Compressive Moduli of the Human Medial Meniscus in the Axial and Radial Directions at Equilibrium and at a Physiological Strain Rate

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Received 5 January 2007; accepted 8 October 2007

Published online 12 February 2008 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jor.20573

ABSTRACT: The axial and radial compressive moduli of the human meniscus are important material properties in tibiofemoral joint models, but they have not been determined previously for fresh-frozen tissue. Our goals were to measure the moduli at equilibrium and at a physiological strain rate, to determine whether the axial and radial compressive moduli are equal for each type of loading, and to determine whether they depend on the region (i.e., anterior, middle, posterior) of the meniscus. Samples from each region from 10 fresh-frozen human medial menisci were tested in unconfined compression at four strain levels (3%, 6%, 9%, and 12%) at 32%/s, a strain rate determined to be physiologically relevant to walking, and then allowed to reach equilibrium in stress relaxation. At equilibrium, the axial and radial compressive moduli at 12% strain were 83.4 kPa and 76.1 kPa, respectively ($p = 0.58$), whereas at the physiological strain rate, the axial and radial compressive moduli at 12% strain were 718 kPa and 605 kPa, respectively ($p = 0.61$). At the physiological strain rate, the modulus increased with increasing strain (79.2 kPa at 3% strain vs. 662 kPa at 12% strain) and the modulus in the anterior region (1,048 kPa at 12% strain) was significantly greater than that in the posterior region (329 kPa at 12% strain) ($p = 0.04$). Our study supports a plane of isotropy for the material properties of meniscal tissue. However, the material behavior is strongly nonlinear because the compressive modulus is several orders of magnitude smaller than previously reported values for tensile modulus. Further, the compressive modulus depends on the activity of interest (i.e., static such as standing or dynamic such as walking) due to viscoelastic effects, the strain level, and the region of the tissue. © 2008 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res

Keywords: meniscus; compression; modulus; material property; strain

Information regarding the material properties of the human meniscus is critical in the study of tibiofemoral contact,[1,2] the effect of meniscal injury on tibiofemoral contact,[3] and the selection criteria for meniscal replacements.[4] Mathematical models demonstrated that the contact pressure distribution is sensitive to the axial, radial, and circumferential moduli.[1,2] Because stresses in the circumferential direction are predominantly tensile[5] when the tibiofemoral joint transmits compressive loads and because the circumferential tensile modulus was determined previously for human tissue,[6–8] the present work focused on the axial and radial moduli.

Meniscal material properties should be determined from human specimens. Properties vary not only between species, but also among topographical locations within a species.[9] No known animal model is an appropriate substitute for human meniscal material properties.

For accurate mathematical modeling, material properties should reflect physiological loading. Axial and radial stresses are compressive throughout most of the cross section, with small tensile values in the extreme peripheral region.[10] Tensile properties for human menisci are known in the radial and circumferential directions,[8] but properties of articular cartilage, another tissue with an ultrastructure of collagen fibers, are different in compression and tension.[11] Material properties may also be different for the menisci in compression versus tension.

No study has satisfied the criteria of using human menisci and physiological loading conditions. Compressive properties of human meniscus have been measured through indentation and confined compression tests, but only in the axial direction.[9,12,13] Leslie et al.[14] tested human tissue in unconfined compression in axial, radial, and circumferential directions, but the biomechanical properties were changed by storing specimens in formaldehyde prior to testing.

Our first objective was to measure the axial and radial compressive moduli of human meniscus at equilibrium to characterize the material properties of the solid phase of the tissue. Our second objective was to measure these same moduli at a physiological strain rate representative of human walking. Our third objective was to determine whether the axial and radial moduli are equal under either or both loading conditions, suggesting a plane of isotropy (i.e., transversely isotropic). Such a relation is necessary for a mathematical model to provide valid contact pressure distribution in the tibiofemoral joint when compared to experimental data.[1,2] Because material properties in compression are affected by the region of the meniscus from which samples are taken,[9,15] a fourth objective was to determine whether any regional dependence exists in axial and radial moduli at equilibrium and at the physiological strain rate.

