Chronic in vivo load alteration induces degenerative changes in the rat tibiofemoral joint

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SUMMARY

Objective: We investigated the relationship between the magnitude and duration of sustained compressive load alteration and the development of degenerative changes in the rat tibiofemoral joint.

Methods: A varus loading device was attached to the left hind limb of mature rats to apply increased compression to the medial compartment and decreased compression to the lateral compartment of the tibiofemoral joint of either 0% or 100% body weight for 0, 6 or 20 weeks. Compartment-specific assessment of the tibial plateaus included biomechanical measures (articular cartilage aggregate modulus, permeability and Poisson’s ratio, and subchondral bone modulus) and histological assessments (articular cartilage, calcified cartilage, and subchondral bone thicknesses, degenerative scoring parameters, and articular cartilage cellularity).

Results: Increased compression in the medial compartment produced significant degenerative changes consistent with the development of osteoarthritis (OA) including a progressive decrease in cartilage aggregate modulus (43% and 77% at 6 and 20 weeks), diminished cellularity (38% and 51% at 6 and 20 weeks), and increased histological degeneration. At 20 weeks, medial compartment articular cartilage thickness decreased 30% while subchondral bone thickness increased 32% and subchondral bone modulus increased 99%. Decreased compression in the lateral compartment increased calcified cartilage thickness, diminished region-specific subchondral bone thickness and revealed trends for reduced cellularity and decreased articular cartilage thickness at 20 weeks.

Conclusions: Altered chronic joint loading produced degenerative changes consistent with those observed clinically with the development of OA and may replicate the slow development of non-traumatic OA in which mechanical loads play a primary etiological role.

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Introduction

Requisite mechanical loading is essential for the maintenance of healthy articular cartilage while aberrant loading is implicated in the development of degenerative changes1. Multiple risk factors for the initiation and progression of osteoarthritis (OA) of the knee affect the mechanical environment of the joint including alignment, occupational and sporting activities, body weight (BW), and injury2. However, the thresholds of chronic load alteration that initiate changes in the structural and material properties of joint tissues have yet to be clearly identified. Furthermore, the mechanisms by which cartilage and subchondral bone respond to non-traumatic levels of chronic load alteration and contribute to the initiation and progression of OA remain unknown.

Commonly used animal models of OA initiate degenerative changes via transection of ligaments or the meniscus resulting in progressive degenerative changes over several weeks3,4. In these models, the amount of load alteration in the joint is typically unquantified and uncontrolled. Animal models that transect internal joint structures with joint capsule disruption may better replicate the development of OA secondary to acute injury (i.e., anterior cruciate ligament injury or meniscal tears)5 rather than the slower development of non-traumatic OA in which altered mechanical loading plays a primary etiological role. Research assessing the differences between the development of primary and...
secondary OA suggests distinct disease subsets in humans\textsuperscript{6,7} and animal models\textsuperscript{8,9}.

In previous work, we have developed a varus loading device (VLD) and applied it to small animals to isolate and study the effects of in vivo chronic load alteration on the tibiofemoral joint. Chronic increased load of 44% BW applied to the rabbit knee for 12 h/day over 12 weeks resulted in increased articular cartilage thickness and permeability with minimal fibrillation of the articular surfaces\textsuperscript{10}. While increased load magnitudes (50% and 80% BW) and durations (12 and 24 weeks; exposure: 12 h/day) in the medial compartment of the rabbit knee produced early degenerative changes including fibrillation, chondrocyte hypertrophy, and decreased cellularity\textsuperscript{11}. When altered loading was applied to the rat knee (80% BW; 12 h/day; 12 weeks), load-induced changes in tissue thickness were most prominent in the lateral compartment which experienced decreased loading\textsuperscript{12} without significant alteration of vertical ground reaction force\textsuperscript{13}. These results indicate that the response to chronic load is magnitude, duration, and species dependent. It remains to be determined if early load-induced changes progress to joint degeneration with increased duration of loading or accelerate with increased daily exposure to load alteration. Furthermore, debate remains regarding the early temporal response of joint tissues during the onset of joint degeneration. This served as the motivation for this study which investigates the relationship between chronic load alteration and the development of degenerative changes to the tibiofemoral joint.

