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Changes in In Vitro Compressive Contact Stress in the Rat Tibiofemoral Joint with Varus Loading

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Abstract

Increased compression of the tibiofemoral joint, due to increased body mass or malalignment, is a risk factor for the onset and progression of osteoarthritis. This work investigates compressive stresses and contact areas in the articular cartilage of the rat tibiofemoral joint during standing with different applied varus loads.

Cadaver rat knees underwent loading of the extensors combined with varus loading (0%, 50% or 100% of bodyweight) of the tibiofemoral joint. Articular cartilage contact stress was evaluated using stereophotogrammetric measurements of biplanar radiographs, high-resolution micro-computed tomography and discrete element analysis. Random coefficients regression models were used to analyze the relationship between peak and spatially averaged contact stresses and contact areas as a function of increasing varus loadings.

The contact stresses increased linearly in the medial compartment. Peak stress significantly increased 0.042 MPa ($p=0.006$) and spatially averaged stress significantly increased 0.029 MPa ($p=0.045$) for each 10% increase in varus loading. There was a trend for a small decrease in contact areas in the lateral compartment with varus loading.

This is the first report of the contact stresses in a rat tibiofemoral joints under simulated weight bearing conditions. The 0.42 MPa increase in peak contact stress at the cartilage-cartilage interface of the medial compartment with 100% bodyweight varus load is similar to the reported change in peak contact stress associated with development of symptomatic knee osteoarthritis in humans. Determination of contact stresses in rat tibiofemoral joints allows comparison to contact stresses in humans with the development of osteoarthritis.

INTRODUCTION

Increased compression of the tibiofemoral joint, due to varus malalignment, valgus malalignment, or increased body mass, is a risk factor for the onset and progression of

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CONFLICT OF INTEREST

The authors of this work have no competing interests.

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osteoarthritis (OA) (Brouwer et al., 2007; Felson et al., 1988; Reijman et al., 2007; Sharma et al., 2001; Sharma et al., 2012). Segal et al. (2009) reported that cases presenting with symptomatic tibiofemoral OA at 15 month follow-ups had 0.54 MPa significantly greater peak articular compressive contact stress at the cartilage-cartilage interface compared to controls. More recently, they demonstrated that increased spatially averaged and peak contact stresses were risk factors for knee degeneration (Segal et al., 2012).

Contact stresses in diarthrodial joints can be estimated experimentally using pressure sensitive films or mats, analytically for simple geometries, or computationally using the finite element method or discrete element analysis (DEA) (Brand, 2005; Volokh et al., 2007). The small size of the rat tibiofemoral joint and invasiveness of the pressure sensors make data collection under *in vivo* conditions difficult. DEA has been used to estimate contact stresses in hip (Armiger et al., 2009; Genda et al., 2001; Yoshida et al., 2006), ankle (Haraguchi et al., 2009), patellofemoral (Elias et al., 2004; Elias et al., 2010), and tibiofemoral (Miller et al., 2009; Segal, 2009; Segal, 2012) joints. DEA results have been confirmed against finite element analyses and pressure measurements in ankles (Anderson et al., 2010) and pressure measurements in tibiofemoral joints (Miller, 2009). Since DEA is a simpler and more efficient computational approach, it was chosen for this study.

A representative review of 13 different healthy articular joints in 33 human and 3 nonhuman mammalian studies found similar peak and spatially averaged contact stresses across joints and species (Brand, 2005). Thus, determination of *in vivo* contact stresses in animal models will allow comparisons with contact stresses in humans with progressive OA.

METHODS

This work investigates compressive contact stresses and areas in the rat tibiofemoral joint across the cartilage-cartilage interface with static loading during stance without and with applied varus loadings. The varus loads were applied using a previously developed varus loading device (VLD) (Fig. 1) (Roemhildt et al., 2010a,b; Roemhildt et al., 2012a,b). The VLD allows application of varus loadings to the tibiofemoral joint without disrupting the joint capsule while maintaining normal range of knee motion.

