Deformational behaviour of knee cartilage and changes in serum cartilage oligomeric matrix protein (COMP) after running and drop landing


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SUMMARY

Objective: To investigate (1) the effect of running and drop landing interventions on knee cartilage deformation and serum cartilage oligomeric matrix protein (COMP) concentration and (2) if the changes in cartilage volume correlate with the changes in serum COMP level.

Methods: Knee joint cartilage volume and thickness were determined using magnetic resonance imaging (MRI) as well as COMP concentration from serum samples before and after in vivo loading of 14 healthy adults (seven male and seven female). Participants performed different loading interventions of 30 min duration on three different days: (1) 100 vertical drop landings from a 73 cm high platform, (2) running at a velocity of 2.2 m/s (3.96 km), and (3) resting on a chair. Blood samples were taken immediately before, immediately after and 0.5 h, 1 h, 2 h and 3 h post intervention. Pre- and post-loading coronal and axial gradient echo MR images with fat suppression were used to determine the patellar, tibial and femoral cartilage deformation.

Results: Serum COMP levels increased immediately after the running (+30.7%, pre: 7.3 U/l, 95% confidence interval (CI): 5.6, 8.9, post: 9.1 U/l, 95% CI: 7.2, 11.0, P = 0.001) and after drop landing intervention (+32.3%, pre: 6.8 U/l, 95% CI: 5.3, 8.4; post: 8.9 U/l, 95% CI: 6.8, 10.9, P = 0.001). Cartilage deformation was more pronounced after running compared to drop landing intervention, with being significant (volume: P = 0.002 and thickness: P = 0.001) only in the lateral tibia. We found a significant correlation (r² = 0.599, P = 0.001) between changes in serum COMP (%) and in cartilage volume (%) after the drop landing intervention, but not after running.

Conclusions: In vivo exercise interventions differentially regulate serum COMP concentrations and knee cartilage deformations. The relation between changes in COMP and in cartilage volume seems to depend on both mechanical and biochemical factors.

Introduction

The morphology and composition of healthy mature articular cartilage is optimized to enable its main function of force transfer and distribution. Understanding this functional behaviour of intact articular cartilage in vivo is important to increase the knowledge regarding the pathogenesis, diagnosis and therapy of osteoarthritis (OA). Even though a number of in vitro studies have investigated the mechanical properties and deformational behaviour of cartilage explants with various methods like confined2,3 or unconfined compression4,5 as well as indentation tests7,8, they allow only limited conclusions about the function of cartilage under in vivo environmental conditions. Recently, high-resolution magnetic resonance imaging (MRI) has been established as a precise, accurate and reproducible method to determine cartilage morphology in vivo8-11. Several studies have also shown that this technique can be used to quantify the deformational behaviour of articular cartilage in vivo12-15. It has been demonstrated that short-term (<10 min) mechanical loading, e.g., cycling, walking,
and squating, leads to a dose- and site-dependent deformation of the patellar cartilage. After knee bends, patella cartilage volume decreased by approximately 6%, whereas deformation of femorotibial cartilage was relatively small. Long-lasting mechanical loading of 30 min running resulted in a significant deformation of medial (5.3%) and lateral (4.0%) femoral cartilage whereas at the tibia only the cartilage volume of the lateral condyle (5.7%) was reduced. After 1 h running Kersting et al. (2005) also found a significant reduction in cartilage volume of the femur and lateral tibia.