Our primary hypothesis was that increased compressive loading in the medial compartment would initiate degenerative changes analogous to OA in the joint (as quantified by histological measures and cartilage material properties) that would increase with increasing load duration (0, 6, 20 weeks). A secondary hypothesis was that decreased compressive loading in the lateral compartment would result in diminished material properties, but less severe structural changes.

Methods

Animal model

Twenty-five, 9-month-old, male, Sprague–Dawley rats (weight: 666 ± 32 g) were randomly assigned to one of five groups: 0% BW 0 week (baseline; n = 5), 0% BW 6 week (n = 5), 0% BW 20 week (n = 4), 100% BW 6 week (n = 5), 100% BW 20 week (n = 6). Rats were housed in single cages (19(w) × 32(l) × 19(h) cm), fed chow (Prolab RMH 3000, Purina) and water ad libitum, and maintained on a 12:12 h light:dark cycle. Procedures were carried out in accordance with the Institutional Animal Care and Use Committee. All animals underwent surgery to attach transcutaneous bone plates to the lateral aspect of the left tibia and maintained on a 12:12 h light: dark cycle. Procedures were performed for each rat. Sections were deparaffinized and stained with Safranin-O and Fast Green (SOFG) or Hematoxylin and Eosin (H&E) prior to examination under a light microscope (BX50, Olympus Inc.) fit with a digital camera (RET-2000R-F-CLR-12-C,
QImaging) for acquisition of digital images (1,200 × 1,600 pixels). Custom written MatLab code was used to facilitate histological measurements. Region-specific outcome measures were calculated by dividing each compartment into three equally spaced regions (peripheral, central and midline).

**Thickness measures**
Articular cartilage, calcified cartilage and subchondral bone thicknesses were measured across each compartment. The articular surface, tidemark, calcified cartilage-subchondral bone junction, and inferior boundary of the subchondral bone plate were identified on digital images of SOFG slides.

**Degenerative scoring**
Cartilage degeneration was evaluated on three SOFG stained sections utilizing the Osteoarthritis Research Society International recommendations for the rat, which include parameters of cartilage matrix loss width (MLW), area of cartilage degeneration, total and significant cartilage degeneration widths, zonal depth ratio of lesions, and osteophytes as described in Supplemental Table S1.

**Cellularity**
Chondrocytes with visible nuclei were identified on digital images of H&E stained sections collected using a 10× objective and counted for each region of each compartment. Cellularity was determined as the number of chondrocytes/articular cartilage area.

**Statistical analyses**
For each outcome measure, analyses of variance were used to test for differences between experimental conditions. Because the factorial design was not complete (i.e., no 100% load, 0 week duration condition), one-way analyses of variance were performed in conjunction with linear contrasts. The homogeneity of variance and normal assumptions associated with the analyses of variance were examined using Levene’s tests and normal probability plots of residuals respectively. Contrasts were constructed to test for main effects of load (0% BW vs 100% BW), duration (6 vs 20 weeks) and their interaction. To control type I error experiment wise, Fisher’s protected least significant difference (LSD) was used to perform pairwise comparison only if significant main effects of load and/or duration, or a significant interaction was detected (P < 0.05). Significance was based on statistical significance at P < 0.05. All statistical analyses were performed using Statistical Analysis System (SAS) statistical software (SAS Institute, Cary, NC). Means and summary statistics are presented in the results section with dot plots of data included in supplemental Figs. S7–S12.