Articular cartilage contact stresses of the rat tibiofemoral joint were estimated using five steps (Fig. 2). Cadaveric male Sprague-Dawley rats ($n = 4$, mean ($\pm$ SD) age = 8.75 ± 1.89 months, mean ($\pm$ SD) bodyweight = 8.29 ± 2.11 N) were used. Transcutaneous bone plates were attached to the left hind femur and tibia and fit with the VLD (Roemhildt, 2010a,b). At least four 0.5 mm diameter tantalum beads were implanted in the femoral and tibial bones of the left legs (Fig. 1). Beads were placed in the medial and lateral aspects of the tibiofemoral joint following preparation with a 0.508 mm diameter drill and secured with cyanoacrylate adhesive. All major joint structures including the menisci remained intact throughout data collection. Rats were positioned to replicate *in vivo* standing posture (tibiofemoral flexion angle $\sim 80^\circ$) using polyurethane foam blocks within the stereoradiograph calibration cage (Fig. 2A). Muscle and bodyweight forces across the tibiofemoral joints were simulated by applying a 4.4 N load to the patella via a nylon suture that was placed through the center of the patella. The 4.4 N patella force represents approximately half bodyweight (BW). The patella force was aligned parallel with the long axis of the femur by drilling a hole in the proximal end of the femur and was applied in all trials. Stereoradiographs were collected for each animal without and with applied varus loadings with up to two replicates. Based on a free body analysis of the tibiofemoral joint (Roemhildt, 2010), the VLD applied varus loadings were targeted to alter the compressive loadings in the compartments by 0, 50, or 100% BW (Fig. 1). Each loading condition was applied for 15 minutes prior to collection of the radiographs to ensure tissue creep had reached equilibrium (Roemhildt, 2012a,b). The

three-dimensional positions and orientations of the femur and tibia were measured from the stereo radiographs using Roentgen stereophotogrammetric analysis (RSA) (UmRSA, RSA Biomedical, Umeå, Sweden). The mean translational and rotational accuracies of our RSA system were 50 μm and 0.3° (Beardsley et al., 2007).

Joints were dissected and high-resolution micro-computed tomography (μCT) (Locus, GE Healthcare, Ontario, Canada) scans of the proximal tibia and femoral condyles were obtained at 80 kVp, 450 μA and 400 ms exposure time with (20.5 μm)³ voxel size. To determine the geometry of the femoral and tibial subchondral bone and bead surfaces, MicroView software (Version 2.2, GE Healthcare, London, Ontario, Canada) was used to threshold the surfaces and output the resulting triangulated surfaces (Fig. 2B). Bony surfaces were registered with the RSA determined joint position using the common bead positions and rigid body transformations (Challis, 1995) using custom Matlab code (MathWorks, Natick, MA USA) (Fig. 2C).

Using the RSA measured positions and orientations of the femur and tibia as inputs (Tashman and Anderst, 2003) and following the DEA methodology (Chao et al., 2010), the separation distance between the tibia and femur surfaces were calculated in Matlab by finding where the tibial surface normal vectors intersect the femoral surface (Fig. 2D). The compressive strains (ε) were calculated as

$$\varepsilon = \frac{t - \frac{d}{2}}{t} \quad \text{Equation 1}$$

where t is the femoral or tibial cartilage thickness and d is the separation distance. Only positive strains were considered since negative strains imply no contact between the femur and tibia. The compressive contact stresses (σ) were calculated as

$$\sigma = E\varepsilon \quad \text{Equation 2}$$

where E = elastic modulus of the cartilage (Fig. 2E).

