Osteoarthritis and Cartilage 27 (2019) 1851–1859

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Effects of treadmill running and limb immobilization on knee cartilage degeneration and locomotor joint kinematics in rats following knee meniscal transection

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ARTICLE INFO

Article history:
Received 9 February 2019
Accepted 7 August 2019

Keywords:
Osteoarthritis
Knee injury
Rehabilitation
Computed tomography
X-ray motion analysis

SUMMARY

Objective: This study examined the effects of reduced and elevated weight bearing on post-traumatic osteoarthritis (PTOA) development, locomotor joint kinematics, and degree of voluntary activity in rats following medial meniscal transection (MMT).

Design: Twenty-one adult rats were subjected to MMT surgery of the left hindlimb and then assigned to one of three groups: (1) regular (i.e., no intervention), (2) hindlimb immobilization, or (3) treadmill running. Sham surgery was performed in four additional rats. Voluntary wheel run time/distance was measured, and 3D hindlimb kinematics were quantified during treadmill locomotion using biplanar radiography. Rats were euthanized 8 weeks after MMT or sham surgery, and the microstructure of the tibial cartilage and subchondral bone was quantified using contrast enhanced micro-CT.

Results: All three MMT groups showed signs of PTOA (full-thickness lesions and/or increased cartilage volume) compared to the sham group, however the regular and treadmill-running groups had greater osteophyte formation than the immobilization group. For the immobilization group, increased volume was only observed in the anterior region of the cartilage. The treadmill-running group demonstrated a greater knee varus angle at mid-stance than the sham group, while the immobilization group demonstrated greater reduction in voluntary running than all the other groups at 2 weeks post-surgery.

Conclusions: Elevated weight-bearing via treadmill running at a slow/moderate speed did not accelerate PTOA in MMT rats when compared to regular weight-bearing. Reduced weight-bearing via immobilization may attenuate overall PTOA but still resulted in regional cartilage degeneration. Overall, there were minimal differences in hindlimb kinematics and voluntary running between MMT and sham rats.

Introduction

Traumatic knee injuries, such as ligamentous and meniscal tears, increase the risk of osteoarthritis (OA) by at least 10-fold[1]. Symptoms of post-traumatic OA (PTOA) can manifest at 35–50 years of age, which is 10–20 years earlier than those with naturally developing OA[2,3]. Early onset PTOA threatens the long-term health of individuals with knee injuries, many of whom are relatively young and otherwise physically active. Although patients with knee injuries often require prolonged rehabilitation, current rehabilitation mainly focuses on safe and early return to pre-injury activity participation. Little is known regarding whether certain elements of the post-injury rehabilitation (e.g., strengthening, exercises, etc.) can delay PTOA progression.

Mechanical stimuli through proper joint loading are required to maintain the health and function of the chondrocytes in the articular cartilage[4,5]. Alterations in knee loading following joint injuries are implicated as a prevailing mechanism leading to PTOA[1,6–8]. Both reduced and elevated loading at the knee have been observed in patients after knee injuries[7,8]. Such conflicting

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https://doi.org/10.1016/j.joca.2019.08.001
1063-4584/

information makes it difficult for the clinical community to make any strong inferences, yet modifying knee loading (e.g., weight-bearing restrictions, weight-bearing exercises) has been suggested to be a key element during post-injury rehabilitation[9–11]. However, the effect of either increasing or reducing joint weight bearing on PTOA progression has not been fully evaluated.

Before assessing clinical interventions, there is a strong need to investigate the responses of cartilage health and knee function to weight-bearing modulation in a preclinical model. Therefore, the primary objective of this study was to examine the progression of PTOA, and the changes in both locomotor joint kinematics and voluntary activity under contrasting rehabilitative principles of weight bearing (elevated vs reduced) implemented in rats following a surgically-induced meniscal injury. Elevated weight bearing was simulated via daily treadmill running post-surgery, while reduced weight bearing was achieved via immobilization of the operated limb. Given that both reduced loading and elevated loading at the knee have been thought to be associated with PTOA after knee injuries[7,8], we hypothesized that both elevated and reduced weight bearing post-injury would accelerate cartilage degeneration. We further hypothesized that the accelerated cartilage degeneration due to weight-bearing modulation would be accompanied by a greater deviation in locomotor joint kinematics and reduction in voluntary activity.

Methods

Induction of knee degeneration and assignment of weight-bearing conditions

All animal study procedures were conducted in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines. Twenty-one male Lewis rats (weight: 324.3 ± 54.0 g) were subjected to a medial meniscal transection (MMT) surgery of the left hindlimb according to published procedures[12,13]. Briefly, in anesthetized animals, an incision was made through the skin overlying the knee and the medial collateral ligament (MCL) was exposed by blunt dissection. The MCL was then transected to reflect the meniscus toward the femur. The meniscus was cut through the full thickness at its narrowest point to simulate a complete tear[12,13]. Four weight- and age-matched male Lewis rats (weight: 322.5 ± 81.8 g) were subjected to a sham surgery where the MCL was transected, but the meniscus was left intact. Following surgery, rats were given 1 week of recovery before any further research activities/interventions. All rats were followed up for a total of 8 weeks post-surgery. Our prior research has shown that initial features of PTOA develop by 3 weeks post-MMT with further progression at 6 weeks post-MMT[13,14]. It was thus anticipated that a longer follow-up time (i.e., 8 weeks) might result in greater differences among varying weight-bearing conditions. The use of an 8-week follow up is also consistent with a previous study examining the effects of treadmill running on PTOA post-MMT[15].

