Osteoarthritis and Cartilage

OARSI
OSTEOARTHRITIS
RESEARCH SOCIETY
INTERNATIONAL

Check for updates

Dose-response relationship between ambulatory load magnitude and load-induced changes in COMP in young healthy adults

S. Herger † ‡, W. Vach †, A.-M. Liphardt §, C. Egloff †, C. Nüesch † ‖, A. Mündermann † ‖ *

† Department of Orthopedics and Traumatology, University Hospital Basel, Basel, Switzerland
‡ Department of Exercise and Health Sciences, University of Basel, Basel, Switzerland
§ Department of Internal Medicine 3 – Rheumatology and Immunology, Friedrich-Alexander-University Erlangen-Nuremberg (FAU) and Universitätsklinikum Erlangen, Erlangen, Germany
‖ Department of Biomedical Engineering, University of Basel, Basel, Switzerland

ARTICLE INFO

SUMMARY

Objective: To determine the dose-response relationship between ambulatory load magnitude during a walking stress test and load-induced changes in serum concentration of cartilage oligomeric matrix protein (sCOMP) in healthy subjects.

Design: sCOMP was assessed before and after a 30-min walking stress test performed on three test days by 24 healthy volunteers. In each walking stress test, one of three ambulatory loads was applied in a block randomized crossover design: normal body weight (BW) (100%BW = normal load); reduced BW (80%BW = reduced load); increased BW (120%BW = increased load). Knee kinematics and ground reaction force (GRF) were measured using an inertial sensor gait analysis system and a pressure plate embedded in the treadmill.

Results: Load-induced increases in sCOMP rose with increasing ambulatory load magnitude. Mean sCOMP levels increased immediately after the walking stress test by 26.8 ± 12.8%, 28.0 ± 13.3% and 37.3 ± 18.3% for the reduced, normal or increased load condition, respectively. Lower extremity kinematics did not differ between conditions.

Conclusions: The results of this study provide important evidence of a dose-response relationship between ambulatory load magnitude and load-induced changes in sCOMP. Our data suggests that in normal weight persons sCOMP levels are more sensitive to increased than to reduced load. The experimental framework presented here may form the basis for studying the relevance of the dose-response relationship between ambulatory load magnitude and load-induced changes in biomarkers involved in metabolism of healthy articular cartilage and after injury.

Introduction

Articular cartilage is found in every arthrodial joint of the human body and facilitates joint motion with low friction, force distribution across the articulating surfaces and force transmission to the subchondral bone[1]. Because of its unique structure, healthy articular cartilage is resistant against high cyclic loads and shear stress and shows almost no signs of degeneration throughout many decades of the lifespan in healthy adults[2,3]. However, once the degeneration process has been initiated—for instance, through injury—its layered architecture, slow metabolism and the chondrocytes' low potential for replication make it difficult to regenerate.[3,4]

Chondrocytes produce and maintain the extracellular matrix of articular cartilage[3–6] and their activity is influenced by mechanical and chemical stimuli that trigger changes in the production and release of growth factors and soluble cytokines[7–9]. Concentrations of these enzymes, of structural proteins and their fragments can be measured in serum and reflect upon the tissue status and metabolism. For instance cartilage oligomeric matrix protein (COMP) is a cartilage constituent that may be involved in cartilage degeneration and has been classified as biomarker for osteoarthritis in the

* Address correspondence and reprint requests to: A. Mündermann, Department of Orthopedics and Traumatology, University of Basel Hospital, Spitalstrasse 21, 4031 Basel, Switzerland. Tel.: 41-61-3285445.
E-mail addresses: simon.herger@stud.unibas.ch (S. Herger), werner.vach@usb.ch (W. Vach), anna-maria.Liphardt@uk-erlangen.de (A.-M. Liphardt), christian.egloff@usb.ch (C. Egloff), corina.nueesch@usb.ch (C. Nüesch), annegret.muendermann@unibas.ch (A. Mündermann).

Burden of Disease, Investigative, Prognostic, Efficacy of Intervention and Diagnostic (BIPED) classification[10–12]. COMP is a 524 kDa non-collagenous homopentameric glycoprotein produced by the chondrocytes and plays an important role in extracellular matrix organization[13,14]. COMP binds to collagen I/II and IX and procollagen I/II and thereby has a role in collagen fibril organization[15,16]. In early stages of fibril formation, it raises collagen I and II fibrillogenesis and facilitates collagen interaction by holding them tightly together[17]. When articular cartilage is degenerated, components of the extracellular matrix—and hence also fragments of COMP—are effused into the synovial fluid and to the blood.[18]

COMP has been proposed as mechanosensitive biomarker. For instance, previous studies examining the effect of different physical activities and exercise on cartilage biomarkers reported increased serum COMP (sCOMP) levels after the activity burst[19–29]. Specifically, serum concentration of cartilage oligomeric matrix protein (sCOMP) levels were distinctly higher during and after a marathon (up to 1.6-fold) or ultramarathon run (up to 3-fold) than before the marathon[22–24,28,30]. Moreover, during 14-day and 21-day bedrest sCOMP levels were 15% and 20% lower than before bedrest[31,32]. These previous results indicate that sCOMP is not only sensitive to physical activity and to inactivity but that the magnitude and direction of response depends on the type or duration of the activity and possibly on the load magnitude.

