

Original Paper

Nocturnal Changes in Knee Cartilage Thickness in Young Healthy Adults

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Cartilage · Knee joint · Deformation · Magnetic resonance imaging

Abstract

Magnetic resonance imaging (MRI) allows one to analyze cartilage physiology *in vivo*. Cartilage deforms during loading, but little is known about its recovery after deformation. Here we study ‘nocturnal’ changes in knee cartilage thickness and whether postexercise deformation differs between morning and evening. Axial magnetic resonance (MR) images were acquired in the right knees of 17 healthy volunteers (age 23.5 ± 3.0 years) after a normal day, and then after 30 deep knee bends. Coronal images were additionally acquired in 8 of these volunteers after a normal day and then after 2 min of static loading of the leg with 150% body weight. The volunteers then remained unloaded overnight and the same protocol was repeated in the morning. A significant increase ($p < 0.01$) in cartilage thickness was observed between evening (preexercise) and morning (preexercise): +2.4% in the patella, +8.4% in the medial tibia and +6.2% in the lateral tibia. Deformation in the morning (−6.8/−4.6/−5.1%) was generally greater than that in the evening (−5.4/−3.2/−3.7%), but this difference did not reach statistical significance. No significant difference in the nocturnal thickness increase (or postexercise deformation) was observed between men and women. We conclude that knee cartilage

(thickness) recovers overnight by approximately 2–8%, independent of sex. Given the lack of ‘predeformation’ after nocturnal periods of unloading, morning postexercise deformation of the cartilage may have a greater magnitude than evening postexercise deformation.

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Introduction

The metabolism of chondrocytes is known to react to changes in the biomechanical environment [Sah et al., 1989; Urban, 1994; Kim et al., 1995; Wong et al., 1997; Quinn et al., 1998; Buschmann et al., 1999; Ofek et al., 2010] and knowledge on mechanical tissue ‘strains’ occurring ‘*in vivo*’ can thus enhance the understanding of mechanobiological interactions. Such knowledge can also guide mechanical cell stimulation of tissue culture (i.e. bioreactor). *In vitro/ex vivo* studies only provide an

Abbreviations used in this paper

| | |
|-----|----------------------------|
| BMI | body mass index |
| MR | magnetic resonance |
| MRI | magnetic resonance imaging |

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approximation of the in vivo deformation of cartilage since the joint loads occurring in vivo are largely unknown and because joint pressure is unevenly distributed throughout cartilage surfaces.

Magnetic resonance imaging (MRI) permits the non-invasive measurement of deformational behavior in situ and in vivo [Eckstein et al., 2001, 2006b]. Under in situ (ex vivo) conditions, patellar cartilage deformed little during the first minutes of static loading [Herberhold et al., 1999] due to hydrostatic pressurization of the tissue [Ateshian et al., 1994]. Even after several hours of loading, the cartilage continued to deform without reaching equilibrium and recovery from static loading required several hours [Herberhold et al., 1999]. In vivo deformation of the patellar cartilage amounted to 5–6% after deep knee bends [Eckstein et al., 1998], with a period of 90 min being required to recover from deformation [Eckstein et al., 1999]. A ‘dose-dependent’ magnitude of patellar cartilage deformation was observed after various physiological activities that scaled with assumed load severity [Eckstein et al., 2005]. In vivo deformation of femorotibial cartilage, however, has been studied to a lesser extent and the magnitude of deformation observed was smaller than that for patellar cartilage under similar loading, i.e. knee bends, and a somewhat more homogeneous response (across participants) was seen under static loading [Eckstein et al., 2005].

Waterton et al. [2000] showed that no significant diurnal changes occur in healthy femoral cartilage volume, but subtle differences in the regional distribution of femoral cartilage thickness were described between the morning and evening. However, because the participants of the above study walked to the MRI unit in the morning and deformation may have occurred between getting up and the MR image acquisition, this observation does not preclude that recovery (thickness increase) of knee cartilage may occur overnight from mechanical loading during the day. MRI has previously been used to study nocturnal vertebral volume (and fluid flow) in humans [Paa-janen et al., 1994; Roberts et al., 1998; Malko et al., 2002; Hutton et al., 2003], but, to our knowledge, the nocturnal changes of healthy cartilage have not yet been studied. The purpose of this study, therefore, was to employ MRI in vivo in order to explore:

- The magnitude of ‘nocturnal’ changes in knee (patellar and tibial) cartilage thickness, i.e. differences in cartilage thickness after a normal day and physiological unloading overnight
- Whether postexercise deformation of the cartilage is greater in the morning (after a long period of unloading) than in the evening (after a normal day)

- Whether there are sex differences in the nocturnal cartilage thickness changes, or in postexercise deformation between men and women

Methods

The right knees of 17 healthy young subjects were examined. Eight volunteers were female (age [mean \pm SD] 22 ± 2.7 years, body mass index (BMI) 22.23 ± 2.20) and nine male (age [mean \pm SD] 24.3 ± 3.2 years, BMI 21.5 ± 2.1). The volunteers had no history of symptoms, signs, trauma or surgery at the knee joint, had a normal BMI (<25) and were not professional or amateur athletes. Leg dominance and knee alignment were not determined. The study was ratified by the local ethics committee (University of Munich). Written informed consent was obtained from all volunteers prior to the study.

Axial MR images of all participants were obtained at 1.5 T (Magnetom Vision; Siemens, Erlangen, Germany), using a circumferentially polarized knee coil. The idea of also studying the femorotibial joint emerged throughout the study and coronal MR images were additionally obtained in the last 8 volunteers recruited. Of these, 4 were women (age 23.3 ± 1.3 years, BMI 23.8 ± 0.90) and 4 men (age 25.0 ± 3.0 years, BMI 22.5 ± 2.6). Image acquisition was performed using a 3D T_1 -weighted 3D-FLASH sequence with water excitation (repetition time = 17.2 ms, echo time = 6.6 ms, flip angle = 20° , slice thickness = 1.5 mm, in-plane resolution = 0.31×0.31 mm, axial acquisition time = 3 h 47 min, coronal acquisition time = 6 h 19 min [Eckstein et al., 2005]) that has been previously validated for the purpose of cartilage thickness measurement [Graichen et al., 2004]. No fluid-sensitive sequences were acquired to exclude potential bone marrow, meniscus or early cartilage lesions.

The participants were invited to the MRI unit at 8 p.m. after a normal weekday. They had been instructed not to perform strenuous physical activity or sports they were unfamiliar with in the 48 h preceding data acquisition. After arrival, axial (and coronal) baseline data sets of the right knee were obtained. The knee was then repositioned in the scanner and a second axial (and coronal) acquisition was performed. The participants were then asked to perform 30 deep knee bends (up to 120° knee angle) within 90 s outside the MRI scanner; they were then immediately repositioned in the magnet and another axial acquisition was obtained at 90 s after finishing the knee bends. Eight volunteers were additionally asked to then stand on their right leg on a commercial bathroom scale for 120 s, with the knee flexed to 15° [Eckstein et al., 2005]. Weights equivalent to 50% of their body weight were added to their back (i.e. one-legged stance with 150% body weight). The knee was then again positioned in the magnet and the post-loading coronal data acquisition was started 90 s after the static loading exercise. All volunteers then spent the night at the MRI unit and were not allowed to stand up or walk. When the volunteers had to use the toilet, they were taken by wheelchair and were helped not to step on the right leg. At approximately 5 a.m., after a minimum of an 8-hour nocturnal pause, the same protocol was applied again, starting with two baseline scans (with repositioning in between) and one axial (and coronal) postexercise scan.

Segmentation of the patellar (axial images) and medial and lateral tibial cartilages (coronal images) was performed by one

Table 1. Nocturnal changes in knee cartilage thickness (pre- and postexercise)

| | Pre-ex. evening vs. pre-ex. morning | Post-ex. evening vs. pre-ex. evening | Post-ex. morning vs. pre-ex. morning |
|----|-------------------------------------|--------------------------------------|--------------------------------------|
| P | +2.4 ± 2.2%*** | -5.4 ± 2.2%*** | -6.8 ± 2.7%*** |
| MT | +8.4 ± 2.6%*** | -3.2 ± 3.4%* | -4.6 ± 2.6%** |
| LT | +6.2 ± 3.2%** | -3.7 ± 1.6%*** | -5.1 ± 3.5%** |

P = Patella; MT = medial tibia; LT = lateral tibia; ex. = exercise (30 deep knee bends in case of the patella, and stance on one leg for 2 min with 150% body weight in case of the tibia). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

user (K.H.S.), who had received formal training in cartilage segmentation before starting the study. Image analysis was performed using an Octane Duo workstation (Silicon Graphics, Mountain View, Calif., USA), using a semiautomated segmentation algorithm [Stammberger et al., 1999b]. The readings were done independently for each image, i.e. without uploading other images as a reference. The mean cartilage thickness was determined for each cartilage plate using triangulation and Euclidean distance transformation [Stammberger et al., 1999a].