The deformational behaviour of cartilage is directly linked to the composition of the extracellular matrix (ECM). The synthesis and degradation of ECM molecules affect the mechanical properties of articular cartilage. Several serum biomarkers have been identified to monitor cartilage metabolism. One established biomarker for monitoring cartilage metabolism in relation to joint degeneration in OA, rheumatoid arthritis (RA) and injured knees is cartilage oligomeric matrix protein (COMP). Previous studies have reported an increase in serum COMP concentration immediately after exercise with the dose of mechanical loads determining the magnitude and duration of increased serum COMP concentration. It has been speculated that the elevation of serum COMP reflects the extrusion of COMP fragments from the loaded articular cartilage, but the mechanism whereby mechanical loading through physical activity may lead to an enhanced serum COMP concentration is not completely understood. Kersting et al. (2005) demonstrated a moderate correlation between the changes in serum COMP concentration and total knee joint cartilage volume after a 1 h training run. In explants of porcine cartilage the COMP release increased with the magnitude of dynamic mechanical stress. However, to our knowledge there are no in vivo studies comparing mechanical impact-loading interventions of different frequencies and amplitudes of an equal duration on both in vivo cartilage deformation and serum COMP concentration in combination. Thus, the aims of this study were to investigate (1) the effect of running and drop landing interventions on knee cartilage deformation and COMP concentration and (2) if the changes in cartilage volume correlate with the changes in serum COMP level.

Methods

Subjects

Fourteen healthy young adults (seven male, seven female) were recruited for this study. To meet inclusion criteria, subject had to have an age between 20 and 30 years, a body mass index (BMI) 20–30 kg/m², a sedentary lifestyle (e.g., not being involved in regularly physical activity for the last 3–7 years) and occupation (e.g., desk work). Exclusion criteria were acute injuries, symptoms or pain of the lower extremities; a contraindication for MRI; chronic disease requiring medication; a history of prior trauma or orthopaedic surgery of the knee in the last 10 years. Subject demographics are summarized in Table 1. All subjects participated in occasional leisure physical activity (1.1 h/week) assessed by questionnaires. The local ethics committee approved the study protocol and written consent was obtained from all participants.

Experimental protocol

Each subject completed three different loading interventions on three test days. The experimental protocol was identical on each test day apart from the loading intervention of 30 min duration. Subjects were asked to limit their physical activity to normal levels 24 h prior to a test day and to travel by car or with public transportation to the radiological practice where the study was carried out. Each subject was tested at the same time on each of the three test days. The food was standardized and the same type and quantity of food was served at the same time on each test day. After arriving in the practice, participants rested on a chair for 30 min directly next to the MRI-scanner in order to limit the influence of preceding activity on serum COMP concentration and cartilage volume as well as thickness. After rest, baseline MRI measurement of the knee was taken and the first blood sample was drawn. Thereafter, the participants completed the loading intervention of 30 min duration. Immediately after the intervention, a second blood sample was drawn and a second MRI scan of the previously measured knee was performed (within approximately 4 min). To keep the period of time between the end of intervention and the start of post intervention MRI limited, blood samples were collected immediately after intervention while positioning in the MRI-scanner. Following the second MRI scan subjects had to sit on a chair for the remaining experimental time and further blood samples were drawn 0.5 h, 1 h, 2 h and 3 h after intervention.

Interventions

Subjects had to complete three different interventions each of 30 min duration on the three different test days. One intervention was designed with small frequency and high amplitude (drop landing), another with high frequency and small amplitude (running) and one intervention was performed without loading (resting). The type of intervention, which was performed on a test day, was randomly selected.

The drop landing intervention included 100 vertical drops in 30 min from a 73 cm high platform. Subjects had to step from this height with double-leg landing (knee angle >90°), with hands on the hips and an upright posture. A custom-designed platform with a pneumatic driven lift was used to return the subjects to the height of 73 cm after landing. The jumping intervention was supervised by an instructor. For the running intervention, subjects ran with a speed of 2.2 m/s for 30 min (approximately 4 km). To control the speed and distance the subjects were accompanied by a research assistant using a bicycle with an odometer. In order to count the footfalls during the running intervention a pedometer was attached on the hip of the subjects. Loading frequency was estimated from the number of footfalls during 30 min running (number of footfalls/1,800 s = loading frequency; loading frequency/2 = loading frequency for one leg) and from the 100 vertical drops in 30 min (100 landings/1,800 s = loading frequency). For the resting intervention subjects had to sit on a chair with their legs uncrossed and feet flat on the floor for the 30 min.

