Serum cartilage oligomeric matrix protein: is there a repeated bout effect?

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Abstract

The primary aim of the present study was to investigate if there is a repeated bout effect for cartilage tissue, evident in the marker serum cartilage oligomeric matrix protein (sCOMP). Ten healthy male subjects (26.4±3.14 years) performed two high impact interventions (100 drop jumps with a 30 second interval) carried out at a 3 week interval. After each intervention, sCOMP and muscle soreness were assessed on 8 and 6 occasions respectively. Muscle soreness was determined via a visual analog scale with a maximum pain score of 10. sComp levels did not show a blunted response after the second bout (Bout 1: 12.2±3.3 U/L^{-1}; Bout 2: 13.1±4.0 U/L^{-1}; P>0.05). Remarkably, sCOMP increased from baseline levels by 16% after bout 1 and 15% after bout 2. Muscle soreness was blunted following the second intervention (Bout 1: 5.0±1.8; Bout 2: 1.6±0.8). Unlike the known repeated bout effect for muscle damage markers, sCOMP levels do not show a blunted response after two similar loading interventions. This information on biomarker behavior is essential to clinicians attempting to use this marker as an indicator of cartilage damage associated with the development or progression of osteoarthritis.

Introduction

Cartilage degradation in osteoarthritic joints affects millions of people around the world. In the United States alone, the number of hospital stays for osteoarthritis increased from 418,000 to 921,000 between 1997 and 2009.[1] Due to the irreversible character of osteoarthritis (OA), early recognition of degenerative changes of cartilage tissue is essential for evolving future therapeutic strategies. Therefore, it is not surprising that interest in biomarkers and molecular imaging techniques has increased within the last decade. Since deformational changes of cartilage are associated with changes in the extracellular matrix (ECM),[2] rising serum concentrations of ECM-proteins are used as indicators of cartilage tissue behavior. One such protein is the well-established biomarker, serum cartilage oligomeric matrix protein (sCOMP), physiologically located in the extracellular matrix (ECM) of cartilage tissue.[3] It is assumed that sCOMP changes reflect the extrusion of COMP fragments of loaded cartilage tissue.[4] This is supported by the fact that deformational changes of cartilage have been found to be correlated with COMP levels in blood.[5] However, the exact mechanisms of COMP release from the extracellular matrix into the blood stream still remains unknown.[2]

Increased sCOMP concentrations have been observed in different pathological conditions such as OA or knee injuries[6] and a recently published meta-analysis found that sCOMP is sensitive to OA disease progression.[7] Hunter *et al*.,[8] who investigated the relationship between MRI changes and sCOMP, reported that for each unit increased in sCOMP, the odds of cartilage loss increased six-fold. However, sCOMP has also increased during exercise in a load dependent manner,[4] and decreased after just 24 hours of bed-rest in healthy subjects.[9] Understanding these physiological changes in serum concentration of sCOMP from healthy and mature articular cartilage might reveal a closer insight into biomarker behavior in cartilage pathologies and would therefore help clinicians interpret sCOMP levels.

One phenomenon, known from biomarkers of skeletal muscle and other tissues, is the repeated bout effect (RBE). That is, subsequent bouts of the same high intensive exercise repeated after a few weeks or even months, demonstrate only a blunted increase in muscle damage parameters like creatine kinase or myoglobin.[10] High impact exercise, such as 100 drop jump landings, have also been demonstrated to significantly increase sCOMP from baseline level by about +32.3% (baseline: 6.8 U/L; 95% CI: 5.3, 8.4; post: 8.9 U/L, 95%CI: 6.8, 10.9; P=0.001) and changes have been found to be correlated with deformational changes of cartilage, assessed by MRI.[2] However, it remains unclear if the response of cartilage biomarkers is affected by an RBE. The knowledge of an RBE for sCOMP is crucial to clinicians, as it could obscure cartilage damage and therefore possibly lead to false diagnoses. Therefore, the primary aim of our study was to investigate whether sCOMP increases similarly after the first and second bout of a short but high intensive program of cartilage loading. We hypothesize that the increase in serum COMP levels is blunted after a second bout of high impact exercise, similar to the well known RBE of muscle damage markers following eccentric exercises.

