The Effect of Prior Compression Tests on the Plantar Soft Tissue Compressive and Shear Properties

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Changes in the shear plantar soft tissue properties with diabetes are believed to play a role in plantar ulceration, yet little is known about these properties. Our group recently conducted shear tests on specimens previously tested in compression to fully characterize the tissue under both these loading modes. However, previously tested specimens may not necessarily provide representative mechanical properties as prior testing may have altered the tissue to an unknown extent. Thus, the purpose of this study was to test the effect of prior compression testing on both the plantar soft tissue shear and compressive properties using paired specimens. First, one specimen from each pair was subject to compression using our standard protocol with modifications to compare compressive properties before and after the protocol while the other specimen from each pair was left untested. Then, both specimens (i.e., one previously compression tested and one previously untested) were subject to shear testing. The results indicate that prior compression testing may affect the tissue compressive properties by reducing peak stress and modulus; however, additional testing is needed since these results were likely confounded by stress softening effects. In contrast, neither the elastic nor the viscoelastic plantar soft tissue shear properties were affected by prior testing in compression, indicating that previously compression tested specimens should be viable for use in future shear tests. However, these results are limited given the small sample size of the study and the fact that only nondiabetic specimens were examined. [DOI: 10.1115/1.4024572]

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Introduction

Plantar ulceration in the presence of diabetes can lead to amputation of the affected limb [1]. An understanding of the disease-induced tissue property changes that contribute to plantar ulceration requires first quantifying these changes under relevant plantar loading, i.e., compression and shear. Towards this goal, our group has evaluated diabetic changes in the compressive [2,3] and histomorphological [4] properties of the plantar soft tissue. We also recently obtained the corresponding shear properties from the specimens previously tested in compression [5]. However, it is unclear whether these shear properties are representative since prior compressive testing may have altered the tissue. Furthermore, it would also be useful to know whether the compressive properties are affected by previous testing.

The purpose of this study was to test the effect of prior compression testing on both the plantar soft tissue compressive and shear properties by comparing the results of paired fresh-frozen nondiabetic cadaveric specimens. One specimen from each pair was subjected to compression testing (while examining the pre and post-test compressive properties for changes) before being refrozen/thawed and tested in shear to obtain previously compression tested shear properties. The other untested specimen was subject to the same refreezing/thawing cycle before being tested in shear to obtain previously untested shear properties.

Methods

Four pairs of specimens were isolated per previous methods [2] from the calcaneus and lateral midfoot ($n=8$) from three fresh-frozen, nondiabetic older cadaveric donors (77 ± 4 years, 53 ± 12 kg). Prior to refreezing specimens for shear testing, one from each pair was tested in compression while testing properties before and after (Part A), whereas the other remained untested. Both specimens from each pair were then tested in shear (Part B).

Part A: Compression Testing. Four cylindrical specimens (one from each pair, 19.10 mm diameter, 7.70 ± 2.51 mm thick) were tested in compression using our standard compression protocol [2,3] but modified to include an initial triangle wave test just prior to the standard testing (to obtain previously untested compressive properties) and an identical triangle wave test after the standard testing and recovery period (to obtain previously compression tested compressive properties).

Each of the four specimens (Fig. 1) was placed between sandpaper-covered platens in an environmental chamber ($\sim 100\%$ humidity and 35°C) to approximate in vivo boundary conditions and tested using an ElectroForce 3200 materials testing machine (Bose Corporation, Eden Prairie, MN). Specimens were subject to 30 triangle waves at a frequency of 1 Hz and to 40% strain to obtain previously untested properties before performing the standard compression protocol. Data obtained by performing the standard protocol were not considered as these methods were just used as a treatment. This protocol consisted of ten load control sine waves to reach a target displacement, a displacement control machine tuning period to minimize strain errors, a stress relaxation test (ten preconditioning sine waves, a 0.1 s ramp and 300 s hold to the target displacement), and 30 triangle waves at each frequency of 1, 2, 3, 5, and 10 Hz. After recovering on ice for one hour, specimens were tested using the initial 1 Hz triangle wave test to 40% strain to obtain previously compression tested properties. This entire compression protocol is illustrated in Fig. 2. Specimens were then wrapped in saline-soaked paper towels, vacuum-sealed (in containers to prevent deformation), and refrozen (-20°C).