Table 1: Mean ± SD and (range) of demographic variables for all subjects and by gender

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Age (years)</th>
<th>Body mass (kg)</th>
<th>Body height (m)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>7</td>
<td>22.9 ± 2.5 (18–26)</td>
<td>64.9 ± 8.0 (50.0–72.7)*</td>
<td>1.70 ± 0.06 (1.62–1.80)*</td>
<td>22.3 ± 2.3 (19.2–25.1)</td>
</tr>
<tr>
<td>Males</td>
<td>7</td>
<td>23.4 ± 1.6 (22–26)</td>
<td>74.9 ± 3.7 (70.0–79.3)</td>
<td>1.81 ± 0.06 (1.73–1.89)</td>
<td>22.8 ± 1.3 (20.2–24.0)</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>23.1 ± 2.1 (18–26)</td>
<td>69.9 ± 8.0 (50.0–79.3)</td>
<td>1.76 ± 0.08 (1.62–1.89)</td>
<td>22.5 ± 1.8 (19.2–25.1)</td>
</tr>
</tbody>
</table>

* Significantly (P < 0.050) different from male subject within the group based on independent samples Student’s t-test.

1 BMI = mass (kg)/height (m)².
Serum COMP

All blood samples (each 5 ml) were drawn from the antecubital vein using a short catheter in a serum monovettes (Sarstedt, Germany). Serum was isolated after coagulation by centrifugation (3,500 rpm for 10 min) and stored at −80°C until analysis. Serum COMP was determined using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) (COMP ELISA, Ana- Mar Medical AB, Lund, Sweden) as described earlier. The samples were analyzed in duplicate and random order. All analyses were carried out without knowing the intervention type. A four parameter—regression curve in SigmaPlot 8.0 (Systat Software Inc., San Jose, USA) was used to define the standard curve of light absorbance and serum COMP to calculate the unknown concentration. The intra-assay and inter-assay coefficients of variation were 1.9% and 2.7%, and the detection limit was <0.1 U/l. Differences caused by inter-assay variation were controlled by testing all samples of one subject on the same plate.

MRI and analysis

The MRI measurements before and after all three interventions were performed on the right knee. The only exception was one subject who reported on problems with the right patella tendon more than 10 years ago. In this case, the MRI was acquired in the left knee. The knee joint cartilage deformation (cartilage volume and thickness change) was determined using a 1.0 T MRI-scanner (Philips Intera, Philips, Hamburg, Germany) with a circumferentially extremity coil. The subjects were centred in a supine position and entered the MRI-scanner feet first. A T2-weighted 3D gradient echo sequence with water excitation (WATS) = water only selection) of the knee was performed. First, double oblique coronal scans of femorotibial knee were taken with TR (repetition time) = 35 ms, TE (echo time) = 17 ms, FA (flip angle) = 35° and FOV (field of view) = 160 mm. Subsequently, axial scans of the patella joint were acquired with TR = 24.2 ms, TE = 12.3 ms, FA = 25° and FOV = 140 mm. The acquisition time was 15 min 20 s for the coronal and 2 min 33 s for the axial scan both with a spatial resolution of 1.5 mm × 0.31 mm × 0.31 mm (matrix = 512 × 512).

Segmentation of the total subchondral bone area (tAB) and the cartilage joint surface area (AC) were conducted by manual segmentation on a section using custom software (Chondrometrics GmbH, Ainring, Germany). The knee joint cartilage was divided in patellar, medial tibial, lateral tibial, and medial and lateral central (weight-bearing) femoral cartilage. The segmentations were conducted by four readers. Each reader had to segment the complete data set of a subject while blinded to the order (pre- or post-exercise).

The volume and average mean cartilage thickness over the tAB (VC and ThCrTabMe) were calculated after three-dimensional reconstruction. The ThCrTabMe was calculated as the average of the mean thickness in normal direction of tAB to AC and vice versa. Quantitative data for the entire knee joint cartilage were divided by adding up the volumes and thicknesses of all cartilage plates. The nomenclature of the analysed parameter was based on a previously published proposal and the test—retest precision and sensitivity to chance (in OA) has been evaluated previously.