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Materials and Methods

Subjects

Ten healthy male sport students (age 26.4±3.14 years; body mass index 23.76±1.51 kg×m^{-2}) were included in this study. None of the participants had experience with regular plyometric training, but all of the subjects reported use of resistance training machines and free weights (1-2 hours per week). Exclusion criteria were acute or chronic pain of the lower extremities or back, as well as any other orthopedic, cardiovascular, metabolic, or pulmonary disease, chronic medication, muscular injury within 6 months prior to the study, or any kind of orthopaedic surgery in the subjects' medical history. Subjects were not permitted to take part in physical activity 3 days prior to each intervention and the following 96 hours. The study protocol was approved by the ethics committee of the German Sport University Cologne. All participants were informed of potential risks and gave their written informed consent prior to the investigation.

Interventions

Two equal jump interventions were carried out at an interval of 3 weeks. Both jump interventions were conducted on Monday mornings (between 9-11 a.m.) and participants were instructed to keep their fluid intake constant at 20-25 mL×kg^{-1}×d^{-1}, to reduce variations in

hydration status. Participants were instructed to refrain from physical activity three days prior to the onset and 96 h after each intervention. Between the 96 h after the first intervention and three days prior to the second intervention, subjects were allowed to return to their usual activity level. Before each intervention, subjects performed a standardized warm-up protocol consisting of a 5 minute treadmill run (2 m×s^{-1}, incline 1%) and 3 submaximal countermovement jumps. In order to familiarize themselves with the protocol, the participants performed 2 test jumps from the actual protocol jumping height (see below). The intervention itself consisted of 100 drop-to-vertical jumps (DVJs). The subjects were instructed to step off a 70 cm drop box platform maintaining an upright posture, landing simultaneously with both feet on the hard landing surface (concrete). After cushioning the landing to a knee angle of approximately 90°, they then immediately performed a vertical jump at maximal effort. The knee angle was assessed visually by the investigators and verbal feedback was given to the participants after every jump in order to keep maximal knee angles quite constant throughout the 100 jumps. Participants were advised to keep their hands on their hips and to perform the movement fluidly, without any breaks. After each jump, subjects had to climb three equal steps to reach drop height. Time between each jump was 15 seconds. Subjects were verbally encouraged for maximal performance throughout the entire intervention. The subjects wore their own athletic shoes during both bouts of DVJs, therefore brand and model of footwear differed amongst participants. However, it has been shown previously that footwear does not significantly impact the ground reaction forces during drop jump landings.[11]

Blood samples

Blood samples were taken directly before, immediately after, and 4, 8, 24, 48, 72, and 96 hours after both bouts of the performed DVJs. All samples were drawn by venipuncture from the antecubital fossa region. Blood was collected with the Vacutainer® blood withdrawal system (Becton-Dickinson, Plymouth, United Kingdom). Samples were drawn into Serum Separation Tubes™ (SST) which were first stored for 30 minutes at room temperature and subsequently centrifuged for 10 minutes at 1861 g and 4°C (Rotixa 50, Hettich Zentrifugen, Mühlheim, Germany). Until further analysis, samples were frozen and stored at –80°C. After defrosting, serum levels of COMP were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (AnaMar Medical, Göteborg, Sweden) with a intra-assay variation of 1.9% (mean, 13.1 U/L) and inter-assay variation of 2.7% (mean, 13.1 U/L). The range of the assay is between 0-3.2 U/L. Samples were diluted 1:20 and measured in duplicate.

Muscle soreness

The rating of muscle soreness (MSOR) was assessed by sitting down on a chair from an upright posture and standing up again from this position without using the arms. The subjects were then asked to rate their pain instensity using a 10-cm visual analog scale (VAS) before, immediately after, and 4, 8, 24, 48, 72 and 96 hours after the intervention.

Statistical analysis

Statistical analysis was performed using a statistics software package (Statistica for Windows, 7.0, Statsoft, Tulsa, OK). The results are presented as means and respective standard deviations (SD). For the comparison of within and between-conditions, repeated-measures ANOVA followed by Bonferroni *post-hoc* analysis was used. Statistical differences were considered to be significant for P≤0.05 and marked as *P≤0.05. The effect size *partial η2* was calculated and the thresholds were defined as 0.1, 0.25, and 0.4 for small, moderate, and large effects, respectively. The power (1-ß) was calculated post-hoc for ANOVA repeated measures using α, sample size, and effect size with G*Power Version 3.1.3 (Heinrich-Heine University Duesseldorf, Germany). Kolmogorov-Smirnov and Shapiro-Wilk tests were used to test for normality and residual plots were used to assess linearity and homoscedasticity of data.