To date, only few studies have investigated the effects of different physical loads on sCOMP, and most of these compared different modes of load where several load characteristics (i.e., load magnitude, frequency, range of joint motion, and/or duration) were modified simultaneously[19,25–27,29]. Recently, Denning et al.[33] reported a greater load-induced increase in sCOMP when walking for 30 min with 140% body weight (BW) than with normal BW but observed no difference in load-induced increase in sCOMP between walking with 60% BW and normal BW. However, unloading was facilitated by a pressurized chamber on the lower body which reduces blood flow in the lower extremities reflected by increased

systolic blood pressure[34] and hence likely limit the circulation of COMP in serum during the exercise.[35]

Overall, there is insufficient evidence for a dose-response relationship between physical stress and articular cartilage biomarker concentrations. The purpose of this study was to examine the effect of systematically altered ambulatory load magnitude during a walking stress test on load-induced changes in sCOMP in healthy subjects. Moreover, while controlling for kinematics is very difficult, we tested if the implemented experimental framework is suitable for altering ambulatory load without changing kinematics.

Methods

Participants

Twenty-four healthy persons participated in this study (12 female, mean ± SD, age: 25.7 ± 1.4 years; body height: 167 ± 9 cm; body mass: 62.7 ± 8.4 kg; body mass index (BMI): 22.3 ± 1.6 kg/m^2; 12 male, age: 25.0 ± 2.2 years; body height: 181 ± 8 cm; body mass: 79.1 ± 11.6 kg; BMI: 24.0 ± 2.7 kg/m^2). Inclusion criteria were: age between 18 and 30 years; physically active (>2 times/week); and BMI below 30 kg/m^2. Exclusion criteria were: previous lower extremity injury and neuromuscular conditions that could have affected their gait. The study was approved by the regional ethics board and conducted in accordance with the Declaration of Helsinki, and participants provided written informed consent prior to participation.

Experimental framework

The experimental protocol followed a block randomized cross-over design. Participants performed a 30-min walking stress test on a treadmill (mercury$^®$ 3p, h/p/cosmos sports & medical GmbH, Nussdorf-Traunstein, Germany) on three different days (Fig. 1). In each walking stress test, one of three ambulatory loads was
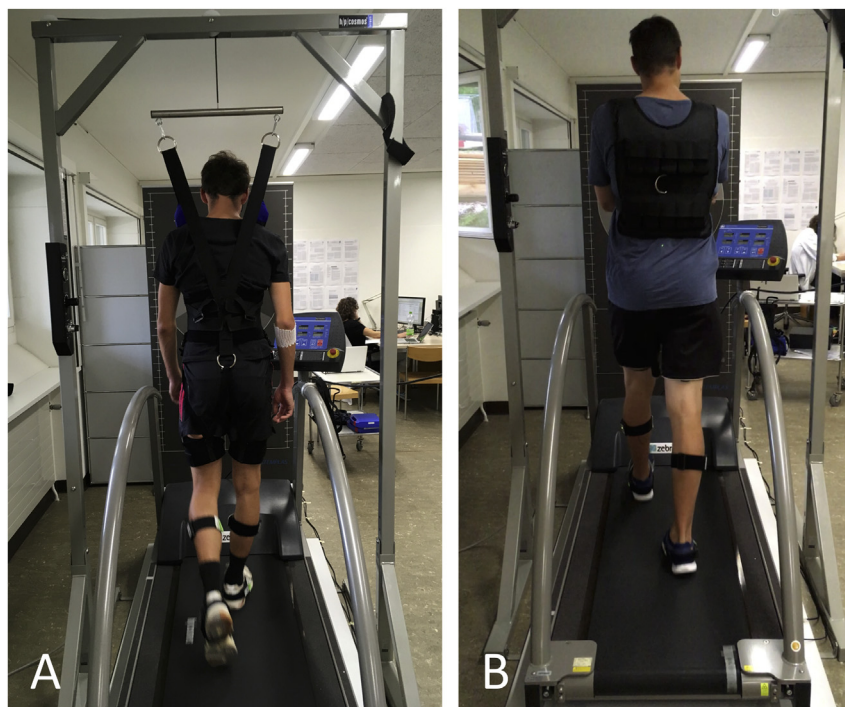
Fig. 1. Photograph of the experimental setup. A, subject walking on the treadmill connected to the dynamic unloading system; B, subject walking on the treadmill while wearing a weight vest.

applied: normal BW (100%BW = normal load); reduced BW (80% BW = reduced load); increased BW (120%BW = increased load; Fig. 1). For the reduced load condition, dynamic unloading was achieved using an h/p/cosmos's airwalk® system (h/p/cosmos sports & medical GmbH, Nussdorf-Traunstein, Germany). This system lowers the bodyweight of a participant dynamically through a harness connected to a pneumatic pulley system set to the intended percentage of bodyweight. The increased load condition was achieved using an adjustable weight vest (CAPITAL SPORTS Monstervest 20 kg, Chal-Tec GmbH, Berlin, Germany) with adjustable weights (1 kg increments) corresponding to 20% body mass placed in pockets on the front and back of the vest.

Procedures

For each participant, the three appointments were all at the same time of day preceded by at least one rest day. Participants were required to refrain from sports and physical activities for 24 h prior to each appointment. On each test day, five venous blood samples were taken 30 min (t_{-1}) and immediately before (t_0) the walking stress test, and immediately (t_1), 30 min (t_2) and 60 min (t_3) after the walking stress test (Fig. 2).