**Results**
Altered compressive loading of the tibiofemoral joint led to extensive changes in outcome measures that were compartment, load duration, and region specific. Fibrillation of the medial compartment of the tibia plateau was observed in the 100% BW loaded groups at 6 and 20 weeks as indicated by India ink staining, while no notable changes occurred in the lateral compartment [Fig. 2(A)]. Qualitatively, periarticular fibrosis was observed in the 100% BW loaded groups, but was not observed in any of the sham (0% BW) groups.

**Mechanical evaluation**
The most prominent alterations in material properties occurred in the medial compartment and included reduced aggregate modulus of the articular cartilage and increased subchondral bone modulus in response to increased compressive loading (Fig. 3).

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**Fig. 2.** (A). Superior view of representative tibial plateaus from each experimental group with India ink staining illustrating increased fibrillation in the medial compartment with increased load magnitude and duration; (B). Safranin-O stained coronal sections from the medial compartment illustrating diminished staining for proteoglycans (#), increased matrix fibrillation and erosion (*), and peripheral chondrophyte/osteophyte (†) with increased loading; (C). H&E stained sections illustrating loss of chondrocytes (#) in the medial compartment with increased loading as indicated by loss of nuclei stained dark purple.
Articular cartilage

In the medial compartment, significant load (P < 0.0001) and duration effects (P < 0.0001) were observed for aggregate modulus. After 6 weeks of loading, the modulus in 100% BW group was 43% lower than the 0% BW group (P = 0.01) and 57% lower than the baseline group (P < 0.0001). At 20 weeks, the aggregate modulus of the 100% BW group was 77% lower than the 0% BW group (P = 0.002) and 87% lower than the baseline group (P < 0.0001). In the lateral compartment, no significant overall load effects were observed for aggregate modulus.
Significant load effects that were dependent on duration ($P = 0.025$) were observed for Poisson’s ratio of the articular cartilage in the medial compartment. Mean Poisson’s ratio of the 100% BW 20-week group was reduced $\sim 47\%$ as compared to all other groups ($P < 0.02$ for each comparison). In the lateral compartment, there were no significant differences in Poisson’s ratio across experimental groups.

No significant differences in articular cartilage permeability were observed in either the medial or lateral compartments as a function of load ($P = 0.95$ and $P = 0.48$, respectively). However, a duration effect was evident in the medial compartment ($P = 0.04$) where mean permeability of both 6-week groups were greater than baseline ($P < 0.03$ each). A similar pattern of duration effect was observed in the lateral compartment ($P = 0.04$).

**Subchondral bone**

In the medial compartment, duration dependent load effects were observed for the subchondral bone modulus ($P = 0.01$). The 100% BW 20-week group was increased $>90\%$ over all other groups ($P < 0.001$ each). No significant load or duration effects were observed in the lateral compartment.

**Thickness measures**

The most evident structural changes in response to altered loading were increased subchondral bone and decreased articular cartilage thicknesses in the medial compartment which were duration dependent and region specific (Fig. 4).

**Subchondral bone thickness**

In the medial compartment, duration dependent load effects were observed for mean subchondral bone thickness ($P = 0.03$). Thickness of the 100% BW 20-week group increased $\sim 30\%$ as compared to all other experimental groups ($P < 0.003$ each). Region-specific changes in subchondral bone thickness were most prominent in the midline region as detailed in Supplemental Fig. S2.

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**Fig. 4.** Thickness of the articular cartilage, calcified cartilage, and subchondral bone of the medial and lateral compartments of the tibia plateau [mean ($\pm 95\%$ CI)]. In the medial compartment, articular cartilage thickness decreased (30%) and subchondral bone thickness increased (32%) with increased compression of 100% BW at 20 weeks as compared to the 0% BW group. In the lateral compartment, calcified cartilage increased with decreased compression; in addition a trend for diminished articular cartilage thickness was observed at 20 weeks. Means not sharing a common letter are significantly different based on Fisher’s LSD procedure ($P < 0.05$). Region-specific plots of these outcome measures are provided in supplemental Figs. S2–S4. Animal-specific data are presented in supplemental Fig. S8.
In the lateral compartment, the mean subchondral bone thickness did not differ across experimental groups ($P = 0.65$). However, region-specific analysis revealed a 29% decrease in peripheral thickness of the 100% BW group compared to the 0% BW group at 20 weeks ($P = 0.025$).