Statistical analysis

All statistical computations were performed using Statistica 7.1 (StatSoft GmbH, Hamburg, Germany). The normal distribution of the variables was tested with the Kolmogorov—Smirnov test. A two-way (time and intervention) analysis of variance (ANOVA) with repeated measurements was used to detect significant differences in serum COMP concentration, cartilage volume and thickness. Duncan’s multiple comparisons test was applied for post hoc analysis. Variables are described as mean, 95% confidence interval (5% CI: lower limit, upper limit). The level of significance was set at a value of α < 0.05. Statistical tests were performed on the absolute values. For graphical presentation data were normalized to the baseline values. In order to investigate if changes in serum COMP concentration reflects changes in cartilage deformation, a correlation analysis was conducted for the percent changes in serum COMP concentration (percent difference between baseline serum COMP concentration and serum COMP concentration immediately after intervention) and the percent changes in total cartilage volume following running and drop landing intervention. Pearson’s Product—Moment Correlation Coefficients and linear regression analysis were used to explore potential linear correlations.

Results

In the 30 min running intervention (2.2 m/s, 3.96 km) subjects performed 4,262 ± 498 footfalls, which resulted in an average stride frequency of approximately 2.4 Hz or loading frequency of 1.2 Hz per leg. During the landing intervention the subjects received 100 foot landings in the 30 min loading period. Therefore, the average loading frequency was 0.06 Hz which was about 20 times lower than the average loading frequency of the running intervention. The total body’s kinetic energy at touchdown for impact was estimated at 0.73 (height of the platform in m) × body weight (BW) Joules when landing after the jumps from the platform. For the average running speed of 2.2 m/s a vertical centre of mass lift of 3.5 cm during the flights can be assumed. This results in a total body kinetic landing energy of 0.035 × BW Joules per step which was about 21 times lower than the pre-impact energy in landing from the height.

Serum COMP concentration

The mean baseline serum COMP concentration was 7.3 U/l (95% CI: 5.6, 8.9) prior to the running intervention, 6.8 U/l (95% CI: 5.3, 8.4) prior to the drop landing intervention and 6.6 U/l (95% CI: 5.4, 7.7) prior to the rest intervention (Table II). No significant difference in the baseline values was observed between the different test days and serum COMP concentrations were in the range of the normal distribution for healthy subjects.

Following rest intervention, serum COMP concentration did not change at the different time points compared to the baseline level [Fig. 1(A)]. Immediately after running, serum COMP concentration increased by 30.7% (pre: 7.3 U/l (95% CI: 5.6, 8.9); post: 9.1 U/l (95% CI: 7.2, 11.0), P < 0.001) and after the drop landing intervention it increased by 32.3% (pre: 6.8 U/l (95% CI: 5.3, 8.4); post: 8.9 U/l (95% CI: 6.8, 10.9), P < 0.001). The average serum COMP concentration remained significantly elevated 1 h after both the running and the drop landing intervention, and then returned to the baseline level.

Table II

Mean (95% CI: lower limit–upper limit) absolute serum COMP concentration after the three interventions resting, running and drop landing (n = 14)

<table>
<thead>
<tr>
<th>Time</th>
<th>Serum COMP concentration (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>6.6 (5.4–7.7)</td>
</tr>
<tr>
<td>Post</td>
<td>7.2 (6.1–8.2)</td>
</tr>
<tr>
<td>0.5 h</td>
<td>6.7 (5.5–7.8)</td>
</tr>
<tr>
<td>1.0 h</td>
<td>6.7 (5.6–7.8)</td>
</tr>
<tr>
<td>3.0 h</td>
<td>6.8 (3.7–7.8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Serum COMP concentration (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting</td>
<td>7.3 (5.6–8.9)</td>
</tr>
<tr>
<td>Running</td>
<td>8.6 (7.1–10.1)</td>
</tr>
<tr>
<td>Drop landing</td>
<td>7.3 (6.0–8.5)</td>
</tr>
</tbody>
</table>

Values are mean (95% CI: lower limit–upper limit).