All rats were exposed to treadmill running for 1 week before surgery to estimate which rats might comply with the treadmill running program post-surgery. After surgery, the MMT rats were assigned to one of the following three distinct loading/weight-bearing conditions: (1) regular weight-bearing (i.e., MMT group; $N = 7$); (2) reduced weight-bearing via left hindlimb immobilization (i.e., MMT + IMB group; $N = 8$); and (3) elevated weight-bearing via treadmill exercise (i.e., MMT + EXER group; $N = 6$). Rats that demonstrated high compliance with the treadmill running before surgery were selected to be assigned to the MMT + EXER group. The remaining rats (i.e., those that did not demonstrate high compliance combined with those showing high compliance but were not selected for the MMT + EXER group) were then randomly assigned to either the regular MMT, MMT + IMB, or sham group.

For the MMT + IMB group, immediately after surgery, the operated left hindlimbs were kept in full knee extension and ankle plantar flexion using bandages to reduce the rats' ability to bear weight with their operated limbs (see Appendix). For the MMT + EXER group, rats were given 1 week of recovery (to avoid damage to the surgical wound) before they were prescribed to run on a treadmill at a speed of 12 m/min, 30 min/day, 4 days/week, for a total of 7 weeks (from the 2nd to 8th week post-MMT)[15,17]. The regular MMT group and the sham group received no intervention and were housed the same way as before surgery. All rats were allowed free access to standard food and water inside the cages.

The primary objective of the study was to determine the effect of varying weight-bearing conditions on PTOA. A priori power analysis based on previous cartilage data in MMT rats[18] indicated that a sample size of 8 rats/group would provide 90% power ($\alpha = 0.05$) to detect a 10% difference in osteophyte formation (i.e., a difference of 0.108 mm^3 with a SD of 0.061 mm^3) among the 3 weight-bearing groups. Given that the effect of MMT on accelerating PTOA has been well-established, to minimize the use of animals, only 5 sham rats were originally included. However, 1 sham rat, 1 rat in the regular MMT group, and 1 rat in the MMT + EXER group were found deceased after the completion of the surgery (unknown reasons). One rat in the MMT + EXER group was found deceased around the end of the 6th week post-surgery (unknown reason; enlarged internal organs were found during necropsy). Thus, data from 4 sham rats as well as 6, 7, and 8 rats from the MMT + EXER, MMT, and MMT + IMB groups, respectively, were available for data analyses.

Assessment of the medial tibial cartilage

At the end of the 8th week post-surgery, rats were euthanized and their hindlimbs were dissected and harvested. The entire hindlimb was then scanned using computed tomography (CT) to establish a hindlimb skeletal model for each rat (to create a CT-based skeletal model for hindlimb kinematics analyses described below). The 3D microstructural and compositional changes in the tibial cartilage of the operated hindlimbs were quantitatively evaluated by re-scanning the limbs using contrast enhanced micro-CT (μCT)[14,18,19]. The proximal end of the tibia with the contrast agent (i.e., Hexabrix 320) was scanned using a microcomputed tomography scanner μCT 40 (Scanco Medical, Brüttisellen, Switzerland) at 45 kVp, 200 μA, 350 ms integration time, 1024 × 1024 pixel matrix, 500 projections, and a voxel size of 14.8 μm[320]. Following scanning, tibiae, joints, and femurs were decalcified in Cal-Ex II (Fisher Scientific, Waltham, MA) for 14 days. Dehydrated samples were routinely paraffin embedded. Coronal sections were cut at 5 μm thickness. Sections were stained with Safranin-O with a Fast Green counter stain for histology.

Since the MMT surgery involved transection of the medial meniscus, the medial tibial plateau becomes more susceptible to the degenerative processes and thus were evaluated in all groups. The Scanco evaluation software was used to assess 3D morphology and composition using previously established protocols[19,20]. Briefly, reconstructed 2D grayscale tomograms were manually contoured along the medial tibial plateau to delineate the cartilage from the subchondral bone and surrounding air[19]. The cartilage was segmented with a fixed lower threshold of 120 mgHA/ccm to separate cartilage from air, and a higher threshold of 340 mgHA/ccm to separate cartilage from bone. 3D images and cartilage thickness heatmaps were then generated.