The schedule of test days was standardized, and only the load condition was modified. Participants rested sitting in a wheelchair for 60 min before and 60 min after the walking stress test. Inertial sensors of the gait analysis system (RehaGait®, Hasomed GmbH, Magdeburg, Germany) were attached to their legs. Subsequently, participants walked for 1 min on the treadmill while the treadmill speed was continually increased to determine each participant's preferred walking speed. This individual preferred walking speed was then used for all load conditions. Then, participants stood next to the treadmill while the RehaGait® sensors and the pressure plate embedded in the treadmill were calibrated and the weight vest or unloading harness was adjusted. For the reduced load condition, after the subject stepped onto the treadmill the harness was attached to the dynamic unloading system and the amount of unloading adjusted to 20% of the subject's BW. During the walking stress test, participants walked on the treadmill at their pre-determined individual preferred speed for 30 min. Immediately after the walking stress test, participants rested for 60 min sitting in the wheelchair.

Gait analysis

During the walking stress test, vertical ground reaction force (GRF) was measured using the pressure plate built into the tread-mill (mercury® 3p, h/p/cosmos sports & medical GmbH, Nussdorf-Traunstein, Germany, with built-in Zebris FDM-T pressure plate, zebris Medical GmbH, Isny, Germany; sampling rate, 120 Hz), and joint kinematics were measured using an inertial sensor based gait analysis system (RehaGait®, Hasomed GmbH, Magdeburg, Germany; sampling rate, 400 Hz). Both systems were calibrated immediately before the walking stress test. Force and kinematic data were recorded in minute 4 of the walking stress test. No additional filtering on the time series extracted from the built-in software was performed. The GRF impulse (area under the GRF curve) and maximum and minimum joint angles at the ankle, knee and hip during stance were calculated for each step and averaged for all steps taken within the 2 min. The total GRF impulse was calculated as the number of steps taken during the 30-min walking stress test times the average GRF impulse. The number of steps was extrapolated from the cadence measured by the pressure plate.

Blood samples

Venous blood samples were taken from the antecubital vein. A vein catheter (Vasofix® Safety PUR 20G, B. Braun Melsungen AG, Melsungen Germany) was placed during the rest period before the first blood sample at t_{-1} (Fig. 2) and remained there for the entire experiment. After every blood sample, the catheter was flushed with 10 ml isotonic saline solution (0.9% NaCl) to prevent plugging through clotting blood. The first 3 ml of every sample were discarded to avoid dilution through the injected saline solution. The blood samples clotted in the blood tubes (S-Monovette® 7.5 ml Z-Gel, Sarstedt AG, Nürnbrecht, Germany) for 30 min. Subsequently, they were centrifuged (Sarstedt AG &Co SMC6) for 15 min at 2016 g and stored in the fridge (4°C) for no longer than 5 h until separation in aliquots and frozen (−20°C). The tubes were transferred to a −80°C freezer within 48 h. sCOMP was measured using a commercial available enzyme-linked immunosorbent assay (Human COMP protein ELISA kit, BioVendor, Modrice, Czech Republic). All samples were analyzed in duplicate. Intra-assay variability was estimated as coefficient of variation between the duplicates and
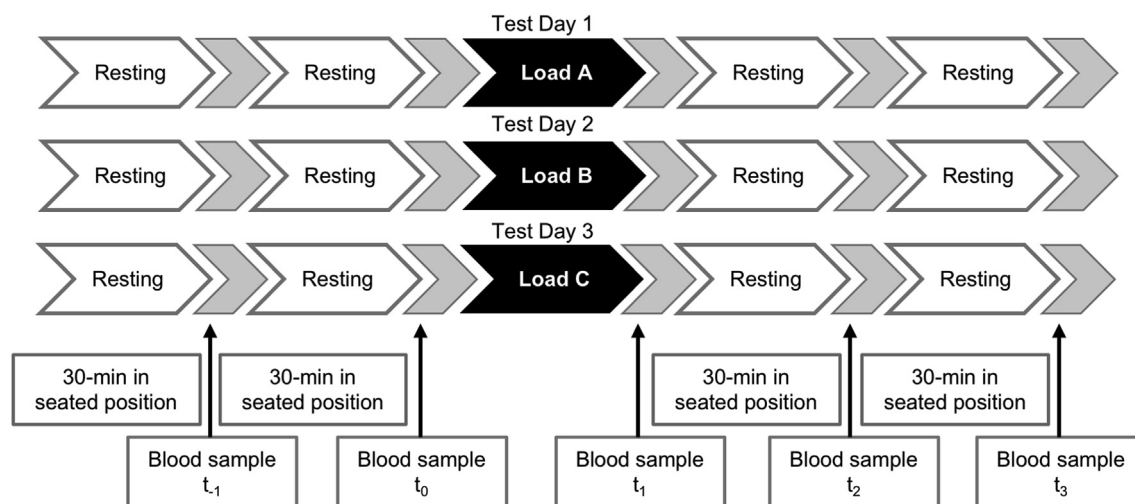

Fig. 2. Illustration of the test protocol. Each participant completed a walking stress test on three separate days after at least one rest day. The order of the load conditions (reduced, normal and increased load) was randomized in a randomized block design.

was 4.2 ± 3.8%. The mean of the duplicates for each time point and condition was used for further analysis. sCOMP concentrations for one participant were above the upper detection limit, and hence data for this participant were not included in the further analysis. Relative changes in sCOMP concentration between two time points t and t' were calculated as (sCOMP(t')-sCOMP(t))/sCOMP(t)*100.