*P < 0.05 vs pre, †P < 0.05 vs previous time point.
Fig. 1. Mean (95% CI) serum COMP concentration before (−0.5 h), immediately after (0 h) and within 3 h after (A) resting and (B) running and drop landing intervention (n = 14). Serum COMP concentrations are presented as percent of baseline serum concentration. *Significantly different from baseline serum COMP concentration; running: immediately after intervention, P < 0.001; 0.5 h after intervention, P = 0.002; drop landing: immediately after intervention, P < 0.001; 0.5 h after intervention, P = 0.012. Significantly (P < 0.001) different from the preceding time point.

Table III

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Thickness (mm)</th>
<th>Volume (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resting</td>
<td>Running</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>2.29 (2.10–2.48)</td>
<td>2.26 (2.07–2.44)</td>
</tr>
<tr>
<td>Post</td>
<td>2.29 (2.09–2.48)</td>
<td>2.13 (1.94–2.31)*#</td>
</tr>
<tr>
<td>Medial tibia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1.87 (1.77–1.98)</td>
<td>1.83 (1.72–1.94)</td>
</tr>
<tr>
<td>Post</td>
<td>1.88 (1.76–1.99)</td>
<td>1.79 (1.68–1.90)*</td>
</tr>
<tr>
<td>Lateral femur</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1.90 (1.70–2.10)</td>
<td>1.90 (1.72–2.09)</td>
</tr>
<tr>
<td>Post</td>
<td>1.92 (1.73–2.11)</td>
<td>1.85 (1.66–2.03)</td>
</tr>
<tr>
<td>Medial femur</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1.91 (1.75–2.08)</td>
<td>1.90 (1.73–2.08)</td>
</tr>
<tr>
<td>Post</td>
<td>1.96 (1.81–2.12)*</td>
<td>1.85 (1.69–2.01)*</td>
</tr>
<tr>
<td>Patella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>2.66 (2.40–2.89)</td>
<td>2.71 (2.47–2.95)</td>
</tr>
<tr>
<td>Post</td>
<td>2.66 (2.43–2.89)</td>
<td>2.63 (2.39–2.86)*</td>
</tr>
</tbody>
</table>

Values are mean (95% CI: lower limit–upper limit).

*P < 0.05 pre vs post, **P < 0.05 run vs drop landing.

[Fig. 1(B)]. Unexpectedly, there was no effect on the magnitude or duration of the serum COMP elevation after drop landing vs running (two-way ANOVA (time and intervention) with repeated measurements, P = 0.253).

**Cartilage deformation**

Cartilage thickness and volume values were in the normal range for young healthy subjects39. After the rest intervention, no significant changes in cartilage volume and thickness were observed at the patella, medial tibia, and lateral tibia and femur. However, medial femoral cartilage volume (+2.8% , P = 0.035) and thickness (+2.9%, P = 0.031) were increased compared to the baseline level (Table III).

Following the running intervention, cartilage volume and thickness were reduced at the patella (−3.5% and −3.1%, P < 0.001 and P < 0.001), medial tibia (−2.7% and −2.2%, P = 0.001 and P = 0.007) and femur (−3.8% and −2.6%, P = 0.003 and P = 0.017), and lateral tibia (−6.1% and −5.8%, P < 0.001 and P < 0.001, Figs. 2 and 3). The drop landing intervention leads to a decrease in cartilage volume and thickness at the patella (−2.8% and −1.9%, P = 0.002 and P = 0.003), medial (−2.5% and −2.2%, P = 0.003 and P = 0.008) and lateral tibia (−1.6% and −1.8%, P = 0.017 and P = 0.019).