Cartilage material properties values for the medial and lateral compartments of unoperated control rat tibias from Roemhildt (2012a) were used for cartilage aggregate modulus and Poisson's ratio and cartilage thicknesses. The aggregate modulus (H_A) and Poisson's ratio (ν) were used to calculate compartment specific elastic moduli using

$$E = \frac{H_A(1+\nu)(1-2\nu)}{(1-\nu)} \quad \text{Equation 3}$$

This results in elastic moduli of 1.56 and 1.28 MPa in the medial and lateral compartments, respectively. The cartilage thicknesses of the central region of the medial and lateral compartment were 325 and 306 μm , respectively. The cartilage of each compartment was assumed to be of uniform and equal thicknesses and have the same material properties for the tibia and femur.

The dimensionless sensitivity of the peak contact stress to variations in cartilage thickness and modulus were calculated as

$$\lambda_t = \frac{\partial \sigma_{peak}}{\partial t} \frac{t}{\sigma_{peak}} = \frac{E d_{min}}{2t^2} \frac{t}{E \epsilon_{peak}} = \frac{d_{min}}{2t} \frac{1}{\epsilon_{peak}} = \frac{d_{min}}{2t} \frac{t}{t - \frac{d_{min}}{2}} = \frac{d_{min}}{2t - d_{min}} \quad \text{Equation 4}$$

$$\lambda_E = \frac{\partial \sigma_{peak}}{\partial E} \frac{E}{\sigma_{peak}} = \epsilon_{peak} \frac{E}{\sigma_{peak}} = \epsilon_{peak} \frac{1}{\epsilon_{peak}} = 1 \quad \text{Equation 5}$$

where σ_{peak} is the maximum contact stress resulting from the peak strain, ϵ_{peak} , at the minimum separation distance between the femur and tibia, d_{min} . The dimensionless sensitivity of the spatially averaged contact stress to variations in cartilage thickness and modulus were calculated using central finite differences with $\pm 1\%$ variation in the parameters.

The relationship between the varus loading and the outcome measures (peak and spatially averaged contact stresses and contact area) in the medial and lateral compartments were analyzed using random coefficients regression models that account for the correlation between observations within animal (Rutter and Elashoff, 1994). Fixed effects of loading were estimated hierarchically with animal-specific intercepts and slopes representing random effects that are assumed to deviate from the fixed population averages. Initial models considered both linear and quadratic effects of load. Because there was no evidence of non-linearity (i.e. quadratic terms were not significant), final models included only a linear term for load. Analyses were performed using SAS, PROC MIXED (SAS Institute, Cary, NC). Statistical significance was determined using $\alpha=0.05$.

RESULTS

Typical distributions of tibial compressive contact stresses in the rat tibiofemoral joint are shown in Fig. 3. In the medial compartment, peak contact stress significantly increased 0.042 MPa ($p=0.006$) and spatially averaged contact stress significantly increased 0.029 MPa ($p=0.045$) for each 10% increase in varus load (Fig. 4; Table 1). The mean ($\pm$ SE) contact area was 3.70 (± 0.29) mm² in the medial compartment and did not differ significantly with varus loading (Table 1). There were trends of decreasing peak and spatially averaged contact stresses and contact area in the lateral compartment with increasing varus loads (Fig. 4; Table 1).

The dimensionless sensitivity of the peak contact stress to cartilage elastic modulus is one (1). Thus, a 10% change in modulus will lead to a corresponding 10% change in peak contact stress. The mean ($\pm$ SE) sensitivity of the spatially averaged contact stress to cartilage modulus was 0.992 (± 0.116) and 0.417 (± 0.032) in the medial and lateral compartments, respectively. The sensitivity of the peak contact stress to cartilage thickness was 0.567 (± 0.118) and 2.267 (± 0.224) in the medial and lateral compartments, respectively. The sensitivity of the spatially averaged contact stress to cartilage thickness was 0.991 (± 0.116) and 2.833 (± 0.240) in the medial and lateral compartments, respectively.