MATERIALS AND METHODS

Testing

Human medial menisci from frozen knees were harvested for sample preparation. Ten knees from cadavers 23–57 years of age (mean = 40.4 years) were obtained frozen from regional tissue banks. The knees were frozen within 72 h of when refrigeration commenced following death; the time between death and refrigeration is unknown. The tissues, including the cartilage appeared white and glossy without visible damage or degeneration. The medial menisci from thawed knees were carefully excised from the knee joint with a scalpel, wrapped in

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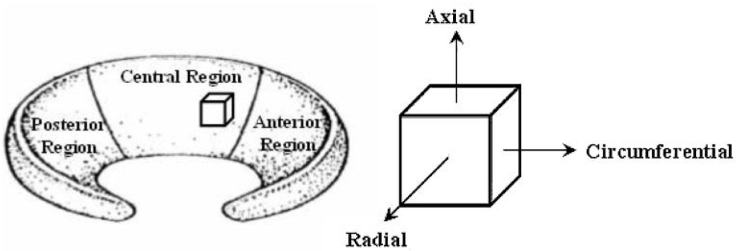

Figure 1. Diagram showing the locations and orientation of test specimens. Two 2-mm cubic specimens were prepared from each region of the medial meniscus and loaded in the axial and radial directions.

Ringer's solution-soaked gauze, and frozen until sample preparation.

For testing, each meniscus was cut into three regions (anterior, central, and posterior) with equal arc lengths in the circumferential direction. Two 2-mm cubic samples were prepared from the middle of each region using a custom cutting device (Fig. 1), consisting of a freezing stage (Hacker Instruments, Fairfield, NJ) and a clamp with a 2-mm cutting guide for parallel cuts. Each specimen started from a radial section of the intact meniscus that was frozen tibial side down on the freezing stage to ensure axial orientation. After the first set of parallel cuts, two more sets of cuts were made by flipping the tissue 90°. The axial and radial directions were marked with waterproof ink.

Unconfined compression testing was performed using a servohydraulic materials testing system (Model 858; MTS, Minneapolis, MN) with a 10-N load cell (Model SMT1-2.2; Interface, Scottsdale, AZ; ±0.05% accuracy). To minimize desiccation, samples were immersed in Ringer's solution during testing. Nonporous Teflon plates were used in the load train to decrease boundary traction between the test platens and the sample.

For testing, samples were thawed at room temperature and allowed to equilibrate in the Ringer's solution at room temperature (74°F) for 30 min.[12,16] One sample from each region was tested in the axial direction and the second sample in the radial direction. Prior to testing, specimen dimensions were measured with a digital micrometer (Mitutoyo, Minato-ku, Tokyo, Japan; resolution = ±0.001 mm) to calculate cross-sectional area and gage length. The dimensions measured with the micrometer were within 0.1 mm of the dimensions measured from an image of the specimen. The image was taken by a video camera with a 50-mm lens and an image sensor containing 768 × 494 elements (model 4910; Cohu, San Diego, CA). Images were captured using a framegrabber card (model LG-3; Scion, Frederick, MD) and processed using Scion Image software (resolution = 0.01 mm).

On each of four consecutive days, the sample was preconditioned at one of four randomized strain levels (3%, 6%, 9%, or 12%) for 10 cycles at a displacement rate of 0.63 mm/s.[17] A stress-relaxation test was then performed at the same strain level as that for preconditioning. Equilibrium was defined as <1% change in stress over 1 min. The 12% strain level was an estimate of a physiological strain experienced in the axial and radial directions.[18] The average circumferential strain in the three regions was added to two standard deviations and then divided by a Poisson's ratio of 0.5 for incompressibility of the tissue. The strain rate of 32%/s was the estimate of 12% strain divided by the time for single-leg stance (0.38 s)[19]; the corresponding displacement rate for a 2-mm sample was 0.63 mm/s.

Displacement and load data were sampled at 250 Hz; the length of each test with preconditioning was 22 min, more than sufficient to reach equilibrium (Fig. 2). Between tests, the sample was wrapped in Ringer's solution-soaked gauze and refrigerated overnight for recovery.