When comparing the magnitude of deformation between running and drop landing, a significantly greater deformation (volume: P = 0.002 and thickness: P = 0.001, two-way ANOVA (time and intervention) with repeated measurements) was observed after running in lateral tibia. Similar trends were seen in other cartilage plates, but these did not attain statistical significance.

**Correlation between serum COMP and cartilage deformation**

There was no significant correlation between serum COMP increase and the total cartilage deformation following running intervention (Fig. 4). In contrast, there was a significant correlation (r = 0.774, P = 0.001) between serum COMP increase and the total cartilage deformation following drop landing indicating that the lesser the decrease of the cartilage volume the higher the increase in serum COMP concentration.

**Discussion**

In the present study we determined the in vivo short-term response of knee joint cartilage to two different impact-loading
Running vs drop landing intervention, drop landing—patella, patella, P = 0.003; drop landing—patella, P = 0.002; lateral tibia, P = 0.017; medial tibia, P = 0.003. #Running vs drop landing intervention, P = 0.002.

The study included kinematic and kinetic analyses of the loading of femur, lateral and medial tibia cartilage following the running and drop landing intervention of 30 min duration (n = 14). Cartilage volumes are presented as percent of pre intervention cartilage volume. *Pre vs post intervention; running: patella, P < 0.001; lateral tibia, P < 0.001; medial tibia, P < 0.001; medial femur, P = 0.003; drop landing: patella, P = 0.002; lateral tibia, P = 0.017; medial tibia, P = 0.003. #Running vs drop landing intervention, P = 0.002.

![Cartilage volume change (%)](image1)

**Fig. 2.** Mean (95% CI) cartilage volume changes of the patellar, lateral and medial femoral, and lateral and medial tibial cartilage following the running and drop landing intervention of 30 min duration (n = 14). Cartilage volumes are presented as percent of pre intervention cartilage volume. *Pre vs post intervention; running: patella, P < 0.001; lateral tibia, P < 0.001; medial tibia, P = 0.001; medial femur, P = 0.003; drop landing: patella, P = 0.002; lateral tibia, P = 0.017; medial tibia, P = 0.003. #Running vs drop landing intervention, P = 0.002.

![Cartilage thickness change (%)](image2)

**Fig. 3.** Mean (95% CI) cartilage thickness changes of the patellar, lateral and medial femoral, and lateral and medial tibial cartilage following the running and drop landing intervention of 30 min duration (n = 14). Cartilage thicknesses are presented as percent of pre intervention cartilage thickness. *Pre vs post intervention; running: patella, P < 0.001; lateral tibia, P < 0.001; medial tibia, P = 0.007; medial femur, P = 0.017; drop landing: patella, P = 0.003; lateral tibia, P = 0.019; medial tibia, P = 0.008. #Running vs drop landing intervention, P = 0.001.

Previous studies have reported a decrease in cartilage volume and thickness after running exercise of various durations and distances. Boocock et al. demonstrated that 30 min running resulted in a reduction of the lateral tibial (−5.7%), medial (−5.3%) and lateral (−4.0%) femoral cartilage volume. Following a 5 km run Kessler et al. showed decreases in patellar (−6.6%) and tibial (−3.6%) cartilage volume. Mosher et al. found a reduction of cartilage thickness at the femur (4%−8%) and tibia (10%−12%) following 30 min jogging in marathon runners and sedentary controls. The extent of cartilage deformation in these studies was higher compared to our results. An explanation for the differences in the magnitude of cartilage deformation maybe that our subjects were relatively untrained (maximum: 1.1 ± 1.2 h/week physical activity) compared to those of Boocock et al. (recreational runners) and Kessler et al. (long distance runners: athletes). Previous animal studies have demonstrated that chronic mechanical loading or exercise can lead to adaptations in the biochemical and/or mechanical properties of articular cartilage. It could be that the cartilage of our relatively untrained subjects has different mechanical properties compared to the cartilage of trained athletes. Moreover, it is likely that the protocol of the MRI cartilage T2 measurements in the study of Mosher et al. could contribute to the discrepancies in cartilage thickness, because they used relatively large voxel dimensions. Moreover, we did not detect a significant deformation of lateral femoral cartilage after running which could be explained through the high inter-individual variability in the decrease of cartilage volume (mean = −3.2% (95% CI −6.39, −0.026), standard deviation (SD) = ±5.5%) or thickness (mean = −2.9% (95% CI −5.98, 0.13), SD = ±5.3%) at this location.

