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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.

Introduction

Requisite mechanical loading is essential for the maintenance of healthy articular cartilage while aberrant loading is implicated in the development of degenerative changes[1]. 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 injury[2]. 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 weeks[3,4]. 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

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secondary OA suggests distinct disease subsets in humans[6,7] and animal models[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[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[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[12] without significant alteration of vertical ground reaction force[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 femur as previously described[10,13]. Following a 2-week recovery, rats were fit with a VLD and the spring torque set to apply compressive load alteration (0 or 100% BW) in addition to the normal compressive forces in the joint produced by muscle forces and ambulation (Supplemental Fig. S1). The VLD increases the compressive load in the medial compartment and decreases the compressive load in the lateral compartment by the target amount (Fig. 1)[9]. The VLD was engaged 24 h per day for 0, 6 or 20 weeks. Animals in the baseline group underwent surgery and 2-week recovery prior to euthanasia at time 0; the start of loading treatment for all groups. Load levels were measured twice daily to ensure application of target load ±10%[12]. Following the experimental loading protocol, animals were euthanized, tibial plateau retrieved, stained with India ink, photographed, and stored at −80°C until mechanical evaluation.

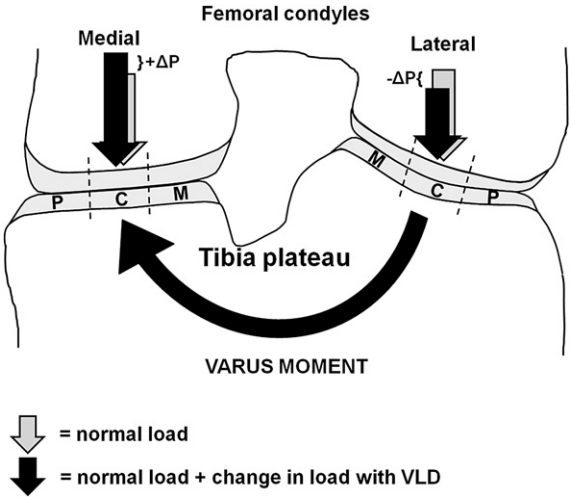

Fig. 1. Anterior–posterior view of the rat tibiofemoral joint. The varus moment applied with the VLD changes the normal compressive loading by increasing the compression (+Δ P) in the medial compartment and decreasing the compression (−Δ P) in the lateral compartment leading to altered compressive loads. Both the medial and lateral compartments were divided into three regions of equal width: peripheral (P), central (C), and midline (M) regions.

Mechanical evaluation

Articular cartilage

The material properties (aggregate modulus, permeability, and Poisson's ratio) of the articular cartilage at central sites in the medial and lateral compartments of the tibial plateau of experimental limbs were evaluated using a biphasic creep-indentation test[14,15] with cartilage thickness determined using the needle probe test as we have previously described[12,16]. A custom materials testing device[12] with a cylindrical, plane-ended, porous, 0.516 mm-diameter indenter tip was used to apply a tare load (0.0255 MPa) to the specimen for 10 min, followed by the test load (0.1249 MPa) until displacement reached equilibrium (<0.1 μm change in displacement over a 300 s period). Following a recovery period, the thickness of the articular cartilage at the testing site was determined using a needle probe test (0.05 mm/s displacement rate; −250 g, three trials)[17]. Material properties of the articular cartilage were determined by curve-fitting the load-displacement response with the biphasic indentation creep solution via a nonlinear regression procedure[15,17].

Subchondral bone

The compressive modulus of the subchondral bone underlying the articular cartilage test sites was determined using a micro-indentation test[18]. Three cycles of repeated loading (2.452 N, 30 s hold) were applied to the osteochondral specimen via a stainless steel needle with a parabolic-shaped tip. The slope of the load-displacement response during the unloading portion of the third cycle was used to determine the modulus of the subchondral bone.

Histological analysis

Specimens were formalin fixed and decalcified with 10% ethylenediaminetetraacetic acid[19]. A 2 mm coronal section of the tibial plateau centered on the collateral ligament attachments was prepared, paraffin embedded, and sectioned (5 μm)[19]. Three sections collected at 200 μm intervals for each tibial plateau were processed for each stain. Sections were deparaffinized and stained with Safranin-O and Fast Green (SOFG) or Hematoxylin and Eosin (H&E)[20] prior to examination under a light microscope (BX50, Olympus Inc.) fit with a digital camera (RET-2000R-F-CLR-12-C,