Nonlinear elastic parameters calculated from the 40% strain triangle data (MATLAB 7.1, The MathWorks, Inc., Natick, MA) included peak strain, peak stress, modulus (slope of the loading stress-strain curve after the inflection), and energy loss (area between the loading and unloading stress-strain curves). Note that parameters were calculated from waves 27, 28, and 29 (the initial

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Fig. 1 Experimental setup with (a) paired specimen locations (only one from each pair was tested in compression) at the lateral midfoot (la) and calcaneus (ca), and (b) sample specimen before skin removal as well as (c) specimen in environmental chamber between sand-paper covered platens (prior to sealing chamber to maintain in vivo conditions of near 100% humidity and 35 °C)

Fig. 2 Illustration of compression protocol showing compressive strain as a function of time starting with (a) 1 Hz triangle waves to 40% strain to obtain previously untested properties followed by (b) the standard compression protocol (specimen-specific load control sine waves to a target strain, machine tuning, preconditioning sine waves, a ramp, and hold, and triangle waves at 1, 2, 3, 5, and 10 Hz) and finally after recovery (c) a repeat of the initial 1 Hz triangle wave test to 40% strain to obtain previously compression tested properties

26 waves were needed to tune the testing software to attain the target strain and for preconditioning, and the final 30th wave was omitted). Linear mixed effects regression was used to test for within specimen differences between treatments, i.e., previously untested (U) versus previously compression tested (C), with specimen and specimen across treatment interaction as random effects. Models were estimated using R 2.11.1 [6].

Part B: Shear Testing. For shear testing, all eight specimens were punched again while frozen (reduced to a 12.7 mm diameter to prevent load cell overload) and cut (to prevent buckling) using a custom guillotine (Fig. 3) to a uniform initial thickness (4.40 ± 0.25 mm). Specimens were placed in an environmental chamber ($\sim 100\%$ humidity and 35 °C) and adhered to the shear tester platens (Mach-1, Biomomentum, Laval, QC) using cyanoacrylate. To emulate biomechanically realistic loading patterns, a 45% static compressive strain was applied prior to shearing the tissue to 81% (based on initial thickness and equivalent to a shear strain angle of 55.7 deg). These compressive and shear strains

were estimated using in vivo fluoroscope images of barefoot running for an adult male [7]. Hence, the 45% static compression was immediately followed by 14 triangle waves at each frequency of 1, 2, and 3 Hz and a 0.25 s ramp and 300 s hold to the 81% target shear strain (Fig. 4).

Similar to Part A, nonlinear elastic parameters were computed except due to differently shaped loading curves, instead of a single modulus, three moduli were computed to accommodate the S-shaped curve [5]: initial modulus (to capture the short initial highly stiff region at low strains), toe modulus (to capture a long low stiffness toe region), and final modulus (to capture a final stiff region at higher strains). Additionally, the relaxation data were used to quantify viscoelastic parameters including the relaxation rate (slope of the force-time curve) at three time intervals, initial ($t = 0-0.5$ s), mid ($t = 10-15$ s), and final ($t = 290-300$ s), and the relative total relaxation (normalized curve area). Again, linear mixed effects regression was used to test for within specimen/within frequency differences in shear elastic measures by treatment [6]. Paired t-tests were used to test for differences in the shear relaxation variables.

Fig. 3 Shear test preparation showing (a)–(b) use of custom guillotine device to ensure uniform thickness before placing (c) specimen in environmental chamber and adhering to bottom platen using sandpaper and cyanoacrylate and (d) then sealing chamber and pumping moist warm air into the testing chamber to maintain in vivo conditions of near 100% humidity and $\sim 35^\circ\text{C}$. Note: the stain on the specimens in (b) and (c) was used to indicate specimen orientation in subsequent work by our group but not in this study (see limitations section of Discussion).

Fig. 4 Illustration of shear protocol showing shear strain as a function of time (after applying static compression) including (a)–(c) triangle waves at each frequency of 1, 2, and 3 Hz, and (d) a ramp and hold to the target shear strain of 81%

Results

Part A: Effect of Prior Compression Testing on Plantar Soft Tissue Compressive Properties. The compressive elastic parameters obtained prior to performing the full compression protocol were considerably different for both peak stress and modulus (Table 1), despite the fact that a one-hour recovery period was used. However, energy loss did not differ between previously untested and previously compression tested tissue (Fig. 5).