After 10 jumps from 40 cm height onto one leg Eckstein et al. found highly significant deformation in the medial (−6.1%) and lateral (−7.2%) tibial cartilage, but not in the medial or lateral femoral cartilage. This is in accordance with our findings following 100 drop landings. Comparing different types of exercise Eckstein et al. reported that patellar cartilage deformation was dose-dependent. More intense loading, such as knee bending, lead to a greater deformation compared to cycling. For the femorotibial cartilage, greatest deformation was observed after impact loading. However, there is no previous study comparing the effect of running and drop landing on the femorotibial cartilage. We detected the greatest deformation at the lateral tibia cartilage following running. Furthermore, this deformation tended to be greater compared to lateral tibial cartilage deformation following drop landing. This indicates that knee joint cartilage deformation may differ following mechanical loading interventions with various amplitude and frequency. Boocock et al. also found the greatest cartilage deformation at the lateral tibia following running and failed to detect cartilage changes in the medial tibia. The study included kinematic and kinetic analyses of the running performance but could not identify a relationship between knee joint loading parameters and cartilage volume changes in the lateral tibia. One can speculate that the loading of the lateral tibia is greater following running compared to drop landing, but based on our data we cannot explain the differences in cartilage deformation following running and drop landing.

The mean average serum COMP concentrations were in accordance with values determined for young healthy adults before and after running exercise. Previous studies demonstrated that physical activity leads immediately to an increase in serum COMP. In our study, serum COMP concentration was also increased immediately after the running and the drop landing intervention. The mechanism for this increase of the serum COMP level is still unknown. It has been speculated that serum COMP
alterations reflect cartilage volume changes. Surprisingly, there was no difference in the magnitude and duration of the COMP increase between impact-loading modes, while we found some difference in the cartilage deformation between the complete running and the drop landing intervention. However, we assumed that the total kinetic energy of the running and drop landing interventions must be nearly equal because the running intervention had a loading frequency approximately 20 times higher and a kinetic energy (per step) of approximately 21 times lower than might be experienced during the drop landing intervention. This might indicate that an elevation of serum COMP concentration did not depend only on the loading frequency, force rate or amplitude but rather on the kinetic energy which was applied.

We were unable to detect a significant difference in the absolute increase or duration of the serum COMP concentration after drop landing vs running. But it should be considered that there was a high inter-individual variability in changes of serum COMP concentrations after the interventions and a relatively small sample size. However, the results showed a remarkable different relationship between changes in COMP and cartilage volume after the two intervention modes. There was a significant correlation between change in cartilage volume and serum COMP following the drop landing, which means that the lesser the cartilage volume changes the higher the changes in serum COMP concentration. In contrast to our study Kersting et al. found a significant negative correlation ($r = -0.487$, $P = 0.0404$) between cartilage volume change and the change of serum COMP in experienced runners following a 1 h run. The authors concluded that if a runner demonstrates a rise of serum COMP after running a greater volume loss is to be expected. In an in vitro study with porcine cartilage explants, the release of COMP into the medium increased in proportion to the magnitude of dynamic mechanical stress when magnitudes higher than 0.025 MPa. Furthermore, Salzmann et al. found that under static and dynamic conditions the distinct regions across the knee joint varied in gene and matrix expression profiles including expression of COMP. Taken together, these results indicate that the characteristic of the mechanical loading had an influence on the release of COMP into the serum in relation to the changes in cartilage volume and it is likely that besides the magnitude of the load the frequency plays an important role for the COMP release. In our study the drop landing had a clearly lower loading frequency (0.06 Hz) compared to the running (1.2 Hz) exercise so that the cartilage might have more time to recover during the intervention. Thus, it might be that COMP was released into the serum during recovery of cartilage volume between the single drop landings, so that the greater the cartilage recovery is the more COMP is released. It seems that drop landing appears to trigger such a reaction in serum COMP concentration whereas running did not. Further experiments are required to determine the exact relationship between mechanical loading and COMP release into the serum.

