a high BMI, may reflect increased rates of collagen synthesis in their joint cartilage and could relate to the previously shown increased risk for disease progression in such patients.

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Several factors have been identified that are associated with an increased incidence and progression of knee osteoarthritis.¹⁻⁹ These risk factors represent underlying but little understood pathological processes in the joint and its different tissues: cartilage, synovium, bone, etc. In an effort to better understand some of these pathogenic processes, previous investigations have examined the relation between osteoarthritis (OA), knee injury, and concentrations of molecular markers in joint fluid and serum of cartilage, synovium, and bone metabolism. Changes in marker

concentrations have been described that relate to diagnosis, OA disease stage, time after injury, maturation, age, and physical activity. The findings suggest, but do not yet prove that such markers can be useful to monitor, for example, disease activity or progression in OA.^{10,11}

Obesity or increased body mass index (BMI), has been identified as a significant risk factor for knee OA, and in particular with an increased rate of disease progression.⁸ The underlying pathogenic mechanism, whether mechanical or metabolic, responsible for the increased disease progression is not well understood.

In this cross sectional study, we have investigated the relation between BMI, OA disease stage, and synovial fluid concentrations of the C-propeptide of type II collagen in patients with primary knee OA. This propeptide has been proposed as a marker of collagen II synthesis.^{12,13} The procollagen II C-propeptide content in human joint cartilage is proportional to the rate of in vitro collagen synthesis and the half life of the propeptide in cartilage is short,¹⁴ suggesting that its release into human joint fluids reflects the rate of collagen type II synthesis in human articular cartilage in vivo.

Methods

The study group included 142 patients with primary knee OA, 118 were women. The median age was 68 (range 42-88) years. The median BMI of the OA group was 24.1 (range 14.7-36.9). OA of the femorotibial joint was classified by examination of standing radiographs into five stages as described in table 1.¹³ Thirty one, 50, 33, 23, and five patients were classified into stages I, II, III, IV, and V, respectively. Concentrations of propeptide were compared with those measured by the same method in a knee healthy reference group ($n = 23$, three women, median age 28, range 20-40).¹⁵ The exact BMI of this reference group is not known, but is retrospectively estimated to be in the range of 22-24.

Joint fluids were aspirated aseptically from the most symptomatic knee joint of patients with knee OA to relieve pain. The fluids were centrifuged at 10 000 rpm for 20 minutes to remove cell and tissue debris, and the supernatants were stored at -80°C. The concentrations of procollagen II C-propeptide were measured by immunoassay.¹⁵ The assay was

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Table 1 Classification of the severity of OA based on radiological evaluation¹³

OA Stage	Bone sclerosis, osteophyte	Joint space narrowing	Bone attrition	Joint subluxation
I	+	—	—	—
II	+	< 50%	—	—
III	+	> 50%	< 3 mm	—
IV	+	Bone contact	3–10 mm	+/-
V	+	Wide bone contact	> 10 mm	+

linear in the interval of 0.2–20 ng/ml. Intra-assay and interassay variability was 4–5%.

For statistical analysis, stepwise regression analysis, Spearman rank correlation, Kruskal-Wallis analysis of variance on ranks, and post-hoc testing with corrections for multiple comparisons (Dunn's method) were used for comparison of groups. *p* Values less than 0.05 were considered significant and all differences and correlations described as such are significant at this level or better. Two tailed tests were used.

Results

The median procollagen II C-propeptide concentration in joint fluid of the OA study group was 3.7 (range 0.2–20) ng/ml. This value is significantly higher than that previously determined by the same method in rheumatoid arthritis patients and in knee healthy volunteers,^{13 15 16} but in the same range as for other patients with primary knee OA or knee injury.¹⁵ The median concentration of procollagen II C-propeptide in joint fluids of healthy volunteers was previously shown to be 1.3 ng/ml (range 0.1–5.7 ng/ml).¹⁵

Multiple stepwise regression analysis of the relations between the propeptide concentrations and the patient sex, age, OA disease stage or BMI, showed that neither sex nor age were significantly related to propeptide concentrations. The lack of relation between age and sex

on the one hand, and propeptide concentrations on the other hand, is consistent with earlier findings, which showed no change with age after maturity.¹⁵

When the propeptide concentrations at different stages of primary knee OA were compared, the median concentrations at stages I, II, III, IV, and V were 2.8, 3.2, 5.2, 5.7, and 3.4 ng/ml, respectively (*p* < 0.001 by Kruskal-Wallis ANOVA on ranks) (fig 1). The correlation coefficient between the degree of radiological joint damage (as assessed by the degree of tibiofemoral joint space narrowing) and propeptide concentrations was 0.343 (*p* < 0.001) (data not shown).