Test-retest precision for independent analysis or repositioned image acquisitions was determined from the preexercise rest-retest scans (in the evening and morning, respectively), by calculating the root mean square coefficient of variation [Glüer et al., 1995]. This was 1.5/1.8% (evening/morning) in the patella, 2.3/1.9% in the medial tibia and 2.3/2.4% in the lateral tibia.

The magnitude of cartilage deformation after exercise was determined as:

$$\frac{[(Th.C_{1preexercise} + Th.C_{2preexercise})/2] - Th.C_{postexercise}}{[(Th.C_{1preexercise} + Th.C_{2preexercise})/2]} \cdot 100 (\%)$$

The statistical significance of differences in cartilage thickness was assessed using a paired t test. Please note that this test was applied in an exploratory context and not for formal hypothesis testing since no previous data existed on which a power calculation could have been based. The statistical significance of potential sex differences in nocturnal changes, or in cartilage deformation after exercise was assessed using a Mann-Whitney U test (patella only).

Results

In all cartilage plates, a significant increase in cartilage thickness between evening (preexercise) and morning (preexercise) was observed. This amounted to +2.4% in the patella, +8.4% in the medial tibia and +6.2% in the lateral tibia (table 1; fig. 1–3).

In the patella, the difference between evening postexercise and morning preexercise measurement was +8.3 ±

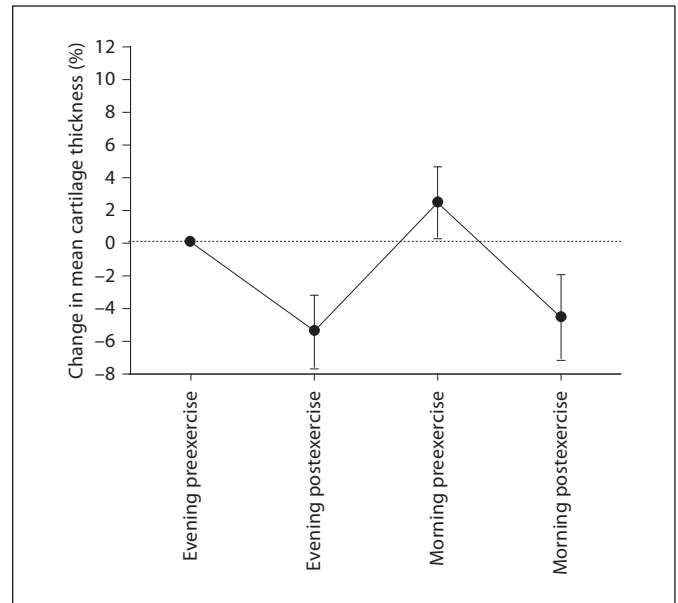


Fig. 1. Patella: changes (% and standard deviation) in mean cartilage thickness after exercise (30 deep knee bends) in the morning and evening, and nocturnal changes in cartilage thickness.

3.5% ($p < 0.001$), and there was a significant decrease in cartilage thickness postexercise, both in the morning and in the evening (table 1; fig. 1). The patellar deformation in the morning (-6.8%) tended to be greater (+28%) than that in the evening (-5.4%), but the difference did not reach statistical significance ($p = 0.15$).

In the medial tibia, the difference between evening postexercise and morning preexercise measurement was +12.0 ± 2.3% ($p < 0.001$). Again, a significant decrease in cartilage thickness was observed after static exercise in the morning and evening. As for the patella, deformation in the morning tended to be greater than that in the evening (+44%; table 1; fig. 2), but the difference did not reach statistical significance ($p = 0.37$).

In the lateral tibia, the difference between evening postexercise and morning preexercise measurement was +10.3 ± 3.9% ($p < 0.001$). A significant decrease in cartilage thickness was observed after exercise in the morning and evening. Again deformation in the morning tended to be greater than that in the evening (+38%; table 1; fig. 3) but the difference did not attain statistical significance ($p = 0.30$).