By quantifying the morphology of the medial tibial plateau, a greater degree of PTOA was defined as (1) increased volume of

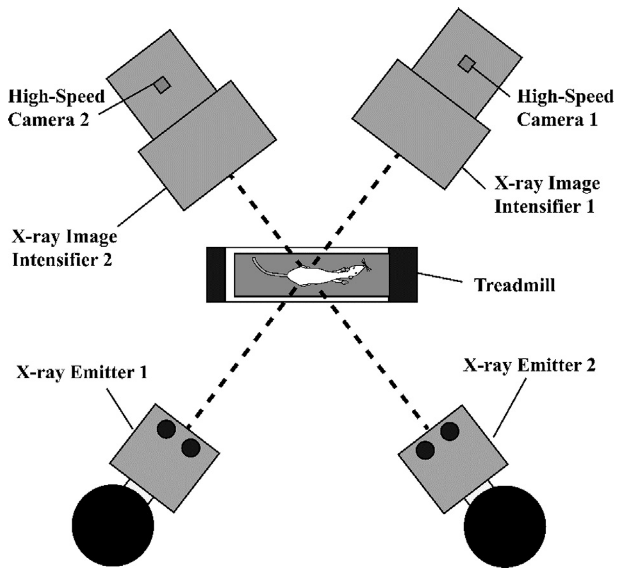

Fig. 1. Setup of the biplanar high-speed video-radiography system to record rats' hindlimb kinematics during treadmill locomotion.

remaining cartilage, (2) increased area of exposed bone (due to full-thickness cartilage lesions that extended to the subchondral bone surface), (3) decreased subchondral bone fraction (due to increased porosity), and/or (4) increased osteophyte formation. Increased cartilage volume has been observed during the degenerative process of articular cartilage[21–24].

Briefly, to quantify cartilage volume and exposed bone area, manual contouring was performed to outline the cartilage and to isolate the cartilage surface from the subchondral bone and surrounding air. The volume of the tibial cartilage was quantified in three sub-regions: the anterior, central, and posterior third of the medial tibial plateau. Exposed bone areas were calculated from vertical projections of thickness maps generated from the contoured cartilage. Projections were imported into ImageJ and the area of exposed bone (any pixel corresponding to 0.0 mm of cartilage height) was measured by manually segmenting the images in Image J and measuring with the area tool.

Contouring and global thresholds for bone were used to segment the entire subchondral bone volume from the tibial cartilage and underlying trabecular bone. Evaluations were run using a threshold of 340 mgHA/ccm to segment the bone and calculations for total subchondral bone volume were generated. Subchondral bone fraction (i.e., ratio of the subchondral bone volume to the total volume) was calculated, and a greater subchondral bone fraction value represents less porosity in the subchondral bone region. Analyses of osteophytes were performed using 2D grayscale tomograms that were rotated to coronal sections. The medial marginal area was then contoured for osteophyte growth. The marginal osteophyte was segmented using two different ranges of fixed threshold values: (1) 120–1000 mgHA/ccm to include both mineralized matrix and cartilage, and (2) 400–1000 mgHA/ccm to include only the mineralized matrix.

Evaluations of voluntary wheel running and joint kinematics during locomotion

The rats' voluntary activity levels were assessed by placing each rat inside a cage mounted with a running wheel (diameter: 14") for 7 consecutive days 1 week before surgery and at 2, 5, and 8 weeks post-surgery. A cycling computer with magnetic sensors (BC8.12, Sigma Sport, St. Charles, IL) was attached to the wheel and the cage to record the daily voluntary running distance and time. To allow enough time for acclimation to the running wheel, only the daily data from days 3–7 (i.e., data from a total of 5 consecutive days) were averaged and used for analysis.

The joint kinematics of each rat's affected hindlimb during treadmill locomotion were evaluated at 8 weeks post-surgery using a high-speed X-ray Motion Analysis (XMA) system consisting of custom biplanar high-speed video-radiography (Imaging System & Service, Painesville, OH) with integrated high-speed digital video cameras (Fig. 1). The use of X-ray technology can overcome the errors in joint kinematics quantification associated with tracking

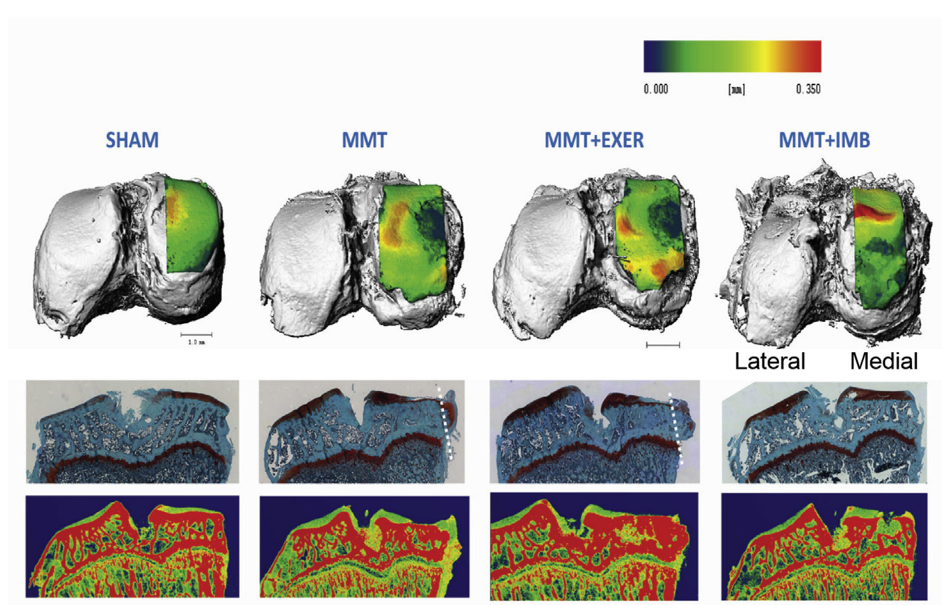

Fig. 2. Representative histology (middle row) and EPIC-μCT (bottom row) coronal sections of the tibial plateau in the sham and 3 MMT groups. Smooth green surface represents the integrity of the tibial cartilage. Cartilage lesions were observed in the 3 MMT rats. Cartilaginous and mineralized osteophytes were observed in the MMT and MMT + EXER rats, respectively.