Statistical analysis

Statistical analysis was performed in STATA Version 14.2 (StataCorp LLC, College Station, TX) and SPSS Version 25 (IBM Corporation, Amonk, NY). Repeated measurements ANOVA was used to compare sCOMP baseline values (t_0), relative decrease in sCOMP during recovery from the walking stress test, and GRF impulse, spatio-temporal and joint kinematic parameters between conditions. Relative load-induced increase in sCOMP was compared using a mixed model with participant as random intercept. Effects of sex and BMI were analyzed by adding them to this model as covariates and reporting the estimated regression coefficients β. Pairwise comparisons were based on Scheffe's method. To assess the difference between two differences in load conditions, we considered the corresponding contrasts. In order to check whether the change we may observe between t_0 to t_1 reflects the biological response to the stimulus of the walking stress test, we also investigated the correlation of this change with the subsequent change between t_1 and t_2. The Pearson's correlation coefficient R was computed to quantify the relationship between the relative load-induced increase in sCOMP and the relative decrease in sCOMP after the intervention. The significance level for all statistical tests was set a priori to 0.05.

Results

Baseline sCOMP

sCOMP concentration at baseline (t_0) were similar on the different test days ($P = 0.274$; Table I) with a pooled mean of 470.5 and a pooled standard deviation of 221.7 ng/ml. Baseline sCOMP levels were similar when comparing when female and male participants (mean ± SD; women: 480.2 ± 267.9 ng/ml; men: 461.6 ± 172.5 ng/ml; $P = 0.731$).

Load-induced changes in sCOMP

Absolute sCOMP levels are listed in Table I. Relative sCOMP increased immediately after the 30-min walking stress test on average by 26.8 ± 12.8%, 28.0 ± 13.3% and 37.3 ± 18.3% for the reduced, normal or increased load condition, respectively ($P < 0.001$; Fig. 3). Pairwise comparisons indicated a statistically significant difference between the increased and normal load condition ($P = 0.010$) as well as between the reduced and increased load condition ($P = 0.003$). The difference between increased and

Table I

Mean (1 standard deviation) sCOMP parameters during the walking stress test for each of the three load conditions ($N = 23$)

Time point	Reduced load	Normal load	Increased load
sCOMPt_{-1} (ng/mL)	502.9 (216.4)	500.9 (219.6)	534.3 (329.5)
sCOMPt_0 (ng/mL)	473.3 (207.8)	449.9 (182.2)	488.2 (273.5)
sCOMPt_1 (ng/mL)	592.5 (237.7)	566.7 (200.8)	646.2 (295.6)
sCOMPt_2 (ng/mL)	492.6 (203.7)	463.4 (161.8)	520.0 (248.4)
sCOMPt_3 (ng/mL)	466.9 (200.5)	442.9 (163.7)	485.1 (244.2)

sCOMP—serum concentration of cartilage oligomeric matrix protein; t_0—immediately before the 30-min walking exercise; t_1—immediately after the 30-min walking exercise; t_2—30 min after the 30-min walking exercise; t_3—60 min after the 30-min walking exercise.
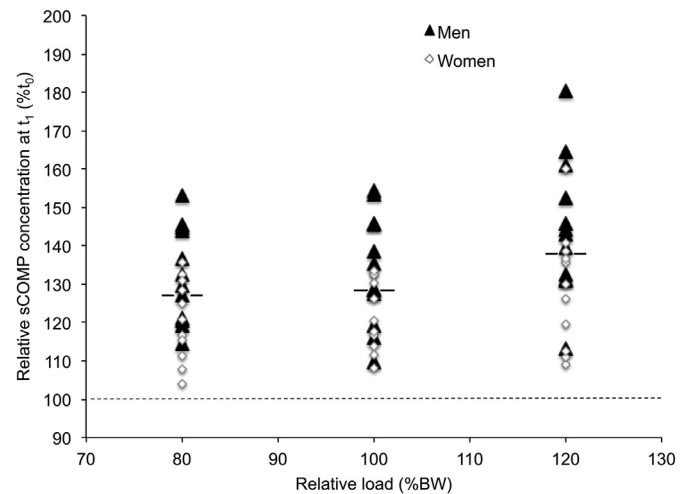
Fig. 3. sCOMP concentration immediately after the walking stress test (t_1) relative to levels immediately before the test (t_0; $N = 23$) for the three ambulatory load magnitudes (reduced load—80%BW; normal load—100%BW; and increased load—120%BW). Horizontal lines represent mean sCOMP concentration for each load conditions; the dotted line represents sCOMP concentration at t_0.

normal load condition (9.28%, 95%CI: [3.30,15.26]%) was distinctly higher than the difference between normal and reduced load condition (1.23%, 95%CI:[-4.75,7.20]%), but this difference was not statistically significant ($P = 0.127$).

The load-induced increase in sCOMP was higher in men than in women (at a statistically significant degree (β = 12.9, 95%CI: [4.6,21.2], $P = 0.002$; Fig. 3). This difference remained statistically significant when adjusting for BMI and baseline values (β = 13.1, 95%CI: [5.7,20.5], $P = 0.001$). The relation to BMI itself was not statistically significant (β = 0.88, 95%CI: [−1.22,2.99], $P = 0.411$).

Thirty minutes after the walking stress test (t_2), sCOMP levels were still higher than at baseline to a statistically significant degree (average ± SD sCOMP at t_2 relative to t_0, reduced load: 105.0 ± 7.1%; normal load: 104.6 ± 8.0%; and increased load: 109.3 ± 10.6%; $P < 0.001$ for all). These concentrations did not differ between conditions at a statistically significant degree ($P = 0.062$). The load-induced increase in sCOMP from t_0 to t_1 correlated with the decrease during recovery from t_1 to t_2 (reduced load: $R = -0.789$, $P < 0.001$; normal load: $R = -0.718$, $P < 0.001$; increased load: $R = -0.734$, $P < 0.001$; Fig. 4). Sixty minutes after the walking stress test (t_3), sCOMP returned to baseline concentrations (average ± SD sCOMP at t_3 relative to t_0, reduced load: 99.9 ± 10.2%; normal load: 99.4 ± 8.0%; increased load: 101.3 ± 9.4%; $P = 0.907$) and did not differ between conditions ($P = 0.734$).