**Articular cartilage thickness**

In the medial compartment, load effects on mean articular cartilage thickness were dependent on load duration ($P = 0.03$). Mean thickness of the 100% BW 20-week group was ~30% lower than all other groups ($P < 0.008$ each). Region-specific decreases in articular cartilage thickness were most prominent in the midline region with a similar pattern of changes of lesser magnitude in the central region (Supplemental Fig. S3).

In the lateral compartment, load effects were not significant ($P = 0.07$), but showed a similar pattern to the medial compartment of reduced articular cartilage thickness at 20 weeks. This pattern was present across the peripheral, central, and midline regions.

**Calcified cartilage thickness**

In the medial compartment, mean and region-specific calcified cartilage thickness did not significantly differ across experimental groups ($P = 0.98$; Supplemental Fig. S4).

In the lateral compartment, significant load effects were observed for mean calcified cartilage thickness independent of duration ($P = 0.02$). At both 6 and 20 weeks, increases of ~20% were observed in the 100% loaded groups as compared to corresponding 0% BW groups. Load-induced increases in calcified cartilage thickness were only observed in the midline region ($P = 0.002$).

**Degenerative scoring**

Increased compression in the medial compartment increased parameters of degeneration (MLW, degenerated cartilage area, and significant cartilage degeneration width), while decreased compression in the lateral compartment produced fewer significant effects. Supplemental parameters of zonal depth ratio of lesions and osteophyte size paralleled these results and are presented in supplemental Figs. S5 and S6.

**Matrix loss width**

In the medial compartment, significant load effects were observed that increased with duration ($P < 0.0001$). The MLW at the surface increased from 11% [95% confidence interval (CI): 3–19%] at baseline to 32% [24–40%] in the 100% BW 6-week group ($P = 0.001$) and 64% [57–71%] in the 100% BW 20-week group ($P < 0.0001$; Fig. 5). Values for surface MLW were greatly increased 100% BW groups compared to the 0% BW groups at 6 ($P = 0.01$) and 20 weeks ($P < 0.0001$).

At the midzone, load effects were evident only after 20 weeks ($P < 0.0001$). The MLW in the 100% BW 20-week group was 15% [12–18%] which was elevated as compared to all other groups ($P < 0.0001$ each). Similar findings were observed at the tidemark, where the MLW was 7% [6–8%] in the 100% BW 20-week group which was elevated compared to all other groups ($P < 0.0001$ each).

In the lateral compartment, the MLW, at the surface, midzone, and tidemark were not significantly different across groups.

**Degenerated cartilage area**

In the medial compartment, significant load and duration effects were observed for the degenerated cartilage area in all regions (Fig. 6). In the midline region, 11% [0–28%] of the cartilage area was degenerated at baseline and increased to 78% [61–95%] in the 100% BW 6-week group ($P < 0.0001$) and 100% [84–100%] in the 100% BW 20-week group ($P < 0.0001$). At both 6 and 20 weeks, degenerated cartilage area of the 100% BW groups was elevated over corresponding 0% BW groups ($P = 0.005$ and $P < 0.0001$, respectively). Similarly, in the central region, 10% [0–27%] of the cartilage was degenerated at baseline, increased to 67% [50–84%] in the 100% BW 6-week group ($P < 0.0001$), and further increased to 100% [84–100%] in the 100% BW 20-week group ($P < 0.0001$). Values in the 100% BW loaded groups at 6 and 20 weeks were elevated over corresponding 0% BW groups ($P = 0.004$ and $P < 0.0001$, respectively). In the peripheral region, 6% [0–21%] of the cartilage area was degenerated at baseline as compared to 44% [29–59%] in the 100% BW 6-week group ($P = 0.002$) and 54% [40–68%] in the 100% BW 20-week group ($P < 0.0001$).