skin markers by direct visualization of bone movements[25]. Specifically, a rat treadmill (Columbus Instruments, Columbus, OH) was positioned such that the locomoting rat was at the center of the intersection of the two X-ray beams creating a 3D motion capture volume. X-Ray images were taken (100 Hz) as the rat exercised on the treadmill at a speed of 30 m/min. This speed was selected based on previous experiments of ensuring a steady state (constant speed) treadmill locomotion in rats[25,26]. An electric grid/coil was attached to the back of the running lane to facilitate running. Five gait cycles of steady locomotion from each rat were identified for analysis. Kinematics data were assessed at three key time points of the stance phase during a gait cycle: (1) paw initial contact with the ground, (2) mid-stance (i.e., the midpoint of the entire stance time/phase), and (3) when the paw came off the ground (i.e., the end of the stance).

The raw X-ray images were first corrected for distortion[27]. The CT-based, 3-D hindlimb skeletal model (obtained from each rat at the end of study) was registered to the corrected biplanar X-ray images using a previously described markerless rotoscoping method (Autoscoper, Brown University, Providence, RI)[28,29]. The resultant tracking data were then used to determine the 3D kinematics of the hip, knee, and ankle joints of the hindlimbs based on the anatomical landmarks and coordinate systems described previously[30,31]. The angles of the knee and the ankle joints were defined as the rotations of the distal bone/segment relative to the proximal bone/segment[27]. Given our inability to generate a CT bone model for the pelvis, hip angles were defined as the angle of the femur relative to a global coordinate system aligned with the treadmill belt.

Prolonged immobilization resulted in joint contracture of the immobilized hindlimb, thereby significantly affecting gait of the rats in the MMT + IMB group. These rats adopted a ‘hopping’ gait even after immobilization was removed (bandage was only removed for the X-ray motion analysis that lasted approximately 5 min). We did not quantify these significant gait deviations because the rats with hindlimb immobilization could not establish a steady state locomotion with multiple gait cycles that are required for analysis and comparison to the other groups. Thus, we only included kinematics data from the sham, MMT, and MMT + EXER groups for subsequent statistical analyses.

Statistical analyses

Variables of interest included the cartilage/bone parameters quantified using μCT (i.e., exposed bone area, remaining cartilage volume, osteophyte volume, subchondral bone volume and fraction), hindlimb joint kinematics, and % change in voluntary daily wheel run time and distance post-MMT. For each variable of interest, the differences among the four groups were examined using a one-way ANOVA with Tukey's HSD test for post-hoc pair-wise comparisons (all significance levels set at $P < 0.05$).

Results

Tibia cartilage microstructure

Representative cartilage histology and maps compared the cartilage and bone structure in the sham and three MMT groups