Gait

Participants walked at an average speed of 1.3 m/s (Table II). The number of steps taken during the 30-min walking stress test and cadence did not differ between load conditions ($P = 0.160$; Table II). The GRF impulses for the reduced and increased load condition were on average 20.7% lower and 18.0% higher than for the normal load, respectively (Table II). We observe only minor differences in maximum and minimum sagittal joint angles between the three conditions ($P \geq 0.081$; Table II) or between sexes ($P \geq 0.109$; all differences in joint angles ≤3°).

Discussion

The purpose of this study was to examine the effect of systematically altered ambulatory load during a walking stress test on
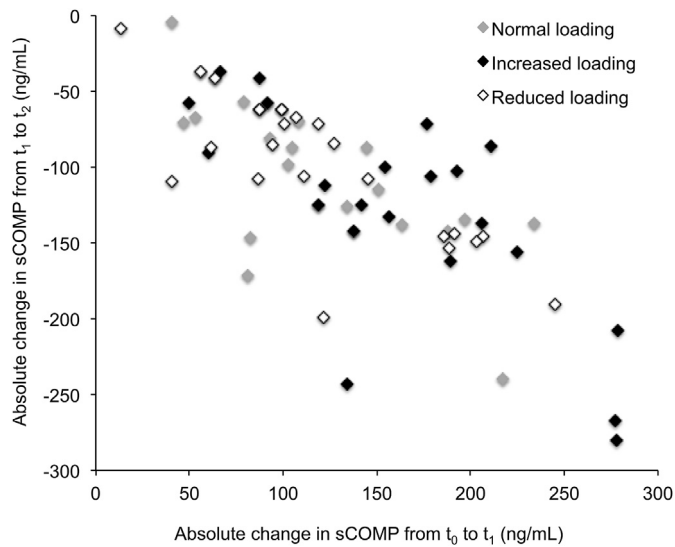
Fig. 4. Correlation between change in sCOMP in the 30 min after the walking stress test (from t_1 to t_2) and change in sCOMP during the walking stress test (from t_0 to t_1) for the three load conditions (reduced load: $R = -0.789$; normal load: $R = -0.718$; increased load: $R = -0.734$). sCOMP—serum concentration of cartilage oligomeric matrix protein.

load-induced changes in sCOMP. Our results showed that the magnitude of the relative increase in sCOMP depends on the magnitude of the applied ambulatory load. These results represent important evidence of a dose-response relationship between ambulatory load magnitude and load-induced changes in sCOMP and clearly show its importance for *in vivo* mechanobiology of articular cartilage.

Baseline sCOMP after 60 min of rest were comparable between test days. These results indicate that participants followed the instructions on maintaining consistent daily routines, and hence boundary conditions were comparable between test days (time of day, food intake, previous physical activity level, etc.). Comparisons of baseline concentrations to those reported in other studies are difficult because different assays were used[19,20,33] or results were reported in other units (e.g., units/liter[25–27,36]) that cannot be converted because some assays target different components or fragments of COMP. Baseline sCOMP levels in our study were similar in men and in women. This result is in contrast to Kersting *et al.*[21] who reported statistically significantly lower baseline sCOMP in female than in male runners. However, Kersting *et al.*[21] neither controlled exercise during the 24 h prior to the exercise nor included a rest period before collecting baseline blood samples, and hence several factors may be responsible for sex differences in

baseline sCOMP in their study. While we observed greater load-induced increases in sCOMP in men than in women, kinematic parameters did not differ between sexes (data not shown). Taking into account the many biological differences between men and women and the potential of the natural exposure to loading to play a role in the evolution of gender differences, we can only speculate about the origin of this sex difference. Further research is needed trying to identify differences in physical or molecular biology between men and women as mediators for this sex difference.

Interestingly, the load-induced response was consistent between participants despite of large variability in absolute concentrations between participants at each time point within load conditions. The average relative load-induced increase with normal BW in our study was greater than those previously reported for similar walking activities despite of comparable walking speeds in all studies. For instance, a 30-min treadmill walking exercise in young adults induced a 10% increase in sCOMP[33], and a 30-min overground walking exercise in healthy young[37] and in healthy older adults[38] induced a 10% and 6% increase in sCOMP, respectively.

The results of this study confirmed our hypothesis of a dose-response relationship between magnitude of ambulatory load and load-induced increase in sCOMP. These results are in agreement with previous observations of greater increase in sCOMP with 40% greater BW applied using weight vest (22% load-induced increase in sCOMP) compared to normal body weight (10% load-induced increase in sCOMP) while walking on a hyperbaric treadmill[33]. Interestingly, Denning *et al.*[33] did not observe a statistically significant load-induced increase in sCOMP with 40% lower BW. These results are particularly noteworthy for two reasons. First, the reduced load condition in the study by Denning *et al.*[33] was achieved by unloading with the hyperbaric treadmill. Being in a hyperbaric chamber increases the pressure acting upon the lower body, and systolic blood pressure increases when walking on a hyperbaric treadmill indicating reduced blood circulation in the lower extremities[34]. Reduced blood circulation presumably also reduces the systemic circulation of blood constituents[35] and hence may affect serum concentrations, possibly explaining the lacking load-induced changes in sCOMP with the reduced load condition reported by Denning *et al.*[33] Second, it is possible that joint load at 40% lower BW is too low to act as a mechanical stimulus.