Part B: Effect of Prior Compression Testing on Plantar Soft Tissue Shear Properties. Initial shear testing parameters revealed no differences between previously untested and compression tested specimens (Table 2). The elastic shear parameters for all specimens did not show any differences between treatments either (Table 3), possibly due to variability between donors as speci-

Table 1 Mean (SE) nonlinear elastic compression data parameters

	U	C	p^*
Peak strain (%)	39.99 (3.6e-3)	39.98 (6.7e-3)	0.3
Peak stress (kPa)	31.6 (6.3)	12.9 (4.5)	0.0002
Modulus (kPa)	267 (72)	87 (35)	0.0031
Energy loss (%)	38.2 (1.4)	37.8 (1.6)	0.7

Note: $p < 0.05$ indicates significance for linear mixed effects regression (12 measures in four specimens per treatment and parameter); U = previously untested, C = compression tested.

Fig. 5 Sample stress-strain hysteresis curve for 1 Hz triangle wave compression testing for the same specimen from one foot (A1) showing large differences between treatments where U = previously untested and C = compression tested

Table 2 Mean (SE) initial shear testing parameters for four paired specimens

	U	C	p^*
Initial thickness (mm)	4.40 (0.17)	4.41 (0.07)	0.9
Compressed thickness (mm)	2.42 (0.10)	2.43 (0.04)	0.9
Static compressive stress (kPa)	348 (105)	218 (92)	0.3

Note: $p < 0.05$ indicates significance for paired t-tests (four measures in four specimens per treatment and parameter); U = previously untested, C = compression tested.

Table 3 Mean (SE) nonlinear elastic shear data parameters across all frequencies

	U	C	p^*
Peak strain (%)	80.9 (1.6e-2)	80.9 (1.6e-2)	0.3
Peak stress (kPa)	9.0 (1.5)	9.9 (2.0)	0.6
Initial modulus (kPa)	58 (19)	61 (16)	0.8
Toe modulus (kPa)	5.4 (0.7)	5.3 (0.9)	0.9
Final modulus (kPa)	21.9 (4.0)	25.4 (5.6)	0.3
Energy loss (%)	48.0 (4.2)	42.8 (4.2)	0.2

Note: $p < 0.05$ indicates significance for linear mixed effects regression (12 measures in four specimens per treatment and parameter); U = previously untested, C = compression tested

mens from two of the three feet tested yielded opposite nonlinear elastic trends (Fig. 6). The viscoelastic shear parameters also demonstrated no differences between treatments (Table 4). Unexpectedly, the mean normalized area under the relaxation curve for the previously tested specimens was negative in magnitude. This anomaly was caused by one potential outlier specimen (B4_C) which experienced a large amount of relaxation that went past the zero loading state and into negative shear (Fig. 7). Although this specimen was not omitted, a separate statistical analysis found that omitting it would not have changed the results due to variability of the data.

Of note, the stress-strain curves in shear and compression differed considerably, with an S-shape rather than J-shape due to an initially high stiffness for low strains (Fig. 6). Comparison of compressive and shear elastic parameters for previously untested specimens showed that the shear final modulus was an order of magnitude smaller than the compressive modulus (22 kPa versus 267 kPa), the shear peak stress was three times less than in compression (9 kPa versus 32 kPa), and shear energy loss was ten percent greater (48% versus 38%).

Discussion

While substantial data are available on the plantar soft tissue compressive properties [2,3,8,9], little is known about the shear properties. This study characterizes any potential changes as a result of prior compression testing in the plantar soft tissue shear or compressive properties.

For the compression study, large differences were found in two of the three measured elastic parameters; although energy loss did not differ, peak stress was more than twice and modulus was three times greater in the specimens before testing using the full compression protocol. The reduction in tissue stiffness after prior testing indicates that multiple compression tests on the same specimen yield variable results. These differences are likely due to stress softening, a phenomenon associated with large reductions in stress during successive cycles at a particular strain level compared to the stress on initial loading [10] and which may be attributed to properties at a cellular/molecular level [11]. Since we used several preconditioning cycles at each strain level, this phenomenon would not be expected to have a large effect. However, for

Fig. 6 Sample stress-strain hysteresis curves in shear showing variability between specimens from two different feet (B1 versus B3). U = previously untested, C = compression tested. Note that the curves for both test groups lay on top of each other for the remaining two pairs of specimens (not shown).