Data Analysis

The compressive moduli at equilibrium in the two directions at the four strains (ε) were determined by calculating stress (σ) from the equilibrium load divided by the initial cross section. A stress-strain curve was plotted using the stress for each strain level. A nonlinear least squares regression using Fung's two-parameter exponential model[20]:

$$\sigma = A^{*}(\exp(\varepsilon^{*}B) - 1) \tag{1}$$

was used to determine the parameters A and B (Fig. 3). The modulus was calculated from A and B and the rate of change of stress with strain:

$$d\sigma/d\varepsilon = B(\sigma + A) \tag{2}$$

The compressive modulus at a physiological strain rate for the four strain levels in the axial and radial directions was determined from the load and displacement data in the ramp portion of the displacement-time curve for the 12% strain level stress-relaxation test. For each time point that data were sampled, stress was calculated as described above and strain was calculated using the initial gage length. From a nonlinear least squares regression of the resulting stress-strain curve (using Equation 1), A and B were determined (Fig. 4). The compressive modulus at each strain level was calculated from Equation 2.

To determine whether the compressive moduli in the axial and radial directions were equal at equilibrium and for the physiological strain rate, and to determine whether the moduli were affected by region, statistical analyses were performed. The acceptance criterion for parameters A and B and the resulting modulus to be used in the analyses was a goodness of fit with an $R^2 > 0.70$. This resulted in 49 of 60 (82%) specimens

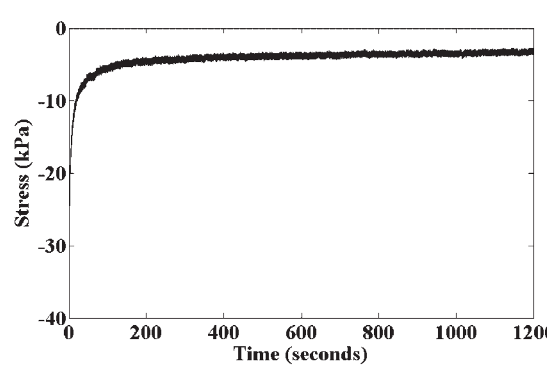

Figure 2. Sample stress-relaxation plot after 10 cycles of preconditioning on an anterior specimen loaded in the axial direction. The stress equilibrium, defined as <1% change in stress over 1 min, was determined for each of the four strain levels for a single specimen and curve-fitted with Fung's exponential to find parameters A and B (Fig. 3).

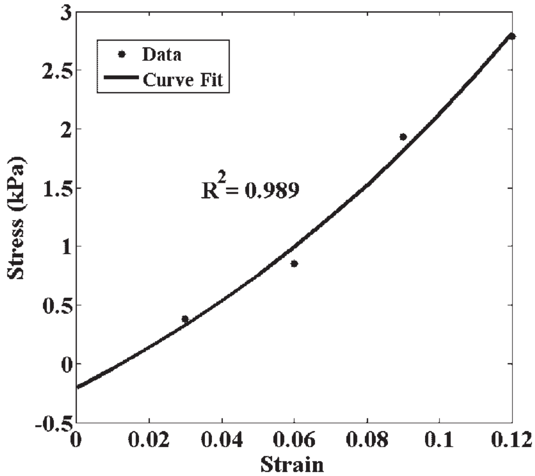

Figure 3. Sample fit of stress equilibrium values for four strain levels (3%, 6%, 9%, and 12%) of an anterior specimen loaded in the axial direction to find parameters A and B of Fung's two-parameter exponential model.

for equilibrium and 53 of 60 (88%) specimens for loading at a physiological rate.

A two-factor repeated measures ANOVA model using SAS software (Cary, NC) was used. Independent variables were direction (axial and radial) and region (anterior, central, and posterior). Because the variance generally increased with increasing modulus, a logarithmic transformation was made on the moduli that resulted in approximately equal variance. The dependent variables were the logarithms (base 10) of the compressive moduli at equilibrium and at the physiological strain rate for 3%, 6%, 9%, and 12% strain. The statistical analysis for each loading condition and strain level was run separately for a total of eight ANOVAs. Preliminary ANOVAs, which included the region $\times$ loading direction interaction term, revealed that all interaction terms were not significant ($p > 0.05$). Accordingly, the interaction term was suppressed to increase the degrees of freedom in the error term. Level of significance was set to 0.05. When differences were detected, Tukey's test was used to determine significantly different treatments.