In our study the difference in the load-induced increase in sCOMP between increased and normal load condition showed a tendency to be greater than the difference in the load-induced increase in sCOMP between the reduced and normal load condition despite of no changes in the environmental pressure. The high correlation between load-induced increase in sCOMP and subsequent return to baseline indicate that the initial increase reflects the response to a biological stimulus present between t_0 and t_1. This

Table II
Mean (1 standard deviation) parameters describing the 30-min walking exercise ($N = 23$)

Parameter	Reduced load	Normal load	Increased load	P-value[a]
Walking speed (m/s)	1.3 (0.1)	1.3 (0.1)	1.3 (0.1)	1.000
Total number of steps	3351.4 (159.3)	3316.4 (179.7)	3331.7 (186.1)	0.160
Cadence (steps/minute)	111.7 (5.3)	110.5 (6.0)	111.1 (6.2)	0.160
Maximum ankle plantarflexion (°)	5.9 (1.8)	7.0 (2.0)	7.6 (2.1)	0.081
Maximum ankle dorsiflexion (°)	8.4 (3.5)	10.3 (4.1)	10.3 (4.3)	0.107
Maximum knee extension (°)	0.5 (0.0)	0.5 (0.0)	0.5 (0.0)	0.211
Maximum knee flexion (°)	19.7 (4.5)	18.4 (4.4)	19.4 (4.9)	0.472
Maximum hip extension (°)	8.9 (3.4)	9.8 (3.4)	8.6 (3.8)	0.196
Maximum hip flexion (°)	20.1 (5.2)	23.4 (4.7)	24.0 (3.9)	0.107
GRF impulse in 1 min (Ns)	28,867.6 (5090.5)	36,473.8 (6780.5)	42,961.6 (7714.2)	<0.001
Relative GRF impulse (% normal load)	79.3 (2.5)	100.0 (0.0)	118.0 (2.9)	<0.001

GRF—ground reaction force; [a]—analysis of variance (ANOVA) for repeated measures with load condition as within subject factor.

stimulus was the walking stress test, and hence the correlations provide further support of our interpretation that the difference in load explains the observed differences between the three conditions. Taking our results and those reported in the literature together suggests that the body is more sensitive to increases than to reductions in BW—at least in normal weight persons—but that hyperbaric pressure may suppress the effects of altered joint load on load-induced increases in sCOMP.

Other approaches for studying the dose-response relationship between ambulatory load magnitude and load-induced increase in sCOMP have included comparisons between different exercise modes such as walking, running, squatting or running and walking at different speeds and different slopes[19,25−27]. For instance, Niehoff et al.[25] reported a load-induced 39% increase in sCOMP after a 30-min run in five healthy young subjects and no changes in sCOMP after 30 min of lymph drainage or deep knee bends. In another study[26], the same group found a load-induced increase in sCOMP of 32% and 30% after 100 drop landings or 30 min running in 14 healthy young subjects. Pruksakorn et al.[27] observed no load-induced increase in sCOMP after a 14 km level walk but a 25% increase after a 14 km uphill walk. However, the activities compared in these studies varied in several load characteristics. For instance, knee deep bends cover a much greater range of motion and do not involve impact load in contrast to walking or running. Moreover, drop landings are performed at a much lower frequency than the typical cadence of running exercises, and uphill walking kinematics differ substantially from level walking kinematics[39]. Finally, the number of load cycles differed between activities within these studies. All of these parameters may potentially influence the load-induced change in sCOMP and hence must be considered in this context.

In our experimental framework, we were able to modulate ambulatory load magnitude without obvious change to joint kinematics. In a study by Denning et al.[19] participants completed different walking and running activities with 4000 steps for each activity and found greater load-induced increases in sCOMP for faster compared with slower activities. Moreover, they reported that not only differences in kinetic but also in kinematic parameters explained more than 60% of variance in load-induced increases in sCOMP. Hence, when comparing the effects of different ambulatory activities on load-induced increases in sCOMP, potentially associated kinematic parameters cannot be neglected. Moreover, the duration of activity may also influence the magnitude of load-induced increases in sCOMP as observed in studies on prolonged running[24,30]. Firner et al.[36] applied a different approach and investigated the effects of modulated external knee flexion moments on load-induced increase in sCOMP increase. However, they did not report any statistically significant differences in sCOMP increase between normal running and running with increased knee flexion moments. Interestingly, they found that changes in knee kinematics affected load-induced increases in sCOMP more than joint moments. These finding represent strong arguments for the relevance of the experimental framework that facilitates modulation of ambulatory load without changing joint kinematics and emphasizes the importance of controlling joint kinematics. Although we did not control for joint kinematics in our study, our experimental setup of treadmill walking at constant speed facilitated modulation of ambulatory load without any changes in joint kinematics. This experimental setup may be useful for studying the relevance of the dose-response relationship between ambulatory load magnitude and load-induced changes in biomarkers involved in articular cartilage metabolism for the initiation and progression of diseases involving articular cartilage.

The results of this study provide important evidence of a dose-response relationship between ambulatory load magnitude and load-induced changes in sCOMP. Yet, our study does not address the metabolic pathway of this relationship. COMP fragments in serum may not only originate from articular cartilage depending on altered effusion caused by altered mechanical load[18] but may also derive from other tissues including tendons, ligaments or menisci[40]. However, in healthy equine carpal joints the content of COMP in intra-articular ligaments and synovial membrane was very low and hence may not largely contribute to the synovial fluid COMP concentration[41]. Moreover, exercise leads to an increase in sCOMP concentration and a decrease in knee synovial fluid COMP concentration whereas rest leads to an increase in synovial fluid and a decrease in sCOMP[20,42]. This evidence suggests that—although COMP is present in other tissues—load-dependent sCOMP likely originate from articular cartilage.