In the lateral compartment, the areas of cartilage degeneration were not significantly different across groups.

**Total cartilage degeneration width**

No significant differences in total cartilage degeneration width, as measured at the articular surface, were observed between groups (Fig. 7).

**Significant cartilage degeneration width**

In the medial compartment, significant load and duration effects ($P < 0.0001$ each) were observed for width of significant cartilage degeneration at 50% depth (Fig. 7). The width of significant cartilage degeneration was 3% [0–13%] at baseline and increased to 53% [43–63%] in the 100% BW 6-week group ($P < 0.0001$) and 79% [70–88%] in the 100% BW 20-week group ($P < 0.0001$) with values in the 100% BW groups elevated over 0% BW groups within each duration ($P < 0.0001$ each).

In the lateral compartment, duration dependent load effects approached significance ($P = 0.051$) with the width of significant cartilage degeneration elevated in the 0% BW 20-week group compared to all other groups.

**Cellularity**

In the medial compartment, significant load ($P < 0.0001$) and duration effects ($P < 0.0001$) were observed for mean cellularity (Fig. 8). At 6 weeks, mean cellularity decreased 38% in the 100% BW group compared to the 0% BW group ($P = 0.007$) and 56% compared to baseline ($P < 0.0001$). At 20 weeks, cellularity of the 100% BW group decreased 51% compared to the 0% BW group ($P = 0.001$) and 68% relative to baseline ($P < 0.0001$). Region-specific decreases in cellularity were prominent in the midline and central regions. In the midline region, the cellularity of the 100% BW 6-week group decreased 63% compared to the 0% BW 6-week group ($P = 0.005$) and 76% compared to baseline ($P < 0.0001$) while the 100% BW 20-week group decreased 95% compared to the 0% BW 20-week group ($P < 0.0001$) and 97% compared to baseline ($P < 0.0001$). Similar changes were observed in the central region where in addition, progression of chondrocyte loss with increased loading duration was observed with the cellularity of the 100% BW 20-week group decreased 80% compared to 100% BW 6-week group ($P = 0.026$).

In the lateral compartment, no significant differences between groups were observed. However, a trend for increased cellularity with decreased load was observed at 20 weeks with the cellularity of the 100% BW group elevated ~40% compared to the 0% BW group across all regions.

**Discussion**

This study investigated the relationship between chronic altered compressive loading and tissue changes that occur with the onset and progression of joint degeneration. We revealed that increased...
Compressive loading of the medial compartment of the tibiofemoral joint over 20 weeks produced progressive degenerative changes of the joint consistent with the development of primary OA in humans including reduced articular cartilage thickness and aggregate modulus, increased subchondral bone thickness and stiffness, decreased cartilage cellularity, increased parameters of degeneration, and fibrosis. Decreased compressive loading applied to the lateral compartment increased calcified cartilage thickness, reduced subchondral bone thickness in the peripheral region and revealed trends for increased cartilage cellularity, diminished articular cartilage thickness, with minimal effects on parameters of degeneration and tissue material properties.