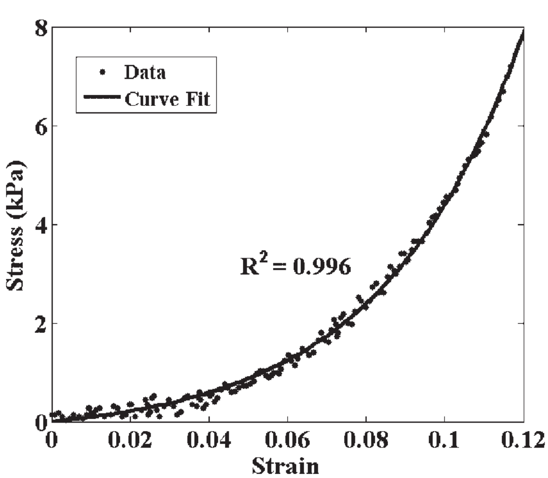

Figure 4. Typical stress-strain curve of the ramp portion of the displacement-time curve for a stress-relaxation test and a least squares fit using Fung's two-parameter exponential model for a posterior region specimen loaded in the axial direction. The parameters A and B determined were used with the measured stress at 12% strain to calculate the compressive modulus at the physiological strain rate.

RESULTS

At equilibrium, the axial and radial compressive moduli were similar ($p \geq 0.22$) except at 6% strain where the compressive modulus in the radial direction was significantly greater than that in the axial direction ($p = 0.03$, Table 1). Averaged over the three regions at 6% strain, the modulus in the radial direction was 47.8 kPa compared to 31.4 kPa in the axial direction. At a physiological strain rate, the axial and radial moduli were similar at all strain levels ($p \geq 0.29$, Table 2). However the modulus at a physiological strain rate was considerably greater than that at equilibrium. Averaged over both loading directions and the three regions, the modulus at a physiological strain rate was 2.6 times greater at 3% strain and 8.3 times greater at 12% strain than the compressive modulus at equilibrium.

The compressive moduli were not significantly affected by region at equilibrium at all strain levels ($p \geq 0.09$), but were significantly affected by region at a

Table 1. Compressive Moduli (kPa) at Equilibrium as Average (Standard Deviation)

	3% Strain		
	Axial	Radial	$p = 0.22$
Anterior	37.3 (34.0)	41.8 (46.9)	
Central	22.9 (15.2)	21.3 (9.2)	
Posterior	25.0 (44.6)	33.9 (34.0)	
$p = 0.45$			
	6% Strain		
	Axial	Radial	$p = 0.03$*
Anterior	52.4 (47.3)	69.1 (62.6)	
Central	30.2 (22.8)	18.7 (3.1)	
Posterior	11.5 (5.8)	55.7 (52.1)	
$p = 0.09$			
	9% Strain		
	Axial	Radial	$p = 0.66$
Anterior	72.9 (77.7)	41.0 (41.7)	
Central	46.0 (28.9)	28.4 (17.5)	
Posterior	36.5 (51.9)	56.3 (78.2)	
$p = 0.82$			
	12% Strain		
	Axial	Radial	$p = 0.58$
Anterior	137.6 (169.8)	102.8 (131.5)	
Central	79.7 (77.6)	29.0 (19.1)	
Posterior	32.8 (46.2)	96.6 (121.3)	
$p = 0.30$			

An * denotes a significant effect ($p < 0.05$).