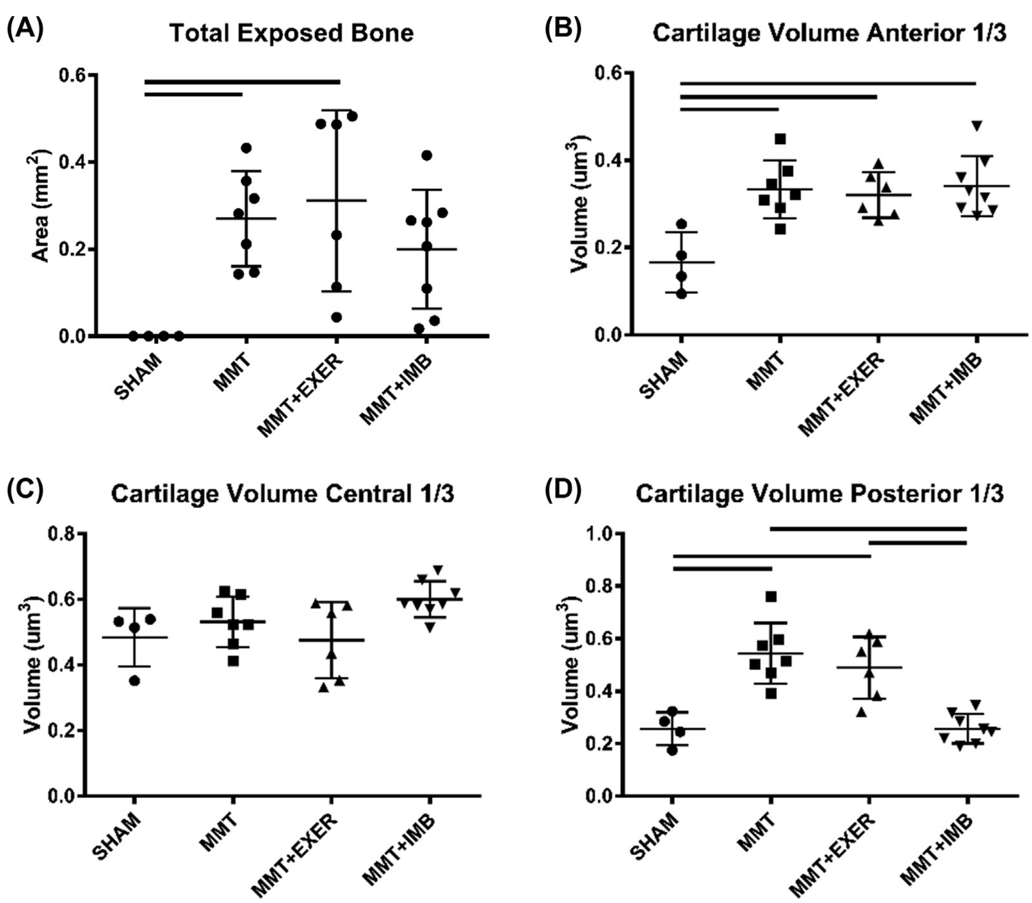

Fig. 3. Area of exposed bone due to cartilage defect (A) and the volumes of the anterior (B), central (C), and posterior (D) one third of the remaining medial tibial cartilage in the sham and the three MMT groups. The horizontal bar denotes a significant difference between the 2 groups connected by the bar.

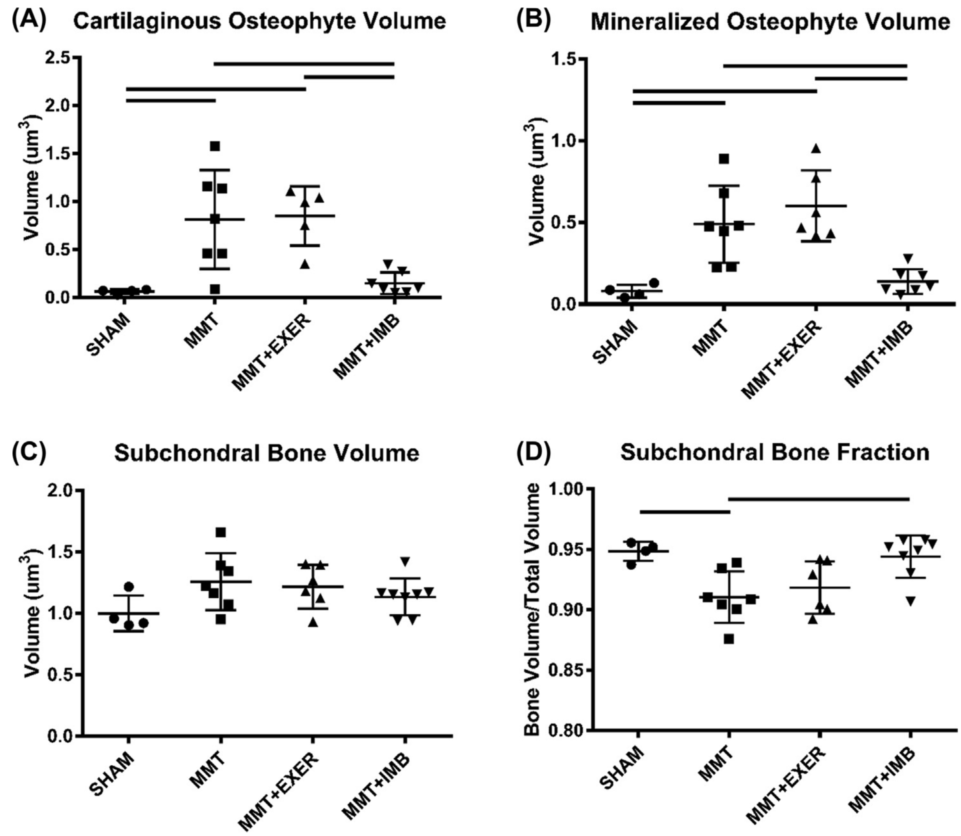

Fig. 4. Cartilaginous osteophyte volume (A), mineralized osteophyte volume (B), subchondral bone volume (C), and subchondral bone fraction (D) of the medial tibial plateau in the sham and the three MMT groups. The horizontal bar denotes a significant difference between the 2 groups connected by the bar.

(Fig. 2). Exposed bone, due to full-thickness cartilage lesions, was seen in all three MMT groups. Minimal to no lesions were observed in the sham group. The exposed bone area in the MMT and MMT + EXER groups was significantly greater than that in the sham group [Fig. 3(A)]. When examining the remaining cartilage, all three MMT groups had a greater volume in the anterior third of the tibial cartilage compared to the sham group [Fig. 3(B)], suggesting an ongoing degenerative process of the remaining cartilage. The MMT and MMT + EXER groups also had a greater volume in the posterior third of the remaining tibial cartilage than the sham and MMT + IMB groups [Fig. 3(D)].

The osteophyte volumes, both cartilaginous and mineralized, in the MMT and MMT + EXER groups were significantly greater than those in the sham and MMT + IMB groups [Fig. 4(A) and (B)]. There was no difference in the total subchondral bone volume [Fig. 4(C)], but the MMT group had a significant lower subchondral bone fraction (i.e., greater porosity in the bone within a given volume) than the sham and MMT + IMB groups [Fig. 4(D)].