The histological and cartilage material properties results support our primary hypothesis that increased compressive loading applied to the medial compartment initiates degenerative changes analogous to OA in the joint that increase in severity with increasing duration. Early changes in response to increased compressive load in the medial compartment at 6 weeks were decreased aggregate modulus accompanied by chondrocyte loss and increased histological degeneration; particularly, matrix loss at the articular surface, degenerated area, and significant degeneration width. These changes progressed at 20 weeks, at which time the thickness and Poisson’s ratio of the articular cartilage were decreased and the thickness and modulus of the subchondral bone were increased with matrix loss at 50% depth. Degenerative changes of the tibial articular cartilage including cartilage loss, increased degenerated area, and decreased cellularity were prominent in the midline and central regions which are uncovered by the meniscus and in direct contact with the articular cartilage of the femur. Degenerative changes observed in the current study using the rat-VLD model, included chondrocyte loss at all depths of the articular cartilage which preceded widespread matrix loss in contrast to transection models in which degeneration emanates at the surface with fibrillation and matrix erosion preceding chondrocyte loss in the underlying depths. Subchondral thickening and load-induced mid- and deep zone changes in articular cartilage prior to surface changes have also been observed in the rabbit. Studies from human explants show that while cartilage decreases

![Fig. 5. MLW at the articular surface, midzone, and tidemark expressed as a percent of the compartment width for the medial and lateral compartments of the tibia plateau (mean ± 95% CI). The MLW increased with increased loading (100% BW) of the medial compartment and was most prominent at the articular surface where matrix loss values increased from 32% at 6 to 63% at 20 weeks. At 20 weeks, significant increases in matrix loss with increased load were also observed at midzone and at the tidemark. Please note the y-axis is expanded on midzone and tidemark plots. There were no significant changes in any of the widths of matrix loss in the lateral compartment with decreased compression. Means not sharing a common letter are significantly different based on Fisher’s LSD procedure (P < 0.05). Animal-specific data are presented in supplemental Fig. S9.](image-url)
in thickness and mechanical integrity with the progression of OA, subchondral bone thickness and stiffness increase\(^2\)\(^7\)\(^8\) similar to changes observed in the rat with increased load.

Our secondary hypothesis was that decreased compressive loading in the lateral compartment would result in diminished material properties and mild structural changes. Increased calcified cartilage thickness and reduced proteoglycan staining, as indicated by increased zonal depth ratio (Supplemental Fig. S5), were observed following 6 weeks of decreased loading, with trends for diminished articular cartilage thickness and increased cellularity at 20 weeks without notable changes in material properties. Articular cartilage thinning has been observed in studies of joint unloading produced by tail-suspension rendering rat limbs non-weight bearing\(^2\)\(^9\), knee distraction in rabbits\(^3\)\(^0\), and immobilization in canine knee joints. Increased calcified cartilage thickness was observed with decreased loading at 6 weeks. Utilization of the rat tail-suspension model led to increased calcified cartilage thickness and advancement of the tidemark at 4 weeks\(^2\)\(^9\); additionally, increased calcified cartilage thickness has been observed in the rabbit VLD model following 24 weeks of diminished loading\(^1\)\(^1\). Increased articular cartilage cellularity has been observed in animal studies of joint unloading produced by knee distraction\(^3\)\(^0\) and osteotomy\(^3\)\(^1\). Advancing of the tidemark may account for the increased calcified cartilage we observed in the lateral compartment. This may also contribute to the reduced articular cartilage thickness given the articular surface remained intact; with compaction of the cartilage matrix elevating cellularity.

