Functionally distinct tendons exhibit architectural differences prior to birth

Theodore J.R. Lownie[1], Samuel P. Veres[1, 2]
[1]Division of Engineering, Saint Mary's University, Halifax, Canada,
[2]Department of Biomedical Engineering, Dalhousie University, Halifax, Canada
Theodore.Lownie@smu.ca, Samuel.Veres@smu.ca

INTRODUCTION: Tendons vary in function, with two main classes currently identified: (i) 'positional tendons' (PTs) that function under low load—chiefly responsible for assisting in the precise positioning of limbs and digits, and (ii) 'energy storing tendons' (ESTs) that have the added role of repeatedly storing and releasing energy under high load to reduce the energy cost of locomotion. Mature PTs and ESTs differ biochemically, structurally, and mechanically.[1] Fetal tendons, in contrast to their mature counterparts, have been proposed to be similar across the two classes.[2] In fact, variance in mechanical loading during early postnatal development has been implicated as a key regulator of class-specific properties.[2] However, multiple structural and mechanical differences between PTs and ESTs at the fetal stage suggest their divergence to instead be intrinsically– rather than mechanically mediated (as an effective absence of inter-tendon variation in mechanical input exists during the prenatal period).[3] Using an anatomically proximate but functionally distinct tendon pair from a large animal model, the present work sought to explore evidence for prenatal structural divergence of PTs and ESTs throughout gestational development.

METHODS: Tendons were dissected from the forelimbs of bovine fetuses (gestational day 100 – 247 of ~283). Consistent with prior studies involving adult tendons, the 'common digital extensor tendon' (CDET) and 'superficial digital flexor tendon' (SDFT) were selected to serve as PT and EST archetypes, respectively.[4] The structures of CDET and SDFT samples taken at different gestational timepoints were compared using 'hydrothermal isometric tension' (HIT) testing, 'transmission electron microscopy' (TEM), and 'polarized light microscopy' (PLM). In HIT testing, force-time-temperature data were collected while samples were heated under isometric constraint. In TEM analysis, mean fibril diameter was determined from cross sections. In PLM, crimp was evaluated from longitudinal cryosections. Statistical analyses were performed using R (version 4.2.2; 2023-01-22).

RESULTS: In HIT testing, 'denaturation temperature' was found to increase at a greater rate in the developing SDFT than in the CDET ($p = 0.002$; $n = 23$ forelimbs, *Figure 1A*). SDFTs also exhibited a higher mean 'temperature of maximum force generation' than did CDETs ($p = 0.04$; $n = 23$, *Figure 1B*). In TEM analysis, CDETs were found to be composed of collagen fibrils with larger mean diameter than SDFTs throughout gestation (*Figure 1C*; ex. 180 day: $p < 0.001$; $n = 10$). In PLM evaluation, crimp appeared better ordered and more prominent in late-stage SDFTs than CDETs (*Figure 1D*) despite being of consistently higher mean wavelength (ex. 245 day: $p < 0.001$, $n = 4$).

DISCUSSION: The present investigation found evidence of architectural idiosyncrasies between the bovine CDET and SDFT during gestation, extending back to early gestational timepoints (close to start of second trimester). The idiosyncrasies largely mirrored class traits in maturity. This suggests that divergence of PTs and ESTs occurs prenatally, and is biologically rather than mechanically mediated.

SIGNIFICANCE/CLINICAL RELEVANCE: Results suggest intrinsic dissimilarity in biological signaling to be a critical mediator of class-specific characteristics. This points to prenatal tendon development as a rich area for future study of the control mechanisms for collagenous tissue structure.

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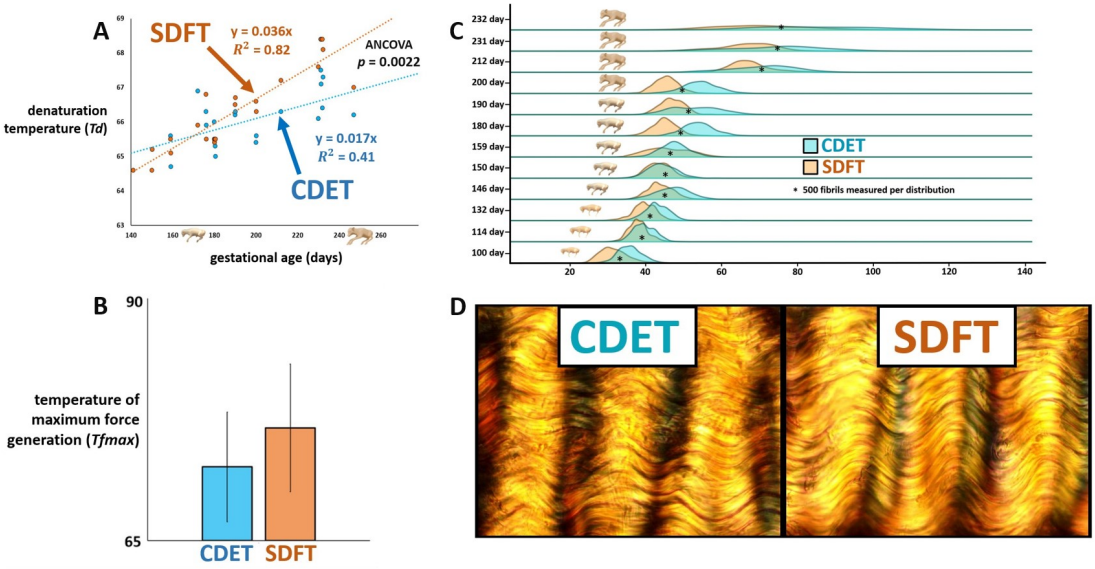

Figure 1: **(A)** The progression of the 'denaturation temperature' (*Td*) values (in °C) of the positional CDET and the energy storing SDFT during the second and third trimesters of fetal development. **(B)** The average CDET and SDFT 'temperature of maximum force generation' (*Tfmax*) values (in °C) of all tendons analyzed. **(C)** Fibril diameter distributions corresponding to twelve fetuses in the second- and third trimesters of development. Central asterisks denote significant results of a 'Kolmogorov-Smirov'(KS) tests ($p < 0.001$). **(D)** Crimp visualized in the positional CDET and energy storing SDFT of a female fetus of age gestational day 247.