DISCUSSION

This is the first report of the contact stresses across the cartilage-cartilage interface in the rat tibiofemoral joint during weight bearing conditions. The peak compressive contact stresses measured in the medial compartment fall within the range of peak contact stresses reported in healthy human and other mammalian tibiofemoral joints (Brand, 2005). Applied varus loading of 100% BW increased peak contact stress 0.42 MPa in the medial compartment, which is similar to the change in peak contact stress of 0.54 MPa associated with

development of symptomatic knee OA in humans (Segal, 2009). In our previous study, varus loading of 100% BW applied up to 20 weeks produced degenerative changes to the rat tibiofemoral joint (Roemhildt, 2012b).

This study replicated the *in vivo* standing posture including tibiofemoral loads. A limitation of this study is absence of knee flexors to balance the applied knee extensor load. This caused a tendency for the cadaver knees to be extended causing contact areas to migrate posteriorly on the tibial plateaus. For simplicity, the patella load was constant for all the rats and not adjusted for body weight. Thus, the compressive load and stresses in the heavier rats may have been underestimated.

Rat subchondral bone modulus is at least a 1000 times greater than cartilage modulus (Roemhildt, 2012b) supporting the assumption that the subchondral bone is rigid. Differences in the subchondral bone surface relative to bead locations may have been produced by differences in thresholds for segmenting the bony surfaces and this would present as differences in the separation distance with similar effects as differences in cartilage thicknesses. In this study, the rat cartilage was assumed to be of uniform thickness, though cartilage thickness varies regionally across the femur and tibia (Roemhildt, 2012a). Based on the sensitivities to cartilage thickness, a 10% change in thickness corresponds to 5.7–28.3% change in the contact stresses. However, variations in cartilage thicknesses should only have minor effects on the relative changes in contact stresses within an animal. The tibiofemoral contact location varies with flexion angle and limb position and may introduce error in the predicted contact stresses. For the heavier rats, there was little or no contact in the lateral compartment. Based on lateral compartment sensitivities, this may be due to the underestimation of cartilage thickness from a study with smaller rats. Due to the sensitivity of the contact stresses to articular cartilage moduli and thicknesses, the accuracy of the contact stresses may be enhanced by using animal specific measures of cartilage material properties and thicknesses.

Due to the difficulty in determining the soft tissue geometry of the menisci, they were not included in the discrete element model. In intact joints with intact menisci, omission of menisci in the DEA of contact stresses in tibiofemoral joints showed a decrease in contact areas with only minor changes in the centrally located peak contact stresses across the cartilage-cartilage interface as compared to including the meniscus in DEA (Anderson, 2010)(Supplement 1). This is in contrast to experimental studies of meniscectomy where removal of the meniscus from the joint results in increased contact stresses in cartilage-cartilage contact regions (Pozzi, 2010; Lee, 2006). By omitting the meniscus in the DEA in our study, the contact areas were underestimated and the spatially averaged contact stresses overestimated for the full contact region (cartilage-cartilage and cartilage-meniscus-cartilage contact regions), but remain valid in the cartilage-cartilage contact region. Since peak contact stresses occurred in areas of cartilage-cartilage contact, the absence of the meniscus in the DEA would not affect predicted peak stresses (Supplement 1 Fig. S1A).

The rat VLD model allows the investigation of varus loadings – a significant risk factor in the development of human primary OA. Increased rat tibiofemoral contact stresses across the cartilage-cartilage interface in the medial compartment produced by increased varus loadings are similar to those leading to degenerative changes in rat and human knees. Determination of contact stresses in rat tibiofemoral joints allow comparison of loading environments in a rat model to those in humans in both normal conditions and during the onset and progression of OA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Rat tibiofemoral joint with the varus loading device (VLD) attached to the lateral side of the left femur and tibia with transcutaneous bone plates. The torque from the VLD torsional spring applies a lateral force (F) to the distal tibia multiplied by the tibia moment arm (L) creates a varus moment (M) about the tibiofemoral joint. Tantalum beads (●) implanted in cortical bone for measuring the joint position are shown on the medial side. At least four additional beads were placed in similar locations on the lateral side. (B) Anterior-posterior view of the left rat tibiofemoral joint. The varus moment (M) changes the normal compressive joint loadings (gray arrows) by increasing the compression ($+\Delta P$) in the medial compartment and decreasing the compression ($-\Delta P$) in the lateral compartment leading to altered compressive loads (black arrows). The target change in compression (ΔP) is equal to the varus moment (M) divided by the intercompartmental moment arm (D) (Roemhildt et al., 2010a, Roemhildt et al., 2012a).