In summary, the results of our study showed that the magnitude of the relative increase in sCOMP depends on the magnitude of the applied ambulatory load. These results represent important evidence of a dose-response relationship between ambulatory load magnitude and load-induced changes in sCOMP and clearly show its importance for in vivo mechanobiology of articular cartilage. The experimental framework presented here may form the basis for studying the relevance of the dose-response relationship between ambulatory load magnitude and load-induced changes in biomarkers involved in articular cartilage metabolism for the initiation and progression of diseases involving articular cartilage such as osteoarthritis.

Author contributions

SH and AM designed the study; SH recruited the participants, collected the data and processed the blood samples; SH, CN prepared the data for statistical analysis; SH, WV, and AM performed the statistical analysis; SH and AM prepared the manuscript; all authors contributed to reviewing and revising the manuscript, and agreed on the final draft.

Competing interest statement

The authors declare no conflict of interest.

Acknowledgements

References

1. Mow VC, Ratcliffe A, Poole AR. Cartilage and diarthrodial joints as paradigms for hierarchical materials and structures. Biomaterials 1992;13:67−97.
2. Buckwalter JA, Mankin HJ. Articular cartilage repair and transplantation. Arthritis Rheum 1998;41:1331−42.
3. Sophia Fox AJ, Bedi A, Rodeo SA. The basic science of articular cartilage: structure, composition, and function. Sport Health 2009;1:461−8.
4. Alford JW, Cole BJ. Cartilage restoration, part 1: basic science, historical perspective, patient evaluation, and treatment options. Am J Sports Med 2005;33:295−306.
5. Dewan AK, Gibson MA, Elisseeff JH, Trice ME. Evolution of autologous chondrocyte repair and comparison to other

cartilage repair techniques. BioMed Res Int 2014;2014: 272481.

6. Woo SLY, Buckwalter JA. Injury and repair of the musculo-skeletal soft tissues. Savannah, Georgia, June 18–20, 1987. J Orthop Res 1988;6:907–31.

7. Buckwalter JA. Activity vs. rest in the treatment of bone, soft tissue and joint injuries. Iowa Orthop J 1995;15:29–42.

8. Buckwalter JA, Mankin HJ. Articular cartilage: tissue design and chondrocyte-matrix interactions. Instr Course Lect 1998;47:477–86.

9. L'Hermette MF, Tourny-Chollet C, Polle G, Dujardin FH. Articular cartilage, degenerative process, and repair: current progress. Int J Sports Med 2006;27:738–44.

10. Bauer DC, Hunter DJ, Abramson SB, Attur M, Corr M, Felson D, et al. Classification of osteoarthritis biomarkers: a proposed approach. Osteoarthritis Cartilage 2006;14:723–7.

11. Bay-Jensen AC, Reker D, Kjelgaard-Petersen CF, Mobasheri A, Karsdal MA, Ladel C, et al. Osteoarthritis year in review 2015: soluble biomarkers and the BIPED criteria. Osteoarthritis Cartilage 2016;24:9–20.

12. Lotz M, Martel-Pelletier J, Christiansen C, Brandi ML, Bruyere O, Chapurlat R, et al. Value of biomarkers in osteoarthritis: current status and perspectives. Ann Rheum Dis 2013;72:1756–63.

13. Hedbom E, Antonsson P, Hjerpe A, Aeschlimann D, Paulsson M, Rosa-Pimentel E, et al. Cartilage matrix proteins. An acidic oligomeric protein (COMP) detected only in cartilage. J Biol Chem 1992;267:6132–6.

14. Tan K, Duquette M, Joachimiak A, Lawler J. The crystal structure of the signature domain of cartilage oligomeric matrix protein: implications for collagen, glycosaminoglycan and integrin binding. Faseb J 2009;23:2490–501.

15. Acharya C, Yik JH, Kishore A, Van Dinh V, Di Cesare PE, Haudenschild DR. Cartilage oligomeric matrix protein and its binding partners in the cartilage extracellular matrix: interaction, regulation and role in chondrogenesis. Matrix Biol 2014;37:102–11.

16. Rosenberg K, Olsson H, Morgelin M, Heinegard D. Cartilage oligomeric matrix protein shows high affinity zinc-dependent interaction with triple helical collagen. J Biol Chem 1998;273: 20397–403.

17. Halasz K, Kassner A, Morgelin M, Heinegard D. COMP acts as a catalyst in collagen fibrillogenesis. J Biol Chem 2007;282: 31166–73.

18. Tseng S, Reddi AH, Di Cesare PE. Cartilage oligomeric matrix protein (COMP): a biomarker of arthritis. Biomark Insights 2009;4:33–44.

19. Denning WM, Becker Pardo M, Winward JG, Hunter I, Ridge S, Hopkins JT, et al. Ambulation speed and corresponding mechanics are associated with changes in serum cartilage oligomeric matrix protein. Gait Posture 2016;44:131–6.

20. Hyldahl RD, Evans A, Kwon S, Ridge ST, Robinson E, Hopkins JT, et al. Running decreases knee intra-articular cytokine and cartilage oligomeric matrix concentrations: a pilot study. Eur J Appl Physiol 2016;116:2305–14.

21. Kersting UG, Stubendorff JJ, Schmidt MC, Bruggemann GP. Changes in knee cartilage volume and serum COMP concentration after running exercise. Osteoarthritis Cartilage 2005;13:925–34.