The concentrations of propeptide increased with increasing BMI (*p* < 0.001, Kruskal-Wallis ANOVA on ranks) (fig 2). For analysis, values were ordered by increasing BMI, and five similar sized groups of patients generated, with median BMI of 20.3, 22.8, 24.2, 25.8, and 28.9, respectively. Median concentrations of propeptide for the five subgroups of increasing BMI were 2.7, 3.2, 3.8, 4.0, and 4.8 ng/ml, respectively. Post-hoc testing with correction for multiple comparisons (Dunn's method) versus the reference group,¹⁵ showed that propeptide concentrations were increased in all OA subgroups (*p* < 0.05). The correlation coefficient between joint fluid concentrations of collagen II C-propeptide and BMI for the OA study group was 0.253 (*p* = 0.002). Interestingly, the correlation was stronger when only early stage OA (I and II) was considered (*n* = 83, *r*_s = 0.404, *p* < 0.001). There was no significant relation between BMI and OA stage (*r*_s = 0.130, *p* = 0.124).

The reference group used here for comparison with knee healthy controls was younger (median age 28 years), and consisted of mostly men, in comparison with the OA study group. However, both the present and previous investigations have shown that age (after maturity) or sex do not influence joint fluid concentrations of procollagen II C-propeptide.¹⁵

Discussion

Obesity, or increased BMI, is one of the strongest risk factors for knee OA, and also increases the risk for disease progression, in particular for middle aged women.^{1 4 5 8 9} It was suggested that obesity represents a risk factor through increased joint loading, and not a metabolic risk factor,⁹ but our understanding of how this translates into specific pathological processes at the cell and tissue level is limited.^{10 11}

The C-terminal propeptide of type II procollagen is released from the procollagen molecule by a specific peptidase during the extracellular formation of the type II collagen fibril.¹⁷ The procollagen II C-propeptide content in human joint cartilage is proportional to the rate of *in vitro* collagen synthesis,¹⁴ suggesting that its release into human joint fluids may reflect the rate of collagen type II synthesis in human articular cartilage *in vivo*.^{13 16} The concentration in human knee synovial fluid of this propeptide

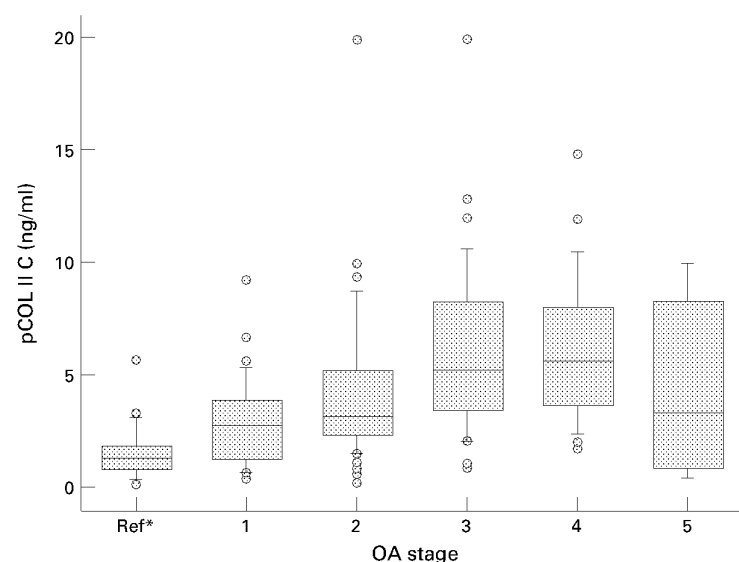


Figure 1 Procollagen II C-propeptide concentrations in knee joint fluids from patients with primary knee OA, related to different OA stages. OA stages were determined by radiology (table 1). The number of patients in the subgroups were 31, 50, 33, 23, and five, respectively. Boxes represent median, 25th and 75th percentiles, bars 10th and 90th percentiles, symbols individual outliers. The box labelled (Ref*) represents data from knee healthy volunteers, *n* = 23.¹⁵