Figure 2.

Articular cartilage contact stresses of the cadaver rat tibiofemoral joint were estimated using five steps: A) implantation of tantalum beads into the femur and tibia, collection of biplanar stereoradiographs and Roentgen stereophotogrammetric analysis (RSA) of the femur and tibia positions with and without applied varus loadings, B) collection of high-resolution micro-computed tomography (μ CT) images of the tibiofemoral subchondral bone and bead surface geometries, C) registration of the μ CT surface geometries based on the common measured bead positions in the RSA and μ CT images, D) calculation of the surface normal vectors and separation distances between the tibia and femur, and E) calculation of the compressive contact stresses based on cartilage properties.

Figure 3.

Typical distributions of tibial compressive contact stresses and the associated peak contact stresses in the medial and lateral compartments of the tibiofemoral joint without and with varus loadings. The varus loadings increase the compression in the medial compartment by 0%, 50%, or 100% body weight (BW) and reduce load in the lateral compartment by an equivalent amount.

Figure 4.

Significant linear increases in peak ($\text{stress}_{\text{peak}}$, $p=0.006$) and spatially averaged ($\text{stress}_{\text{averaged}}$, $p=0.045$) contact stresses in the medial compartment (left) of the rat tibiofemoral joint as a function of varus loading in percent bodyweight (%BW). Different symbols represent different animals. The thick straight lines are the estimated linear relationship as expressed by the parameters in Table 1. The dash lines are 95% confidence bands on the mean contact stresses. There were trends of decreasing peak and spatially averaged contact stresses with varus loading in the lateral compartment (right).

Table 1

Estimated slopes ($\pm$ standard errors) and intercepts ($\pm$ standard errors) for peak ($\text{stress}_{\text{peak}}$) and spatially averaged ($\text{stress}_{\text{averaged}}$) contact stresses and contact area in the medial and lateral compartments of the rat tibiofemoral joint as a function of varus loading in percent bodyweight (%BW). The slopes of the peak and spatially averaged contact stresses show significant increases in the medial compartment with increasing varus loading based on the p-values of the slope estimates. The contact stresses regression lines are shown in Fig. 4.

		Medial Compartment		
		Slope ($\pm$SE)	Intercept ($\pm$SE)	Slope p-value
	Units	(MPa/%BW)	(MPa)	
$\text{stress}_{\text{peak}}$	(MPa)	0.00416 ($\pm$ 0.00058)	0.841 ($\pm$ 0.076)	p=0.006
$\text{stress}_{\text{averaged}}$	(MPa)	0.00287 ($\pm$ 0.00086)	0.456 ($\pm$ 0.024)	p=0.045
		(mm²/BW)	(mm²)	
contact area	(mm ²)	0.00108 ($\pm$ 0.00414)	3.637 ($\pm$ 0.291)	p=0.81
		Lateral Compartment		
		Slope ($\pm$SE)	Intercept ($\pm$SE)	Slope p-value
		(MPa/%BW)	(MPa)	
$\text{stress}_{\text{peak}}$	(MPa)	-0.00167 ($\pm$ 0.00079)	0.331 ($\pm$ 0.146)	p=0.12
$\text{stress}_{\text{averaged}}$	(MPa)	-0.00090 ($\pm$ 0.00045)	0.151 ($\pm$ 0.071)	p=0.14
		(mm²/BW)	(mm²)	
contact area	(mm ²)	-0.00928 ($\pm$ 0.00361)	1.661 ($\pm$ 0.743)	p=0.08