The serum levels of COMP and COMP fragments were aimed to represent a marker of articular cartilage degradation. We did not assume that the increase in serum COMP after our intervention protocols was a result of cartilage destruction but rather a normal turnover or short-term adaptation. The running and drop landing interventions in our study were not so strenuous activities that one would expect a destruction of the cartilage. Long-term intervention studies or more strenuous interventions would be necessary to determine if an increase in the serum COMP concentration after exercise could be partially due to a degenerative process. However, the mechanism how COMP could be released into the serum rapidly in response to mechanical loading through physical activity is not known. COMP is predominantly expressed in cartilage and only in small amounts in high-loaded parts of tendon, in synovial and dermal fibroblasts, meniscus and ligament. While it is unlikely, that other connective tissues are the source of the serum COMP increase, the synovial fluid or the lymphatic system could contribute to the overall COMP levels. In a recently published study moderate mechanical loading of a single knee joint with OA resulted in a local decrease in COMP concentration in the synovial fluid. That indicates that COMP in the synovial fluid was also pressured out into the extraarticular space, the blood or the lymphatic system during mechanical loading of the joint. Even though we cannot further dissect which pathways lead to an increase of serum COMP following physical activity, we found that the relationship between changes in cartilage volume and serum COMP is not a simple one. Besides mechanical factors the release of COMP into the serum seems to depend on the clearance of the COMP molecules from the joint. Furthermore the sandwich ELISA used to analyse serum COMP concentrations does not allow a distinction between full-length COMP and different COMP fragments. Based on our results it remains open if mechanical loading is sufficient to extrude intact COMP from tissue/synovial fluid or to activate proteolytic matrix degradation via so far unknown signals as a short-term reaction to the interventions. The fragmentation of cartilage proteins has been characterized in detail using cartilage explants treated with interleukin-1. However, this latter mechanism is unlikely as previous studies have shown that a significant cytokine (interleukin-1) induced release of proteoglycans from human cartilage needs at least 12 h. In a study from Vilim et al. it was demonstrated that the majority of COMP molecules in serum of OA and RA patients are full-length proteins. This suggests that full-length COMP molecules trigger such a reaction in serum COMP concentration whereas running did not. Further experiments are required to determine the exact relationship between mechanical loading and COMP release into the serum.

![Fig. 4. Linear correlations between the change in serum COMP concentration (%) and changes in cartilage volume (%) following the running and drop landing intervention ($n = 28$).](image-url)
could be released into the serum in direct reaction to mechanical loading of the joint. This study indicates that in vivo knee joint cartilage deformation may differ following mechanical loading interventions with various amplitude and frequency. Furthermore, physical activity induced release of COMP into the serum was influenced by impact-loading mode. However, the relation between the change in COMP and cartilage volume is not a simple one and seems to depend on mechanical and biochemical factors.

Author contributions
G-PB and AN conceived and designed the study; G-PB and AN procured the project funding. LB and MM recruited the participants. LB, FE, MM, AN, UM, TS and FZ collected and processed the data. AN performed the statistical analysis. MM and AN drafted the manuscript. G-PB, LB, TS, FE, UM and FZ contributed to the manuscript. All authors read and approved the final manuscript.

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Conflict of interest
FE is Chief Executive Officer (CEO) of Chondrometrics GmbH, Ainring, Germany and provides consulting services to MerckSerono, Novartis and Sanofi Aventis. Besides, none of the authors has anything to disclose for this manuscript and there are no conflicts of interest.

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