Joint kinematics during locomotion and voluntary wheel running

At 8 weeks post-surgery, the MMT + EXER group showed significantly greater knee varus angles at mid-stance compared to the sham group [Fig. 5(E)]. No statistically significant differences were observed in the other kinematics parameters. As expected, immobilization of the operated hindlimb resulted in a greater reduction in the voluntary daily wheel run time and distance in the MMT + IMB group when compared to the other three groups (Fig. 6). However, only the differences observed at 2 weeks post-MMT reached statistical significance. There was no statistically significant difference among the sham, MMT, and MMT + EXER groups (Fig. 6).

Discussion

The primary objective of this study was to examine the effects of contrasting weight-bearing conditions (i.e., elevated vs reduced) on PTOA progression, locomotor joint kinematics and voluntary activity in rats following a surgery-induced meniscal injury. The present study systematically investigate the effects of both reduced (via hindlimb immobilization) and elevated (via treadmill running) weight bearing on cartilage degeneration in rats following traumatic knee injuries. Exposed bone (due to full-thickness cartilage lesions) and increased cartilage volume were observed in all three MMT groups, indicative of PTOA development compared to the sham group. The observed increase in cartilage volume may be a result of cartilage swelling and/or increased cellularity and hypertrophic chondrocytes that have been reported during early osteoarthritis[21–24]. However, our data did not fully support our hypothesis that elevated or reduced weight bearing following knee injury would accelerate cartilage degeneration and lead to greater deviations in locomotor joint kinematics and reduction in voluntary activity. Based on the variables used to evaluate PTOA progression (i.e., exposed bone area, volume of the remaining cartilage, osteophyte formation, and subchondral bone characteristics), the reduced weight-bearing group via hindlimb immobilization demonstrated less overall cartilage degeneration than the regular and elevated weight-bearing groups.

Reduced knee joint loading has been observed in patients with knee ligamentous injuries and has been associated with early onset of PTOA[11,32,33]. The reduced joint loading observed in patients

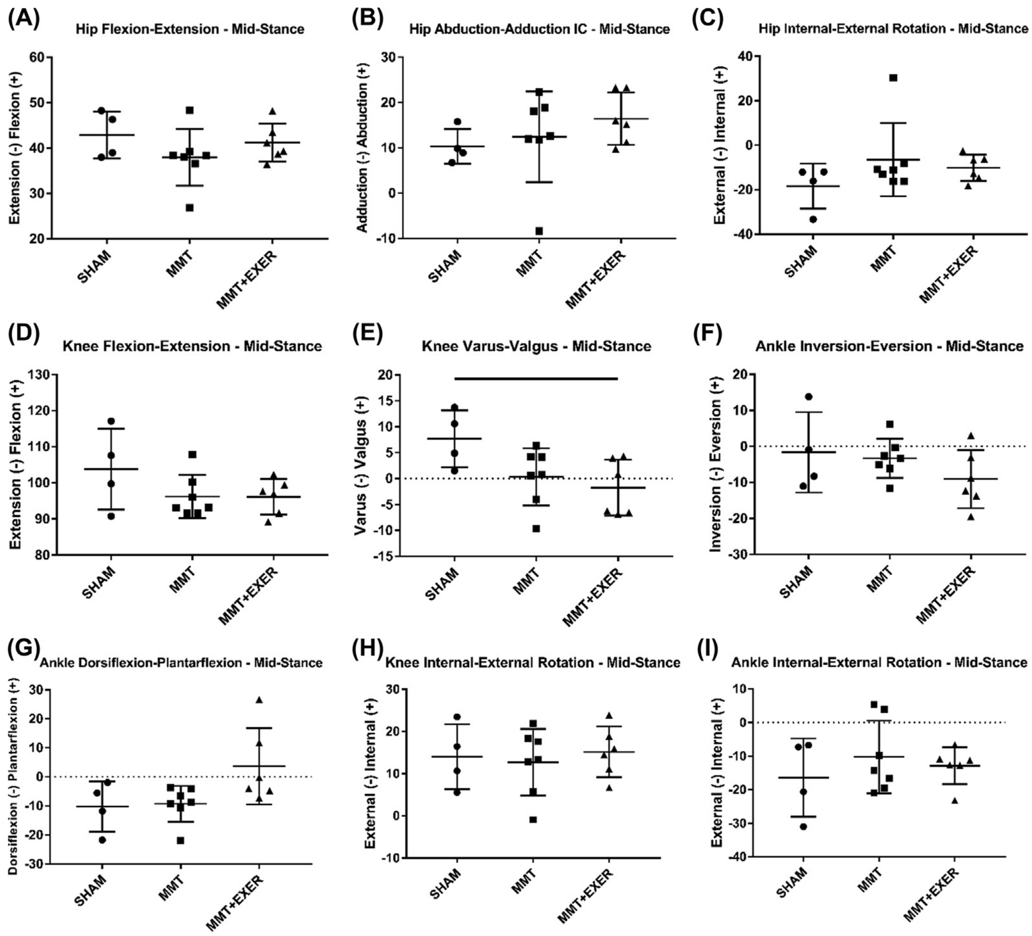

Fig. 5. The 3D joint angles of the hip (A–C), knee (D–F), and ankle (G–I) joints of the surgical hindlimbs in the sham, MMT, and MMT + EXER groups at the mid-stance during treadmill locomotion. The horizontal bar denotes a significant difference between the 2 groups connected by the bar.