Results

As planned, all participants performed 100 DVJs twice, at an interval of three weeks. None of the participants were injured or had joint problems during the intervention period - neither due to the intervention itself nor to any other circumstance. Statistical analyses revealed that the data met the assumption of normality, linearity, and homoscedasticity.

Cartilage oligomeric matrix protein

Mean sCOMP values over all samples taken after the intervention were 12.2±3.3 U/L for bout 1 and 13.1±4.0 U/L for bout 2 respectively. The temporal evolution of sCOMP presented an increase at post-test measurements following both bouts of eccentric exercise and a drop to baseline-levels four hours after the interventions. The mean values immediately after the intervention were 15.0±4.3 U/L for bout 1 and 15.6±5.6 U/L for bout 2 respectively. With 11.0±2.9 U/L (85.2% of baseline value) for bout 1 and 11.3±2.8 U/L (85.2% of baseline value) for bout 2, sCOMP concentration reached its first low point at the 24h-follow up measurement. ANOVA and *post-hoc* Bonferroni testing showed that this decrease in sCOMP at 24 h was statistically significant for bout 1. Values increased from this low mark throughout the remaining three days of follow-up after bout 2 but not bout 1. Although insignificant, sCOMP concentrations did not reach pre-test levels even after 96 h.

There was a significant time effect (1-β=1.0), but no significant bout*time interaction (1-β=0.58). The power-analysis revealed that a total sample size of 75 subjects would have been necessary to achieve a power of 1.0 and 15 subjects for a power of 0.8 regarding the bout*time interaction (Figure 1A).

Muscle soreness

Progress of muscle soreness (MSOR) was similar following both bouts. However, bout 1 values were significantly higher for all post-test measurements, with overall mean of 5.0±1.8/10.0 for bout 1 and 1.6±0.8/10.0 for bout 2. Slight MSOR was perceived by subjects immediately after the exercise protocol and peaked at 48 hours after exercise. Peak values for bout 1 were 7.3±2.2 out of 10, whereas peak values for bout 2 only reached 4.0±1.9/10.0 (P<0.05). Mean follow-up MSOR-rating at 96 hours after the first bout of exercise was at 3.0±1.4/10.0. By contrast, MSOR values already dropped close to baseline levels 96 hours after the second bout of exercise (Figure 1B).

Discussion

The main finding of the present study is that the sCOMP does not present any RBE three weeks after an initial bout of a high impact exercise protocol. Intriguingly, the temporal evolution of sCOMP concentration was not only similar, but almost identical between both bouts for all follow-up blood samples. Against the background of the latter, we are convinced that the hypothesis of an RBE in terms of a blunted sCOMP response following repeated high impact exercises should be rejected, even though the statistical power for the group*time was low (1-β=0.58), which is known to increase the risk of a type II error. Our results further demonstrate an insignificant (P>0.05) trend of increased sCOMP concentrations directly after the high impact intervention at post-exercise measurements and a subsequent drop of values below pre-test levels within 24 h. By contrast, a clear RBE could be found regarding the MSOR data collected from both bouts, with all follow-up values being significantly lower after the second bout. Even though the degree of muscle soreness is not equitable to the magnitude of muscle damage, as the correlations between MSOR

and ultrastructural changes are generally weak,[12] the dampened pain perception likely reflects to some degree a protective adaptation of musculature.

Serum cartilage oligomeric matrix protein pretest values

Even though subjects had been asked to refrain from physical activity three days prior to the onset of the study and blood collection was standardized, the mean baseline values in our study, 12.9±3.4 U/L and 13.7±5.3 U/L for bout 1 and bout 2 respectively, were higher than those previously reported by studies in young healthy adults.[4] The individual levels at baseline ranged between 7.6-19.3 U/L for bout 1 and 8.2-26.3 U/L for bout 2 with, except for one outlier, values being very similar in both bouts for each subject. The latter suggests that the observed values do not reflect measurement errors but are indicative of the true individual sCOMP levels, even though some of them exceed the reference values (<12 U/L) given by the manufacturer manual (AnaMar Medical AB 2003). However, those references may not be suitable for subjects involved in regular sport activities (like the included active male sport students) since this applied to half of the participants.