This study builds on our prior work utilizing the rat-VLD model\(^1\)\(^2\) in evaluating (1) larger magnitudes of altered loading (100% vs 80% BW), (2) increased exposure to altered loads (24 vs 12 h/day), and increased duration of loading (20 vs 12 weeks). The increased load magnitude and duration in the present study produced definitive whole-joint degenerative changes particularly in the medial compartment. In the previous 12-week pilot study, early changes included thickening of the subchondral bone and diminished cartilage cellularity with decreased loading (80% BW;
12 h/day) in the lateral compartment with trends for decreased aggregate modulus and cellularity in the medial compartment with increased loading. Our present findings support previous work showing articular cartilage to be most affected by diminished loading produced by the VLD in the peripheral region of the lateral compartment and increased loading in the midline region of the medial compartment. The nonlinear response of subchondral bone changes with the development of joint degeneration may account for different patterns in subchondral bone thickness in this study as compared to our previous findings as well as a slightly more anterior section of the tibial plateau used for histological analyses in the current study. Decreased cellularity with the onset of degenerative changes was also observed in response to increased loading (80% BW; 12 h/day; 24 weeks) in the rabbit. While loading for 12 h/day may be more similar to altered loads experienced during normal activities of daily living; applying continuous load alteration appears to accelerate the degeneration process; thus increasing the feasibility of utilizing the VLD model to study load-induced OA. This study evaluated one level of chronic load alteration (100% BW) at two select time points (6 and 20 weeks) and unquestionably demonstrates the induction of joint degeneration by altered load. In vitro studies have shown that abnormal mechanical forces can stimulate articular chondrocytes to produce pro-inflammatory mediators, that promote matrix degradation through a variety of metabolic pathways. The use of the VLD model will allow elucidation of the mechanisms of in vivo chronic load-induced joint degeneration in future studies which evaluate early changes following shorter durations of altered loading.

Clinically, osteotomy, joint distraction, bracing and corrective footwear have been used to lower the intra-articular stress and alleviate pain associated with OA. Results of these procedures are often variable, and a better understanding of criteria for physiological levels of loading and the effects and time course of changes resulting from aberrant load levels will further improve the effectiveness of these treatment options.

Commonly used rat models employ transection of joint structures and/or forced exercise to produce OA-like degenerative changes. Although the loading environment of the tibiofemoral joint is altered in these models, this has not been quantified, making it difficult to discern the role of altered loading in these models. Unlike primary OA in humans, which develops over years, transection-based animal models induce rapid degenerative changes within several weeks, and also involve disrupting the joint capsule thus introducing bleeding into the joint. Therefore, these models may be more reflective of OA secondary to acute injury. As clinical OA results from varied etiopathologies, the development of alternative models to study the disease processes will complement the vast body of knowledge already gained from transection models.

Altered compressive loading applied with the VLD produced early to mid-stage histological degenerative changes over a longer time period than commonly used transection-based small animal models of OA. The VLD model may better replicate the slow development of OA in which chronic mechanical loads instead of acute joint injuries play a primary etiological role. In the rat-VLD model, load alteration without disruption of the joint capsule is quantifiable and removable which would allow elucidation of the mechanisms of in vivo chronic loading-induced joint degeneration.
allow future studies to determine whether disease progression may be halted or reversed with removal of altered loads. A comprehensive understanding of the effects of altered loads on articular joints and their relationship to the initiation and progression of primary OA, will aid in the development of methods for the detection of early degenerative changes and treatment methods for addressing the underlying mechanical abnormality in the joint.

**Fig. 8.** Cellularity (compartment mean and region-specific) of the medial and lateral compartments of the tibia plateau in the experimental limb (mean ± 95% CI). Mean cellularity in the medial compartment decreased with increased loading of 100% BW at 6 weeks (38%) and at 20 weeks (51%) as compared to 0% BW groups within time point. Load-induced decreases in cellularity were prominent in the midline and central regions. In the lateral compartment, there were trends for increased cellularity with diminished loading at 20 weeks. Means not sharing a common letter are significantly different based on Fisher’s LSD procedure (P < 0.05). Animal-specific data are presented in supplemental Fig. S12.

**Author contributions**

Roemhildt — Conception and design, analysis and interpretation of the data, drafting of the article, revision and final approval of the article.

Badger — Statistical design, statistical analysis and interpretation of the data, revision of the article, and final approval of the article.
Beynnon — Design, analysis and interpretation of the data, revision and final approval of the article.

Ertem — Analysis and interpretation of the data, revision of the article, and final approval of the article.

Gardner-Morse — Material properties analysis and interpretation of the data, drafting, revision, and final approval of the article.

Gauthier — Histological analysis and interpretation of the data, drafting, revision, and final approval of the article.

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Conflict of interest
The authors of this work have no competing interests.

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