during prolonged rehabilitation is thought to decondition the cartilage and thus reduce the cartilage's ability to sustain loads after the patients return to the pre-injury activities[11]. The rats in the reduced weight-bearing group were immobilized for the entire 8-week follow up and were not re-exposed to physiological weight bearing. Our findings, thus, suggest that while signs of joint degeneration were also observed in rats with reduced weight bearing via immobilization[34], reduced weight bearing alone would not accelerate PTOA progression in rats following a traumatic injury to the knee when compared to regular weight bearing post-injury.

Similar to the findings reported in previous studies[34,35], the MMT + IMB group showed a dramatically altered region-specific pattern of cartilage degradation; elevated cartilage volume was only observed in the anterior third of the tibial plateau. The rats in the MMT + IMB group were immobilized with the knee kept in full extension, potentially localizing/elevating the stress at the anterior portion of the medial tibial cartilage. Future experiments involving other approaches that reduce or eliminate weight bearing (e.g., hindlimb suspension) but allow free joint movements will help determine whether the observed region-specific cartilage degeneration was a result of this specific joint immobilization procedure post-MMT.

As we have reported previously[36], there tends to be thickening and sclerosis of the subchondral bone in MMT rats' tibia that is accompanied by increased porosity (or decreased bone fraction). In this study, we observed a similar significant increase in porosity in the regular MMT group compared to the sham and MMT + IMB groups, which may have been caused by increased remodeling activity both by osteoblasts and osteoclasts. The MMT + EXER group showed a tendency towards increased porosity but this change was not statistically significant; this study was likely underpowered for this parameter and is a limitation of our study.

Previous studies involving rodent OA models have largely focused on the evaluation of cartilage/joint health, and limited information is currently available regarding the associated behavioral changes. While a few studies have evaluated spatial-temporal gait parameters in arthritic rats[37–40], little is known about the specific changes in joint kinematics during locomotion[40]. Using 2D radiography analysis, Boettger *et al.* reported reduced knee sagittal-plane flexion range of motion in rats with antigen-induced OA[41]. Similarly, a decrease in knee flexion (i.e., stiffening knee pattern) is commonly observed in patients with knee injuries[42]. In our model, we did not observe a significant change in knee flexion; this discrepancy between our findings and the reduced knee flexion by Boettger *et al.*[41] may be due to the difference in methods used to induce arthritis (MMT-induced vs antigen-induced), methodology of kinematics calculations (3D vs 2D), and/or the time of gait analysis post-injury (8 weeks vs 21 days). In the present study, we also documented deviations in 3D joint kinematics in rats with PTOA showing increased knee varus angle at mid-stance in the MMT + EXER group when compared to the sham group; this observation is consistent with clinical observations in patients with naturally developing OA[43–45].

A general limitation of our study may have been the low statistical power to detect kinematic differences, particularly given the

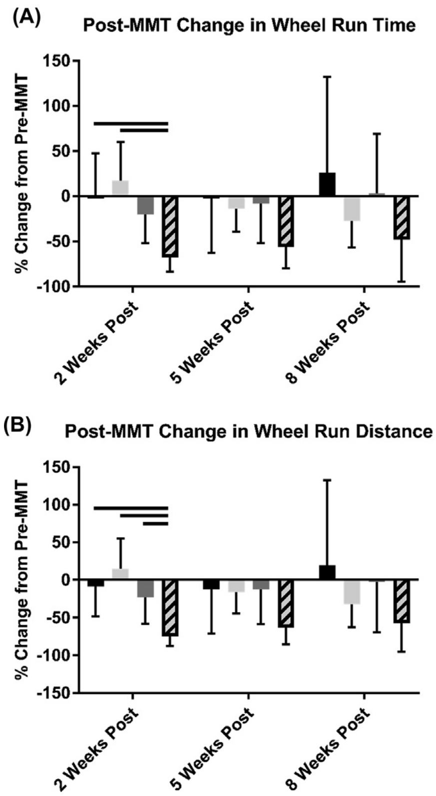

Fig. 6. Changes (i.e., % difference of pre-MMT) in the voluntary daily wheel run time (A) and distance (B) in the sham and the three MMT groups at 2, 5, and 8 weeks post-MMT. A negative % value represents a decrease in voluntary running when compared to pre-MMT running. The horizontal bar denotes a significant difference between the 2 groups connected by the bar.

large variation in joint kinematics. Previous studies examining the changes in spatial-temporal gait parameters in OA rats involved sample sizes ranging from 4 to 8 rats/group[37–39]. Using rats with antigen-induced OA, Boettger *et al.* detected a significant difference in knee flexion between two treatment groups with a sample size of 10 rats/group[41]. While joint kinematics in OA rats are limited in the literature, based on our data and post-hoc power analyses, we estimated that a sample size of 12 and 18 rats/group would be required to achieve a power greater than 80% for detecting a significant group effect on the knee varus and flexion angles, respectively, at 8 weeks post-surgery.

