Multiscale Analysis of Morphology and Mechanics in Tail Tendon from the ZDSD Rat Model of Type 2 Diabetes

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INTRODUCTION

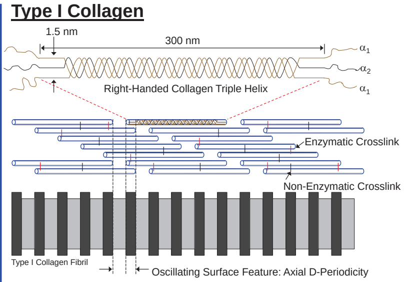
Type 2 Diabetes (T2D)

- Detrimental impacts on multiple systems including the musculoskeletal system
- Chronic hyperglycemia → advanced glycation end product (AGE) formation when reducing sugars react with free amino groups in proteins
- AGEs may stiffen collagen fibrils and impact mechanical properties in collagen-based tissues

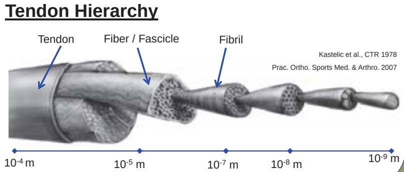
ZDSD Rat as Model of T2D

- Leptin-independent advantage over Zucker Diabetic Fatty (ZDF)
- Gradual diet-induced change simulates human adult-onset diabetes
- Bones have reduced mineral density and mechanical properties (Reinwald et al., 2009)
- ORS 2012: Changes in collagen nanoscale morphology of ZDSD bone and tendon
  - May be important contributor to altered mechanics at larger length scales
- The link between nanoscale changes and tissue structure/function is elusive.

Type I Collagen



Tendon Hierarchy



HYPOTHESIS

Increased stiffness and strength and decreased toughness in ZDSD tendon at fiber level are associated with altered nanoscale morphology and mechanics

MATERIALS AND METHODS

Experimental Groups

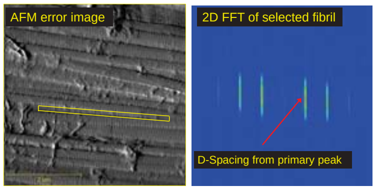
- Female Zucker diabetic Sprague-Dawley rats (ZDSD) and control rats (CD)
- Food diet switched at week 20 from regular chow to high fat test diet for 12 weeks to induce T2D in ZDSD
- Sacrificed at 32 weeks, tail was harvested
- Distal portion used for tensile testing and proximal portion used for AFM imaging and indentation

AFM Imaging and Indentation

- 5 µm images acquired from 2-3 locations in each of 2-3 fascicles per animal
  - All imaging took place fully hydrated
  - Indentation to 20 nN: 5 indents per fibril
  - On average, 70 fibrils indented from each tail for ~300 total indentations per animal.
- All probes calibrated prior to indenting

Collagen Morphological Analysis

- Analysis on 10-15 individual fibrils per location
- D-periodic spacing from 2D Fast Fourier Transform (2D FFT) power spectrum



Nanoscale Mechanical Analysis

- Indentation modulus (E_s): curve fitting from 10% to 70% of unloading curve.
  - Sneddon Model – Indent depth is > than tip radius.
  - Poisson's ratio (ν_s): assume to be 0.35

$$F = \frac{2}{\pi} \cdot \frac{E_s}{1-\nu_s^2} \cdot \tan\alpha \cdot \delta^2$$

Microscale Mechanical Testing

- 10-11 individual fascicles tested from each of 5 CD and 4 ZDSD tails.
- Wet diameter of each fascicle measured at 5 locations along its length at 100X
- Cross sectional area: assumed to be circular
- Displacement control to failure, 0.1 mm/sec.
- Force and displacement recorded at 25 Hz.
- Force normalized by cross sectional area to obtain stress at each data point
- Displacement normalized by original gauge length to obtain strain at each data point.