Table 2. Compressive Moduli (kPa) Measured at a Physiological Loading Rate at 12% Strain Shown as Average (Standard Deviation)

	3% Strain		
	Axial	Radial	$p = 0.29$
Anterior	139.6 (217.0)	100.6 (115.0)	
Central	64.3 (56.4)	51.8 (39.1)	
Posterior	41.2 (60.5)	77.7 (87.4)	
$p = 0.36$			
	6% Strain		
	Axial	Radial	$p = 0.39$
Anterior	276.0 (435.7)	200.6 (232.5)	
Central	128.3 (142.0)	105.1 (80.7)	
Posterior	83.5 (162.3)	112.4 (125.5)	
$p = 0.14$			
	9% Strain		
	Axial	Radial	$p = 0.57$
Anterior	567.4 (876.1)	446.2 (499.6)	
Central	301.9 (369.3)	239.9 (200.3)	
Posterior	184.0 (375.1)	177.9 (194.1)	
$p = 0.09$			
	12% Strain		
	Axial	Radial	$p = 0.61$
Anterior	1,130.3 (1638.4)	965.8 (1,010.0)	
Central	669.0 (797.3)	547.2 (508.0)	
Posterior	356.4 (740.1)	301.0 (310.8)	
$p = 0.04$*			

An * denotes a significant effect ($p < 0.05$).

physiological strain rate at 12% strain ($p = 0.04$). The modulus was significantly greater in the anterior region than the posterior region; averaged for both loading directions, the modulus was about three times larger in the anterior region (1,048 kPa) than that in the posterior region (329 kPa; Table 2). Although not significantly different, the modulus at the three lower strain levels was always greater in the anterior region than the posterior region.

DISCUSSION

The key findings were that the values of the axial and radial compressive moduli at a physiological strain rate were much larger, about a factor of 8 at 12% strain, than the compressive moduli at equilibrium. In general, the axial and radial moduli were not significantly different at equilibrium or at a physiological strain rate at a given strain. However, at the physiological strain rate, the modulus increased substantially as strain increased and was significantly greater in the anterior than in the posterior region.

The measured compressive moduli are useful for tibiofemoral modeling. Haut Donahue et al.[2] determined in a mathematical knee model that the contact pressure distribution of the tibial plateau was sensitive to the axial and radial moduli of the menisci, which were assumed equal. The modulus values in their study were in the range of 15–60 MPa, found from studies which tested bovine and human menisci in tension.[8,21,22] Because the compressive moduli in our study at 12% strain are several orders of magnitudes smaller, they may provide a more accurate contact pressure distribution. The moduli values used in a knee joint model should be based on whether standing or walking is of interest, because these values differ greatly (Tables 1 and 2).

Not only do values of the axial and radial compressive moduli depend on strain rate, but also on the applied stress and/or developed strain. Because Fung's exponential model was fit to the stress-strain data, the modulus computed as the derivative of the exponential model is necessarily linearly related to stress and exponentially related to strain. Accordingly, the modulus can vary widely particularly at the physiological strain rate (Table 2). For example, averaged over both loading directions and the three regions, the compressive modulus varied from 79 kPa at 3% strain to 661 kPa at 12% strain, an almost 10-fold increase.

Our moduli for fresh-frozen human tissue are comparable to those of Gabrion et al.[23] for porcine menisci measured using unconfined compression of samples from the middle region tested at a rate of 10 mm/min. Samples were tested in axial and radial directions to 10% strain, and Fung's model was used to fit the data. The compressive moduli at 2% strain in the radial and axial directions were 0.041 and 0.12 MPa, respectively, comparable to our values at 3% strain of 0.032 and 0.028 MPa at equilibrium averaged over the three regions.

Our coefficients of variation ranged from about 0.5 to 1.5. Gabrion et al.[23] did not report standard deviations; comparable coefficients of variation were seen in other tests using human menisci. Lechner et al.[6] found coefficients for the circumferential tensile modulus ranging from 0.6 to 1.3 for a similarly sized sample as in our study. A source of variation in the tissue might be the condition of the tissue even though no visible damage to either the menisci or articular cartilage was noted. Another source might be a difference in number of circumferential collagen fiber bundles and radial tie fibers in specimens prepared from the same region that caused differences in collagen content.[24]