The large and individual sCOMP values found by the present study challenge the general efficacy and practicality of using this biomarker to identify osteoarthritis or its progression if it is confounded by regular activity. This hypothesis needs to be addressed by future research reinvestigating the reference values for sCOMP in different populations.

Serum cartilage oligomeric matrix protein response to jumping intervention

In some subjects who presented high sCOMP values at baseline, the marker increased beyond >20 U/L, one subject even reached a maximum value of 29 U/L. However, individuals with baseline sCOMP levels <12 U/L stayed in a range between 7.7-14.7 U/L during all follow up measurements. This suggests that the sCOMP response to a defined loading protocol is characterized by a high interindividual variance in terms of high- and low-responders.

To the knowledge of the authors, this is the first study investigating the RBE of a cartilage biomarker in serum. Additionally, information on the time course of sCOMP after a single bout of high impact interventions like drop jump landings are sparse. Niehoff *et al*.,[2] found sCOMP values increased 37% from 6.8 U/L (95% CI:5.3, 8.4) to 8.9 U/L (95%CI: 6.8, 10.9) following 100 DJs, which supports the trend of the present findings. Unfortunately, blood samples in that study were only drawn within the first three hours after the intervention so that it remains unclear if a 24 hour follow-up measurement would have mirrored the observed drop in sCOMP in the present study. The reason for this *late* onset drop also remains unclear. Hypothetically, an altered activity level of subjects following the intervention protocol – possibly due to perceived muscle soreness – could be responsible for this observation. This would be consistent with previously published studies that have shown sCOMP to decrease after a short rest period.[13-15] However, the assumption that increased muscle soreness led to a decrease in physical activity is challenged by the fact that sCOMP values seem to increase again from 24 hours to 48