Statistical Analysis

- CD vs. ZDSD values were compared using non-parametric Mann- Whitney U tests
- D-spacing population distributions:
  - Cumulative Distribution Function(CDF)
  - Kolmogorov-Smirnov(KS) test
- Correlation Analysis
  - Pearson's Product Moment correlational analysis
- p < 0.05 was considered significant

RESULTS

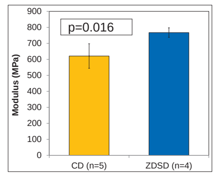
Animal Information

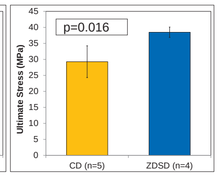
- Significant ↑ final glucose and HbA1 in ZDSD.

	CD(n = 5)	ZDSD (n = 4)	*p* value
Final Body Weight (g)	433± 89	411 ± 30	0.730
Final Glucose (mg/dl)	131 ± 16	472 ± 38	0.016
Final HbA1c (%)	3.9 ± 0.1	103 ± 0.9	0.016

Microscale Mechanical Properties

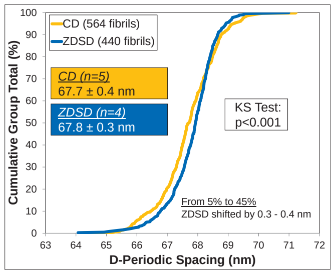
- ↑ Material stiffness and strength in ZDSD





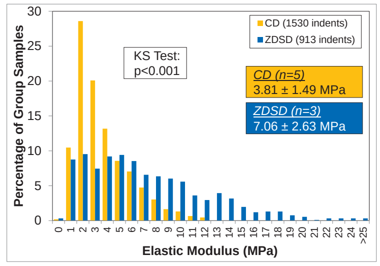
Nanoscale Morphology Changes

- No mean difference in D-Spacing, but significant disease-induced shift ↑ in ZDSD population



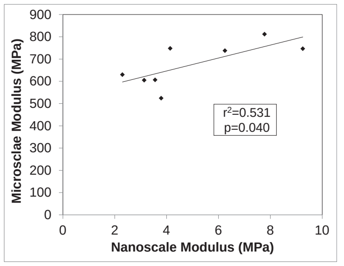
Nanoscale Mechanical Changes

- Nanoscale elastic modulus was more variable and shifted to higher values in ZDSD (mean: p=0.071)



Nano/Microscale Correlation

- Nanoscale and Microscale modulus had a strong and significant positive correlations.



DISCUSSION

- Dietary alteration: ZDSD developed significantly higher blood glucose and Hb1Ac versus CD.
- ZDSD D-spacing distribution was narrower and shifted toward higher spacing values.
  - Both ends of ZDSD population were smaller
  - Bulk of ZDSD population shifted to higher values over majority of its population
  - Odetti et al., 2000: Shift in D-spacing distribution, increased AGEs in diabetic and glucose-incubated tendon
- Swelling study: differential swelling did not significantly contribute to microscale mechanical differences
- ↑ material stiffness and strength in ZDSD, but material underwent less strain before failure.
  - Explained by presence of AGEs in the ZDSD tails?
  - Increased non-enzymatic crosslinking could limit collagen molecules and fibrils from slipping past one another, decreasing overall strain experienced before failure.
  - Same mechanism could increased construct stiffness
  - ↑ D-spacing may be a physical manifestation of AGEs.
- Toughness (energy dissipated) was not altered by the disease state
  - ↑ stiffness/strength enough to offset loss of toughness caused by decreased strain to failure.
- Nanoscale indentation modulus positively and significantly correlated with several microscale measures.
  - Link between indent modulus and microscale strength/stiffness is exciting
  - Suggests that stiffening of fascicles noted in ZDSD rats has its roots at the nanoscale
- AGE quantification and further nanoscale characterizations are needed to understand material changes.

ZDSD rats had no differences in whole fascicle mechanical properties.

Nanoscale changes in collagen morphology and stiffening of individual fibrils in ZDSD are associated with the increase in material-level strength and stiffness.