Table 4 Mean (SE) viscoelastic shear data parameters obtained from stress relaxation curves

	U	C	p^*
Peak strain (%)	80.9 (1.1e-4)	80.9 (2.8e-4)	0.9
Peak stress (kPa)	4.5 (0.7)	4.8 (1.8)	0.8
Initial slope (kPa/s)	-25.5 (3.0)	-26.4 (9.1)	0.9
Middle slope (kPa/s)	-0.041 (0.005)	-0.033 (0.009)	0.6
Final slope (kPa/s)	-0.0043 (0.0021)	-0.0015 (0.0032)	0.2
Normalized area (N.s/N)	35 (16)	-26 (58)	0.3

Note: $p < 0.05$ indicates significance for paired t-tests (four measures in four specimens per treatment and parameter); U = previously untested, C = compression tested.

materials that are subject to stress-softening, any subsequent stress-strain loading paths depend on the maximum strain the specimen experienced previously. Since we did not maintain the same strain level for all portions of testing, it is likely that the observed loss in stress and stiffness is due to the fact that the strain level of 40% used to quantify differences before and after the standard compression protocol was lower than the standard protocol strain level ($53 \pm 5\%$). This is a limitation of the study and further investigation into the extent that straining the tissue to a higher level would reduce the stress measured at a subsequent lower level is needed. The lower 40% strain level was chosen to avoid overstraining the tissue since the specimen-specific higher strain level was only determined later based on biomechanically realistic loading as part of the standard protocol [2,3].

For the shear study, no differences were found between elastic or viscoelastic parameters. Although it did not statistically differ, the static compressive stress was considerably less for the previ-

ously tested tissue; since this is a compressive parameter, this discrepancy is likely due to stress softening. As such, it appears that the shear properties are not affected by prior testing in compression even though the compressive properties were altered. Since the shear properties were all obtained at one strain level, this finding supports the theory that changes in the compressive properties are likely a result of prior strain-level dependent stress-softening. However, a separate and detailed study, e.g., to see if prior shear testing would affect the shear and compressive properties, would be needed to test this theory. Based on the findings of this study, it may seem acceptable to use previously compression tested specimens in future shear tests to obtain a full set of mechanical properties under both loading modes using similar biomechanically realistic strain levels. However, given the small number of specimens, these results should be interpreted with caution. Furthermore, since only nondiabetic specimens were examined, it remains unclear whether these results may be extrapolated to diabetic tissue which may or may not be at greater risk of tissue deterioration and hence alterations in mechanical properties with excessive testing. We stipulate that our results may apply to diabetic tissue since prior compression testing did not diminish the anticipated increased tissue stiffness in shear for diabetic versus nondiabetic tissue [5].

Other limitations of this study include that different rest durations were not investigated as it was estimated that one hour would be sufficient. Increasing recovery time or potentially refreezing specimens before retesting may reduce any reversible effects and should be considered in future studies. Similarly, peak shear stress was less for the relaxation versus the triangle data, also likely due to stress softening effects/insufficient recovery time. Additionally, specimens from all ulcer-susceptible regions were not examined (e.g., the metatarsal heads) due to limitations of being able to obtain paired specimens from the same foot.

Fig. 7 Normalized stress relaxation curves for all four paired specimens on both (a) a log time scale and (b) regular time scale. U = previously untested, C = compression tested. Note: All curves shown are for decimated data for plotting purposes only (all viscoelastic parameters were computed from the original curves with 300,000 data points); B2_C was a potential outlier compared to the other specimens.

Furthermore, frequency test order was not randomized for the shear tests; as such, trends between frequencies were not examined. Since we did not utilize specimens over a wide age range, it is unclear if aging would affect the experimental results. Finally, specimen orientation in shear (anterior/posterior versus medial/lateral) was not controlled which possibly contributed to variability of the data. This contribution should be small given the orientation of the elastic septae and isotropy of the plantar soft tissue in compression [9].

This study demonstrates that prior compression testing of the plantar soft tissue may alter the compressive properties, but further investigation is needed. Of note, the shear properties were not affected by prior testing in compression, indicating that previously compression tested specimens [5] should provide representative shear properties. However, these results do not necessarily apply to diabetic specimens which were not tested in this study.

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