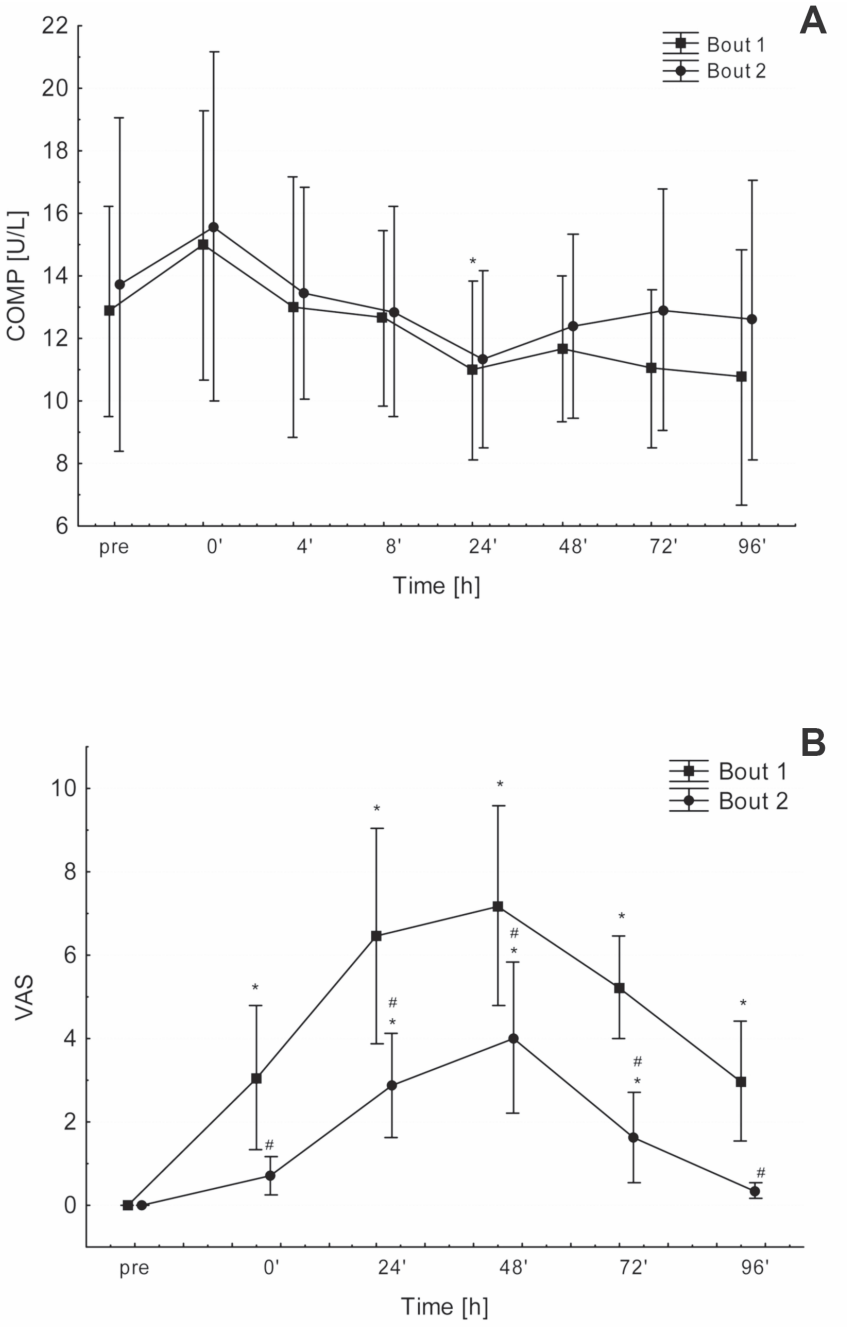

Figure 1. A) Mean absolute serum COMP concentration after the two interventions. Values are presented as mean and respective SD. *P<0.05 *vs* pre value. B) Progress of muscle soreness. Values are mean ±SD. *P<0.05 *vs* pre value. #P<0.05 *vs* bout 1.

hours post, where muscle soreness peaked. Nevertheless, due to the circumstance that daily activity was not recorded in detail, this hypothesis remains highly speculative. Therefore, future studies should include records of physical activity over the full period of blood sampling.

Serum cartilage oligomeric matrix protein clearance from the extracellular space

Irrespective of a potential dose-response relationship, the observed peak of COMP in serum immediately following the intervention clearly points towards a very short diffusion time from its place of origin into the circulation. This is somewhat surprising as COMP is a large pentameric protein with five equally sized subunits of 100 kDa and synovial macromolecules of that size are suggested to exit the joints via the lymphatic system.[16] Uninterrupted basement membranes and tight interendothelial junctions of capillaries within synovium prevent the transepithelial migration of macromolecules with a molecular weight of >1-2 kDa.[17] To enter the circulation, COMP molecules would need to be transported all the way to the left jugulosubclavian venous junction via small lymph vessels and finally to the thoracic duct. From simulated snake bites it is known that transit time from peripheral lymph vessels to systemic circulation usually takes about 1 hour or longer.[18] Further, in an early investigation by Bauer *et al*.[19] it could be shown that macromolecules took one hour to appear in blood after injection in the knee joint of anesthetized dogs whose legs were moved to simulate physical activity.[19] If COMP was transported through the lymphatic system after physical exercise, one might expect a delayed increase of COMP levels in serum. However, this is contrary to what was observed in the present study and as previously described in the literature.[2] Niehoff *et al*.[15] provided indirect evidence against a lymphatic transport for COMP into the circulation by the fact that lymphatic drainage of 30 min duration, which is known to increase lymphatic flow, had no effect on the blood kinetics of sCOMP concentration. The authors stated that in healthy subjects, free COMP fragments are not present at high enough levels to affect sCOMP levels. In conclusion, it remains unclear how the large COMP molecule leaks into the bloodstream that early.[20-23]