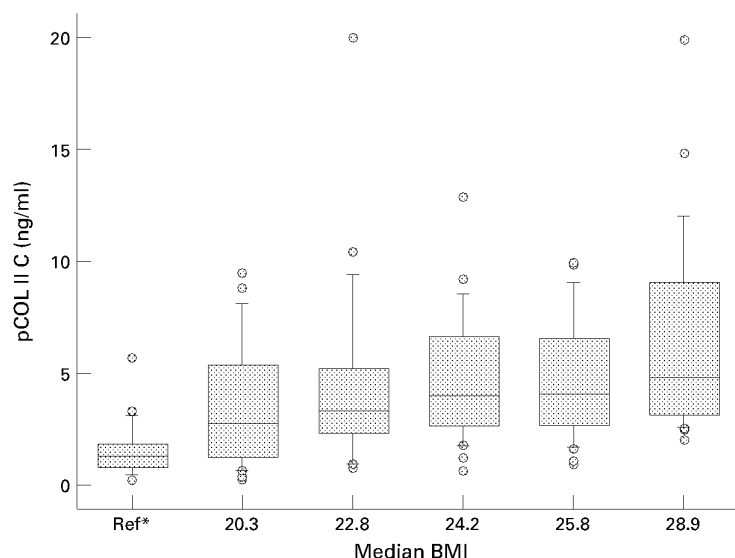


Figure 2 Procollagen II C-propeptide concentrations in synovial fluid from patients with primary knee OA and different BMI. Patients were ordered by increasing BMI, and five subgroups created, with median BMI of 20.3, 22.8, 24.2, 25.8, and 28.9, respectively. The number of patients in the subgroups were 29, 29, 28, 28, and 28, respectively. Median ages of subgroups were 73, 68, 65, 70, 61, and median (mean) OA stages were 2 (2.3), 2 (2.3), 2 (2.3), 3 (2.6), 3 (2.7). See figure 1 for explanation of box plot. The box labelled (Ref*) represents data from knee healthy volunteers, $n = 23$, median age 28.¹⁵

was shown to be increased, compared with knee healthy volunteers, in the growing adolescent,¹⁵ for a time after knee injury,¹⁵ and in early and mid-stage knee OA,^{13 15 16} but to be decreased in rheumatoid arthritis.¹³ These concentration changes are consistent with known alterations in the rate of synthesis of collagen type II in these conditions.

In this cross sectional study, we find, similar to previous studies, that procollagen II C-propeptide concentrations in synovial fluid increase with increasing degrees of radiological OA joint changes, but decrease in end stage disease.^{13 15 16} The decrease in propeptide concentrations in end stage disease is presumably because of a decreased cartilage mass and chondrocyte end stage failure. Although some of the patients studied here were treated with non-steroidal anti-inflammatory drugs at the time of synovial fluid sampling, previous work has shown that these drugs do not inhibit in vitro collagen synthesis in human cartilage at clinically relevant concentrations.¹⁸

We show in addition and for the first time that the concentrations in synovial fluid of a putative molecular marker of joint cartilage type II collagen synthesis increase with increased BMI in primary knee OA. This relation was most evident for patients with early and mid-stage OA, the type of patients where obesity has been identified as a risk factor for disease progression.⁸ The underlying reasons for the apparent increase in propeptide concentrations with increasing BMI are not understood, neither are the mechanisms by which they may relate to the known risk increase in disease progression, but we suggest that it is associated with an increased rate of type II collagen synthesis in the knee joints of these patients. This increase could, for example, be caused by other metabolic changes in these joints, by an increased joint loading, or

a combination thereof. Oscillatory loading of joint cartilage in vitro causes an increase in synthesis of both proteoglycans and collagen.¹⁹ Of further interest is that the procollagen II C-propeptide has been found in high concentrations in osteophytes in human knee OA.¹⁶ The increase in propeptide concentrations could be secondary to an increased disease activity in the overweight patients. Finally, it should be noted that changes in BMI with time and differences between groups may represent a confounding factor in studies of molecular markers of joint tissue metabolism.

The results presented in this cross sectional study thus suggest that differences in knee joint cartilage metabolism in primary knee OA are related to differences in BMI, and that such differences may be reflected by metabolic markers in knee synovial fluid. It would thus be of interest to perform a controlled, prospective intervention study in overweight patients with primary knee OA. This study could investigate whether a decrease in BMI results in changes in markers of turnover of cartilage and other joint tissues, and whether such changes relate to changes in joint structure and OA symptoms.

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