The immobilization in MMT rats also provide interesting insights into the relation between kinematics and PTOA progression. In the present study, a physically imposed kinematic constraint to the operated hindlimbs appeared to lead to a dramatically different degeneration pattern than all the other MMT rats. Yet, given our cross-sectional experimental design, causal relationship cannot be established between the observed deviations in knee joint kinematics and PTOA progression in rats post-MMT. Longitudinal studies, particularly with the data obtained before surgery/injury, are needed to further understand whether altered joint kinematics may facilitate the development of knee osteoarthritis in rats post-MMT[46].

Voluntary wheel running is often used as a functional outcome for rodent injury/disease models, including arthritis. Decreased wheel running in the MMT + IMB group was expected due to the inability to use the immobilized hindlimb. However, the observed significant differences in cartilage microstructures between the sham and the regular (i.e., MMT) or elevated weight-bearing group (i.e., MMT + EXER) were not accompanied by any statistically significant difference in voluntary wheel running. In acute, chemically-induced rodent arthritis models, an immediate reduction followed by quick recovery within 3–5 days in wheel running was observed[47,48]. The MMT model is typically a slower developing and less destructive OA model that is associated with less pain compared to the chemically-induced OA. It is thus possible that the use of wheel running might not be sensitive enough to reflect the effect of PTOA on overall limb function in MMT rats.

We did not observe any statistically significant differences in cartilage microstructures between the MMT and MMT + EXER groups. Thus, the addition of treadmill running as early as 1 week after surgery did not seem to have any further negative effects on cartilage degeneration in MMT rats. In recent studies, Iijima and colleagues implemented a similar treadmill running protocol (i.e., 12 m/min, 30 min/day, 5 days/week) for the first 4 weeks post-MMT (cartilage evaluated at the end of the 4th week post-MMT)[17] and from the 5th to 8th week post-MMT (cartilage evaluated at the end of the 8th week post-MMT)[15]. They observed less degenerative changes when the treadmill running protocol was implemented[15,17]. However, they also observed accelerated PTOA when MMT rats ran at a faster speed (i.e., 21 m/min) for the same daily and weekly running duration[15]. In addition to our implementation of a longer treadmill running intervention (i.e., 7 weeks vs 4 weeks), the post-traumatic response of the knee cartilage to treadmill running is likely dose dependent and potentially animal specific.

Another confounding variable and potential limitation in our study may be the use of voluntary wheel running. The free access to wheel running at 2, 5, and 8 weeks post-MMT altered the total amount of running compared to the treadmill intervention alone in the MMT + EXER group. On average, rats in the MMT and MMT + EXER groups ran 1300–3300 m/day when given access to the running wheel. The treadmill running regimen was only 360 m/day (only 4 days/week), substantially less than the wheel running distance achieved by MMT rats. Thus, when compared to the much greater voluntary wheel running (4–9 times greater than treadmill running), the additional treadmill running used in the present study may not be enough to result in distinguishable PTOA progression between the regular MMT and MMT + EXER groups. The rationale of selecting proper treadmill running parameters/protocols for rodent OA models are currently lacking. More standardized/quantifiable approaches (e.g., percent increase/decrease based on pre-surgery activity levels) are needed to better understand the effect of weight-bearing activities on PTOA progression.

In conclusion, our implementation of elevating weight bearing via treadmill running at a slow/moderate speed did not accelerate PTOA in MMT rats. Reduced weight bearing via immobilization may delay PTOA, but kinematic joint constraint due to immobilization potentially led to a different joint loading regime within and thus resulted in regional cartilage degeneration. The presence of PTOA in MMT rats was not accompanied by significant changes in voluntary running post-MMT, and no differences in hindlimb joint kinematics between the regular MMT group and sham group were identified. Future studies involving varying degrees of weight-bearing conditions and longitudinal gait analysis will better our understanding in the influence of altered weight bearing and/or joint kinematics on PTOA progression in rats post-MMT. Similar to the majority of previous studies implementing the MMT model[13–15,17,39], only male rats were used in the present study. Future studies including both male and female rats will help determine whether the influence of weight-bearing conditions on PTOA is sex dependent.

Author contributions
Conception and design: LCT, GLW, YHC, and NJW.
Collection and assembly of data: LCT, ESC, KMH, GLW, YHC, and NJW.
Analysis and interpretation of data: LCT, ESC, KMH, YHC, and NJW.
Drafting of the article: LCT.
Critical revision of the article for important intellectual content: All authors.
Obtaining of funding: LCT, YHC, and NJW.
All authors take responsibility for the integrity of the data analysis.

Conflict of interests
All authors have no conflict of interests to declare.

Funding sources
This study was supported by the Alliance for Regenerative Rehabilitation Research and Training (AR^{3}T) pilot grant and Department of Veterans Affairs (1 I21 RX002372-01A1). The funding sources were not involved in the design, writing, and/or publication of the present study.

Acknowledgments

The authors acknowledge the efforts of Dr. Caroline R. Waters and Dr. Katelyn N. Corbin for their assistance with data acquisition.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.joca.2019.07.016.

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