Increased Tendon Fiber Modulus and Strength and Decreased Failure Strain in a Rat Model of Type 2 Diabetes

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INTRODUCTION

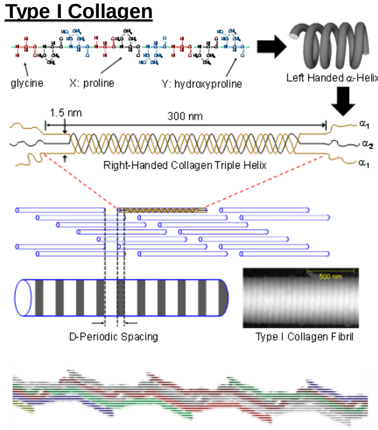
Type 2 Diabetes (T2D)

- 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 in Type I collagen may stiffen collagen fibrils and impact mechanical properties in collagen-based tissues

ZDSD Rat as Model of T2D

- Gradual diet-induced change simulates human adult-onset diabetes
- Bones previously shown to have reduced mineral density and mechanical properties
- ORS 2012: Demonstrated 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



Orgel et al., PNAS, 2006; 103 (24): 9001-9005

SIGNIFICANCE AND HYPOTHESIS

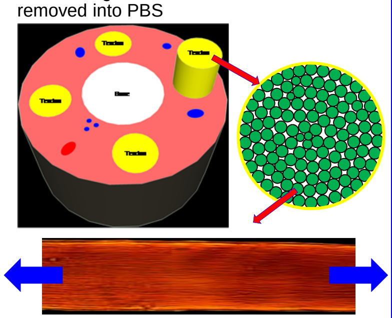
The purpose here is to investigate mechanical properties of individual tail tendon fascicles from normal and diabetic rats

Increased stiffness and strength and decreased toughness will accompany the increased variability that is known to exist in collagen nanoscale morphology of diabetic rat tail tendon.

MATERIALS AND METHODS

Animals and Sample Preparation

- Female Zucker diabetic Sprague-Dawley (ZDSD) and control rats (CD) used
- 20 weeks: diet switched from regular chow to high fat test diet for 12 weeks to induce T2D in ZDSD rats
- All animals sacrificed at 32 weeks.
- 90 mm lengths of individual tendon fascicles removed into PBS



- Wet diameter of each fascicle measured at 5 locations along its length at 100X
  - Mean width calculated from each fascicle
- Cross sectional area was calculated using this width (assuming circular cross section)

Mechanical Testing and Analysis

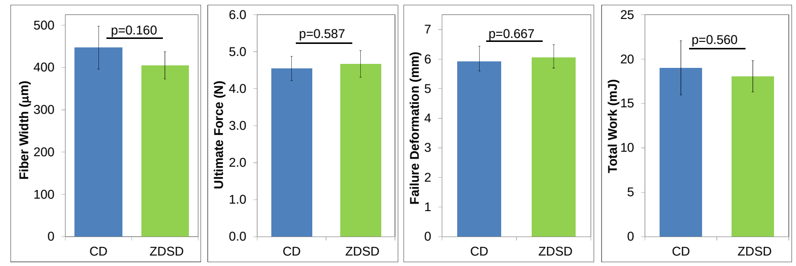
- Fascicle ends sandwiched between 180 grit sandpaper and placed within the grips of a mechanical testing system.
- 10-11 individual fascicles were tested from each of 5 CD and 5 ZDSD tails.
  - Average gauge length of 69 ± 11 mm.
- Testing to failure proceeded in displacement control at 0.1 mm/sec.
- Force and displacement recorded at 25 Hz.
- Force was divided by cross sectional area to obtain stress at each data point.
- Displacement was divided by original gauge length to calculate average strain across the gauge section at each data point.

Statistical Analysis

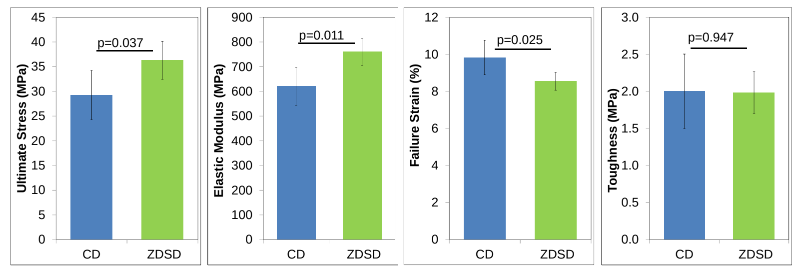
- Width and mechanical property values from all fascicles from each rat were averaged to provide a single value for that animal
- CD vs. ZDSD values (n=5 per group) were compared using Student t-tests
- $p < 0.05$ was considered significant
- All data shown as mean ± standard deviation

RESULTS

- A total 56 CD and 59 ZDSD fascicles were tested from 5 tails from each group.
- At the whole fascicle level, no mechanical properties differed between the 2 groups



- Significant differences existed at the level of the tissue (↑ stress, ↑ modulus, ↓ strain to failure)



DISCUSSION

Previous Work in ZDSD Bone and Tendon

- ↓ mineral density and ↓ structural mechanical properties in the femur and L4 vertebral bodies
  - Also tissue-level changes in vertebrae including ↓ strength, modulus and toughness.
- ORS 2012: To uncover structural mechanism for these changes, atomic force microscopy used to investigate nanoscale morphology in collagen from ZDSD bones and tendons.
  - D-periodic spacing differences in collagen were more pronounced in tendon
  - CD tendons: 74% of all fibrils fell within the mean ± 1 standard deviation (SD) of the CD group versus about 55% for the ZDSD tendons over the same range.
  - ZDSD population was shifted to significantly higher D-spacings.
- Linking changes back to the structural level hindered by the length scale disparity and the tissue hierarchy between the nanoscale and macroscale.

Mechanical Testing of Individual Fascicles

- ↑ material stiffness and strength in ZDSD, but the material underwent less strain before failure.
- Could be explained by the presence of AGEs in the ZDSD tails.
  - Increased non-enzymatic crosslinking could limit collagen molecules and fibrils from slipping past one another, decreasing the overall strain experienced before failure.
  - This same mechanism could increase the stiffness of the construct.
  - ↑ D-spacing in collagen fibrils previously noted could 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.
- AGE quantification and nanoscale characterizations are needed to understand material changes.

Stiffness and strength of individual tendon fascicles were increased in the ZDSD model of T2D, but at the expense of total failure strain.

Studies investigating nanomechanical mechanism and verifying the presence of AGEs in these tissues are underway.