The compressive modulus at a physiological strain rate was considerably greater than at equilibrium due to the tissue's biphasic behavior.[25] The movement of fluid through the permeable solid matrix creates drag forces contributing to the load bearing with the solid matrix. The tissue should be stiffer at a physiological rate because the high strain rate causes large drag forces that help support the load while at equilibrium stresses are supported solely by the solid matrix. The same is true for articular cartilage; indentation tests showed an increase in stiffness with increasing strain rate from $5 \times 10^{-5}\ s^{-1}$ to $1 \times 10^{3}\ s^{-1}$.[26]

Our results support a constitutive relation with a plane of isotropy, which was used for meniscal tissue in a previous tibiofemoral loading model.[1,2] Both at equilibrium and at a physiological strain rate, the loading direction was insignificant to the modulus, and the moduli in the axial and radial directions were comparable. The anisotropy expected by the presence of radial collagen fibers thought to tie the circumferential fibers together to prevent longitudinal splitting of the tissue did not significantly affect compressive modulus.[24] Our results do not fully validate a plane of isotropy for material properties of meniscal tissue because other properties (e.g., Poisson's ratios) were not a subject of our study.

While our results support a plane of isotropy, this does not imply a linear constitutive relation as a mathematical model for the stress-strain behavior. Such a relation requires that the modulus be same in tension and compression for a given direction. Our compressive moduli are orders of magnitude smaller than the tensile moduli in the same direction.[8,21,22] This large disparity is likely important in tibiofemoral joint models.

The axial and radial compressive moduli were significantly greater in the anterior region than in other regions particularly at the physiological strain rate. A similar regional dependence was previously reported in confined compression tests of human tissue[9] and is likely due to the higher proteoglycan content in the anterior region.[27] The fixed charge density causes charge–charge repulsive forces and Donnan osmotic pressure, so a higher content would increase the resistance to deformation under compressive load.

In unconfined compression tests, friction occurs at the specimen–platen interface that can affect the load-deformation response of soft tissues.[10,28] The friction effect is influenced by the aspect ratio of the specimen and the friction coefficient of the platen material. To minimize the effect, the aspect ratio of our specimens was near one and Teflon was used for the platen.

To verify that the friction developed at the specimen-platen interface did not affect the load-deformation response of our specimens, a pilot study measuring the transverse strain at the middle of the specimen and the transverse strain at the boundary between the specimen and platen using a CCD camera was conducted. Two test samples were prepared from each region of three menisci and one sample from each region was tested in the axial direction, the other in the radial direction. The average difference between the middle and the boundary strain as a percent of the middle strain was 0.10%.

Freezing the tissue once may have changed the material properties from that of fresh tissue, but it is unlikely that multiple freeze–thaw cycles further affected the material properties. The specimens necessarily had multiple cycles of freeze and thaw due to storage, harvest, and specimen preparation. In a previous study, freezing porcine articular cartilage once and thawing caused significant changes in the aggregate compressive modulus and the viscoelastic behavior.[29] However, another study found that five cycles of freeze and thaw after two initial cycles did not significantly alter either viscoelastic behavior or compressive properties of the temporomandibular joint (TMJ) disk.[30] Strong similarities exist between the TMJ disk and the meniscus regarding the relative amount (~80% dry weight) and primary collagen type (Type I), organization of collagen fibers (primarily circumferential), and the relative amount (~3%–5% dry weight) and type of glycosaminoglycans (primarily chondrotin sulfate).[31] Similar to the TMJ disk, multiple freeze–thaw cycles likely had little effect on meniscal compressive properties.

In summary, the axial and radial compressive moduli in unconfined compression are comparable at equilibrium and at a physiological strain rate at a given strain, but are substantially larger (nearly factor of 10 at 12% strain) at the physiological strain rate. Hence, our results support a plane of isotropy for the material properties of meniscal tissue. Owing to large differences in the modulus in tension versus compression, however, the material behavior is strongly nonlinear. Due to viscoelastic effects, the compressive moduli depend on the activity of interest (i.e., static vs. dynamic). For dynamic activities, the compressive moduli also depend on the applied stress and/or developed strain and the regions of the tissue.

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