22. Kim HJ, Lee YH, Kim CK. Biomarkers of muscle and cartilage damage and inflammation during a 200 km run. Eur J Appl Physiol 2007;99:443–7.

23. Kim HJ, Lee YH, Kim CK. Changes in serum cartilage oligomeric matrix protein (COMP), plasma CPK and plasma hs-CRP in relation to running distance in a marathon (42.195 km) and an ultra-marathon (200 km) race. Eur J Appl Physiol 2009;105: 765–70.

24. Neidhart M, Muller-Ladner U, Frey W, Bosserhoff AK, Colombani PC, Frey-Rindova P, et al. Increased serum levels of non-collagenous matrix proteins (cartilage oligomeric matrix protein and melanoma inhibitory activity) in marathon runners. Osteoarthritis Cartilage 2000;8:222–9.

25. Niehoff A, Kersting UG, Helling S, Dargel J, Maurer J, Thevis M, et al. Different mechanical loading protocols influence serum cartilage oligomeric matrix protein levels in young healthy humans. Eur J Appl Physiol 2010;110:651–7.

26. Niehoff A, Muller M, Bruggemann L, Savage T, Zaucke F, Eckstein F, et al. Deformational behaviour of knee cartilage and changes in serum cartilage oligomeric matrix protein (COMP) after running and drop landing. Osteoarthritis Cartilage 2011;19:1003–10.

27. Pruksakorn D, Tirankgura P, Luevitoonvechkij S, Chamnongkich S, Sugandhavesa N, Leerapun T, et al. Changes in the serum cartilage biomarker levels of healthy adults in response to an uphill walk. Singap Med J 2013;54:702–8.

28. Shin KA, Kim AC, Kim YJ, Lee YH, Shin YO, Kim SH, et al. Effect of ultra-marathon (308 km) race on bone metabolism and cartilage damage biomarkers. Ann Rehabil Med 2012;36: 80–7.

29. Harkey MS, Blackburn JT, Hackney AC, Lewek MD, Schmitz RJ, Pietrosimone B. Acute serum cartilage biomarker response after walking and drop landing. Med Sci Sports Exerc 2018;50: 1465–71.

30. Mündermann A, Geurts J, Hugle T, Nickel T, Schmidt-Trucksass A, Halle M, et al. Marathon performance but not BMI affects post-marathon pro-inflammatory and cartilage biomarkers. J Sports Sci 2017;35:711–8.

31. Liphardt AM, Mundermann A, Andriacchi TP, Achtzehn S, Heer M, Mester J. Sensitivity of serum concentration of cartilage biomarkers to 21-days of bed rest. J Orthop Res 2018 May;36(5):1465–71, https://doi.org/10.1002/jor.23786. Epub 2017 Nov 17.

32. Liphardt AM, Mundermann A, Koo S, Backer N, Andriacchi TP, Zange J, et al. Vibration training intervention to maintain cartilage thickness and serum concentrations of cartilage oligometric matrix protein (COMP) during immobilization. Osteoarthritis Cartilage 2009;17:1598–603.

33. Denning WM, Winward JG, Pardo MB, Hopkins JT, Seeley MK. Body weight independently affects articular cartilage catabolism. J Sports Sci Med 2015;14:290–6.

34. Lee KY, Han JY, Kim JH, Kim DJ, Choi IS. Physiological responses during the lower body positive pressure supported treadmill test. Ann Rehabil Med 2016;40:915–23.

35. Macias BR, D'Lima DD, Cutuk A, Patil S, Steklov N, Neuschwander TB, et al. Leg intramuscular pressures and in vivo knee forces during lower body positive and negative pressure treadmill exercise. J Appl Physiol 1985;113:31–8. 2012.

36. Firner S, Willwacher S, de Marees M, Bleuel J, Zaucke F, Bruggemann GP, et al. Effect of increased mechanical knee joint loading during running on the serum concentration of cartilage oligomeric matrix protein (COMP). J Orthop Res 2018 Jul;36(7):1937–46, https://doi.org/10.1002/jor.23859. Epub 2018 Feb 13.

37. Mündermann A, Dyrby CO, Andriacchi TP, King KB. Serum concentration of cartilage oligomeric matrix protein (COMP) is sensitive to physiological cyclic loading in healthy adults. Osteoarthritis Cartilage 2005;13:34–8.

38. Mundermann A, King KB, Smith RL, Andriacchi TP. Change in serum COMP concentration due to ambulatory load is not related to knee OA status. J Orthop Res 2009;27:1408–13.

39. Hong SW, Leu TH, Li JD, Wang TM, Ho WP, Lu TW. Influence of inclination angles on intra- and inter-limb load-sharing during uphill walking. Gait Posture 2014;39:29–34.

40. Muller G, Michel A, Altenburg E. COMP (cartilage oligomeric matrix protein) is synthesized in ligament, tendon, meniscus, and articular cartilage. Connect Tissue Res 1998;39:233–44.

41. Skioldebrand E, Lorenzo P, Zunino L, Rucklidge GJ, Sandgren B, Carlsten J, *et al.* Concentration of collagen, aggrecan and cartilage oligomeric matrix protein (COMP) in synovial fluid from equine middle carpal joints. Equine Vet J 2001;33: 394–402.

42. Helmark IC, Petersen MC, Christensen HE, Kjaer M, Langberg H. Moderate loading of the human osteoarthritic knee joint leads to lowering of intraarticular cartilage oligomeric matrix protein. Rheumatol Int 2012;32:1009–14.