When stratifying the results between both sexes, the increase in patellar cartilage thickness between evening (preexercise) and morning (preexercise) was similar in men (+2.6 ± 2.2%) and women (+2.1 ± 2.3%), with no

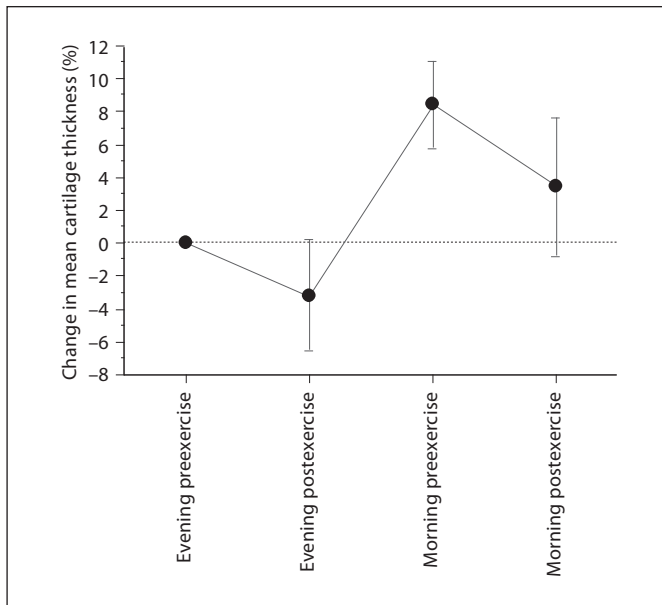


Fig. 2. Medial tibia: changes (% and standard deviation) in mean cartilage thickness after exercise (150% body weight, stance on one leg for 120 s) in the morning and evening, and nocturnal changes in cartilage thickness.

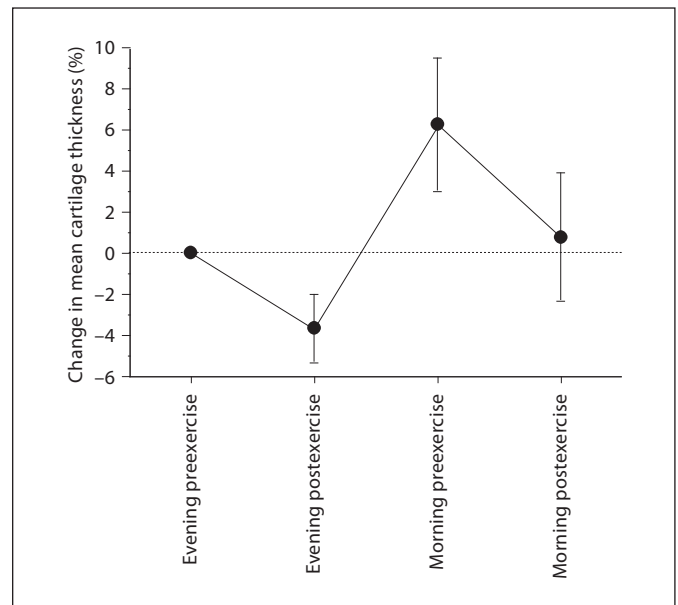


Fig. 3. Lateral tibia: changes (% and standard deviation) in mean cartilage thickness after exercise (150% body weight, stance on one leg for 120 s) in the morning and evening, and nocturnal changes in cartilage thickness.

significant difference in the increase between sexes ($p = 0.56$). Similarly, no relevant sex differences were identified for morning and evening postexercise deformation values (data not shown).

Discussion

This is the first study to explore nocturnal changes in (knee) cartilage thickness *in vivo*. As previous studies have shown that mechanical stimuli can enhance cell metabolism [Sah et al., 1989; Urban, 1994; Kim et al., 1995; Wong et al., 1997; Quinn et al., 1998; Buschmann et al., 1999; Ofek et al., 2010], the current findings may provide some guidance on regimens of mechanically stimulating cells in tissue culture, preparing them for the physiological *in vivo* demands in the target tissue to where they are transplanted. Although one must be careful in extrapolating these results from the healthy knees of young volunteers to osteoarthritic knees of elderly participants of clinical trials in a reciprocal manner (i.e. changes from morning to evening), the data may also provide clues as to whether baseline and follow-up measurements need to be matched for ‘daytime’ in clinical studies exploring cartilage loss (i.e. in osteoarthritis) over time [Eckstein et al., 2006a, 2009].