Repeated bout effect

Based on the COMP data of the present study, cartilage tissue seems not to feature any exercise induced protection in the form of an RBE. It might be speculated that a single intervention is not sufficient to induce a protective effect for cartilage tissue, yet there seems to be an effect after 12 weeks of running, as reported by Celik *et al*.[24] Recalling that COMP is an extracellular biomarker of the cartilage matrix and not of the chondrocytes itself, it remains unclear from the present data if there is a bio-positive adaptation on a cellular level in cartilage tissue comparable to that assumed for the muscular tissue. It should be noted that the RBE in the present study was tested three weeks after the initial bout of eccentric exercise. Therefore, a blunted sCOMP response following a shorter period of time cannot be excluded from the present data. Additionally, it is possible that a more periodic blood sampling in the minutes after the intervention would have revealed an RBE. Niehoff *et al*.[2] recently showed that sCOMP values already decrease 30 min after an intervention, underlying the importance of blood sampling close to the intervention. Further, it could be speculated that the applied impact was inappropriate to induce desired adaptations. The type of loading strongly influences the increase in sCOMP levels after exercise. One might expect that impact loading induces the highest deflection, which in fact, does not seem to be the case. 100 drop-to-vertical jumps, as presented here, led to moderate serum COMP changes of +16% and +15% from baseline values for bout 1 and 2 respectively, even though one has to consider that baseline values had already been exceptionally high. However, Niehoff *et al*.,[2] who investigated COMP responses following 100 drop landings, found similarly low COMP increments (32.3%), when compared to the marked sCOMP deflections that can be found following high volume sport activities. sCOMP values measured after an ultramarathon race of 200 km caused a 90% increase from pre-race levels.[25] Furthermore, Brüggemann *et al*.[26] demonstrated that 30 min of running at 2.2 $m*s^{-1}$ resulted in a significantly greater cartilage deformation than 100 drop landings from 73 cm of height. Even moderate walking activities over 30 min led to an increase in sCOMP concentration of 9.7% compared to baseline.[4] These findings suggest that impact loading might not induce severe cartilage damage or an sCOMP increase. However, the minor increase following short but high intensive loading interventions contrasts with the fact that sports including rapid acceleration and deceleration moments are hypothesized to increase the risk of osteoarthritis more than high volume endurance activities.[27]

Dose-response relationship

If there is a dose-response relationship underlying the RBE for sCOMP, as observed for markers of muscle damage,[28] it could be speculated that the level of perturbation induced by high volume activities, is needed to provoke this kind of tissue protection. Given the mechanical behavior of cartilage, duration/volume of activity is likely a major contributor. Thus, the relatively short duration of the DVJs in combination with three weeks of recovery time may be responsible for not detecting an RBE for sCOMP. Therefore, it remains to be determined by future research if an RBE for cartilage tissue can be induced by prolonged activities. However, a repeated bout effect could be shown for MSOR indicating that the intervention as performed in the present study elicits protective effects in other tissues.

Limitations

The present study is limited by the fact that we did not investigate a non-exercise control group and were therefore not able to compare the sCOMP deflections with the physiological variation of this biomarker. Further, it needs to be taken into account that small amounts of COMP are present in other tissues like ligament, tendon, and meniscus.[29] That is, the observed increases of sCOMP may originate from tissues other than cartilage. However, as stated by Andersson *et al*.,[16] there is compelling evidence that serum COMP is primarily derived from cartilage tissue. As stated by Niehoff *et al*.,[2] the synovia and the lymphatic system may also contribute to the overall sCOMP levels. In other words, COMP fragments that have been released from cartilage at an earlier time point are likely to be present in these fluids. These fluids may leak into the circulation during strenuous exercise due to an increased lymphflow and pressing out the synovia. As we did not measure the actual cartilage damage in the present study, it is impossible from the present data to correlate such changes with the observed sCOMP values. Therefore, an important question for future studies is to clarify if an RBE is detectable by modern molecular imaging of cartilage and to what extent these changes are correlated with cartilage biomarkers, such as sCOMP.

Conclusions

The present findings substantially expand current knowledge on cartilage biomarker behavior in response to exercise. While it is well described that sCOMP is sensitive to different kind of loading pattern,[2,4,6,9,16,24,26] little is known about the response of this biomarker after a second bout of exercise. Following two bouts of high-impact intervention protocols (100 DVJs) of the present study almost identical sCOMP changes were found. This is, to our knowledge, the first study investigating the repeated bout effect for sCOMP concentration after a high intensity intervention. Although further studies with more statistical power are needed to confirm the present findings, our study indicates that sCOMP, unlike biomarkers from other tissues, such as skeletal muscle, do

not show a repeated bout effect following a short but high intensive exercise protocol. This information on biomarker behavior is essential to clinicians, attempting to apply this biomarker as an indicator of cartilage damage associated with osteoarthritis development or progression.

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