Limitations of the study include the small number of participants, particularly of the tibial cartilage deformation tests. Nocturnal changes in cartilage thickness (and changes after loading in the morning and evening), however, were statistically significant in all cartilage plates (patella, medial tibia and lateral tibia), showing that the study was sufficiently powered to address these. Although we could not statistically confirm with 95% confidence that morning postexercise deformation was greater than evening postexercise, the observations point in this direction and are physiologically plausible (see below). However, the preliminary observations from this exploratory study should be confirmed in larger cohorts. Further, they should be extended to participants with different degrees of knee osteoarthritis in order to explore to what extent the observations are clinically relevant.

A strength of this study is that postexercise acquisitions were not compared to a single preexercise acquisition, but to the mean of two preexercise acquisitions. Preexercise test-retest precision was evaluated for independent (nonpaired) readings and this compared favorably with the literature [Eckstein et al., 2006a]. This helped to reduce precision errors for the preexercise measurements. Therefore, the deformation measures in this study should be more robust than those based on single preexercise

acquisitions [Eckstein et al., 2005]. The magnitude of patellar cartilage deformation observed in this study was similar to that seen in previous investigations using similar technology [Eckstein et al., 1998, 1999, 2000; Hudelmaier et al., 2001; Eckstein et al. 2005]. The tibial cartilage deformation observed after loading with 150% body weight was greater than that seen with MRI during static loading of one limb with 50% body weight, using an MR-compatible loading device [Cotofana et al., 2011]. However, it was similar to that seen after high-impact loading, and somewhat greater (and more uniform) than that previously reported after a static loading exercise with similar loading magnitudes to here [Eckstein et al., 2005]. This may be due to: (1) the tibial cartilage being measured after a combined exercise of deep knee bends and static loading; (2) a different setup of the static loading experiment incorporating the standing position which requires more balance and muscular cocontraction, and/or (3) smaller precision errors in the current study, due to the averaging of two preexercise acquisitions.

A previous study found no diurnal variation in the overall volume or thickness of femoral cartilage, but identified a thickness decrease in specific locations [Waterton et al., 2000]. Because the femur has relatively large surfaces that are not in contact with the tibia (or patella) during standing, the current study was confined to the tibial cartilage plates, of which relatively larger areas are mechanically loaded during knee bends or static loading. Our current findings extend previous observations [Waterton et al., 2000] in showing that knee cartilages undergo thickening overnight. Knee cartilage thickness in our study was compared after a normal day and directly after unloading overnight, whereas the above authors studied knees in the morning after the participants had walked from home to the MRI unit. The results of our study agree with those on intervertebral disc fibrocartilage that have shown an increase in height and volume of approximately 11% overnight during rest [Paajanen et al., 1994; Roberts et al., 1998; Malko et al., 2002; Hutton et al., 2003].

As cartilage contains 70–90% fluid, the thickening overnight is explained by fluid flow from the joint (or

subchondral bone) to the interstitial matrix, whereas fluid is expelled from the matrix during loading [Mow et al., 1993; Ateshian et al., 1994; Eckstein et al., 1999; Herberhold et al., 1999]. Previous MRI-based studies were able to relate changes in hydration (and other compositional change) to cartilage deformation [Lusse et al., 2000; Mosher et al., 2005; Souza et al., 2010]. With more fluid being present in the matrix after periods of physical rest, there is also potential for a greater magnitude of exercise-related deformation than in situations where the cartilage has just been loaded. Although patellar cartilage was between 2 and 3% thicker in the morning than in the evening, previous studies have shown that simply walking leads to about 3% of patellar cartilage deformation [Eckstein et al., 2005]. Therefore, one may assume that it does not make a relevant difference whether patellar cartilage is imaged in the morning or evening in a longitudinal study, as long as the participants are not imaged after longer periods of unloading or severe exercise. Tibial cartilage, however, showed greater thickening (fluid influx) overnight and smaller deformation than the patella after loading (i.e. exercise). Therefore, the time of day of acquisition may play a stronger role in the assessment of femorotibial than patellar cartilage thickness in longitudinal studies [Eckstein et al., 2008]. This should be assessed by further experimental studies imaging femorotibial cartilage in the morning (after normal activity) and then again in the evening after a normal day.

In conclusion, this study shows that knee cartilage (thickness) recovers overnight by approximately 2–8%, independent of sex. Given the lack of ‘predeformation’ and potentially greater hydration after nocturnal periods of unloading, morning postexercise deformation of the cartilage may have a greater magnitude than evening postexercise deformation.

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