

# Athletic training alters equine superficial digital flexor tendon interfascicular matrix mechanics, elastin and lubricin

Sushmitha Durgam<sup>1</sup>, Caitlin Moreno<sup>1</sup>, Sidney Long<sup>1</sup>, Garrett Hoffman<sup>1</sup>, Emily McKinley<sup>1</sup>, Heidi Reesink<sup>3</sup>, Tanya Garcia<sup>3</sup>, Matthew Stewart<sup>2</sup>, Susan Stover<sup>3</sup>  
<sup>1</sup>The Ohio State University, Columbus, OH, <sup>2</sup>University of Illinois, Urbana, IL, <sup>3</sup>University of California, Davis, CA  
 durgam.3@osu.edu

**Disclosures:** SD (N), CM (N), SL (N), GH (N), EM (N), HR (Calyx Biosciences Inc.), TG (N), MS (N), SS (N)

**INTRODUCTION:** The tensile strength and elasticity of the human Achilles and equine superficial digital flexor tendons are conferred by the hierarchical structure consisting of type 1 collagen-rich fascicles interspersed with the interfascicular matrix (IFM).<sup>1</sup> The IFM is composed of non-collagen fibrous proteins, glycoproteins and proteoglycans, mainly, elastin and lubricin with elasticity and interfacial gliding properties, respectively. Although IFM thickness, elastin content and mechanical extensibility of the equine superficial digital flexor tendon (SDFT) decreases in the context of aging;<sup>2,3</sup> to date, the impact of exercise/athletic training on tendon hierarchical structural-compositional dynamicity and on tendon function as they relate to tendon injury mechanisms have not been established. The **objectives of this research** were to a) assess the hierarchical structure b) quantify and immuno-co-localize elastin, desmosine and lubricin in the whole tendon and IFM, respectively, c) determine whole tendon, intact- and elastase-digested fascicle and IFM tensile mechanical properties, and correlate with athletic training data from 2-, 3- and 4-year-old Thoroughbred racehorses.

**METHODS:** Bilateral mid-metacarpal SDFTs from 2- (n=18,10M8F; when training is initiated), 3- (n=14;8M6F) and 4-year-old (n=17;10M7F; training since 2yo and raced >6 times) Thoroughbred racehorses with documented athletic training data, necropsied through CHRB were collected within 48 hours of death/euthanasia under an approved IACUC. **Tendon and fascicle CSA and IFM thickness** were measured in paraffin-embedded, H&E-stained, transverse, and longitudinal SDFT sections (6µm), respectively, by 3 blinded evaluators via ImageJ. **IFM elastin and lubricin area fraction (AF) %** were quantified in immunostained (rabbit anti-elastin primary mAb and FITC secondary; mouse anti-lubricin primary mAb and AF546 secondary) longitudinal SDFT cryosections (15µm) imaged via concurrent immunofluorescence-SHG microscopy and subsequent ImageJ analyses (8-bit image conversion and thresholding for AF%). **Total tendon elastin, desmosine (%DW; dry weight) and lubricin (%WW; wet weight) concentrations** via FASTIN™ absorbance measurement in oxalic acid-digested, equine-specific sandwich ELISA and custom sandwich ELISA in hyaluronidase-extracted SDFTs, respectively.

**Biomechanical evaluation:** Whole tendons and replicate (n=3/horse) fascicle and IFM subunits (dissected via stereomicroscope visualization) underwent 50-cyclical tensile loading cycles to 16 MPa (~5% strain) and then loaded to failure in tension at a strain rate of 2%/second. Specimen CSAs were determined via CT and laser scanner image stacking and MATLAB processing. Yield stress (MPa), yield strain (%), elastic modulus (MPa), maximal stress and strain measurements were derived. **Athletic training data** were gathered via a public database (InCompass Jockey™). **Statistics:** The effect of racehorse age alone on SDFT structure-function parameters was assessed using a mixed-model ANOVA. Whole tendon, intact- and elastase-digested fascicle and IFM tensile stress, strain and moduli, age, athletic training variables, histomorphometry, biochemical composition were analyzed via univariate, and PCA score-guided multivariate linear regression in SPSS. Significance was set at p ≤ 0.05.

**RESULTS:** Fascicle CSA in the core of 3-yo SDFT was 30% decreased (p=0.01) compared to 2-yo racehorses. In contrast, core IFM thickness in 3-yo SDFTs was 26% (p=0.01) increased compared to 2-yo racehorses (Fig 1). IFM elastin AF% decreased (p<0.01) between 2- and 3-yo (Fig 2A,B). Lubricin immunostaining was predominantly restricted to the IFM (Fig 2C); elastin-lubricin colocalization and AF% analyses are ongoing. There were no significant associations between SDFT pepsin/acid soluble collagen %DW and athletic training, however, proteoglycan %DW was inversely related to 'career days' (R=-0.365;p=0.02). Univariate and multivariate analyses demonstrated that elastin (%DW; p=0.03) and desmosine (ng)/mg

elastin (p=0.034), an elastin degradation marker, concentrations exhibited inverse relationships with 'furlongs raced per month' (Fig 3). Additionally, lubricin (ng)/mg tendon %WW was positively correlated with IFM thickness and negatively correlated with 'furlongs raced per month' (Figure 2). Whole tendon pre-yield modulus (r=0.455; p=0.009) and yield stress (r=0.4; p=0.021) were positively correlated with increasing racehorse age. SDFT core (r=-0.302; p=0.05) elastin and lubricin (r=-0.476; p=0.005) contents negatively correlated with number of races per year of active training. Mean±SD of intact and elastase-treated IFM and fascicle yield strain (mm/mm), yield stress (GPa), pre-yield modulus (GPa), ultimate strain (mm/mm), ultimate stress (GPa), and post-yield modulus (GPa) in 2-, 3-, and 4-yo THB racehorse SDFT are compiled in **Table 1**. Elastase treatment significantly decreased the yield strain and failure strain properties of IFM by approximately 60% (p<0.001).

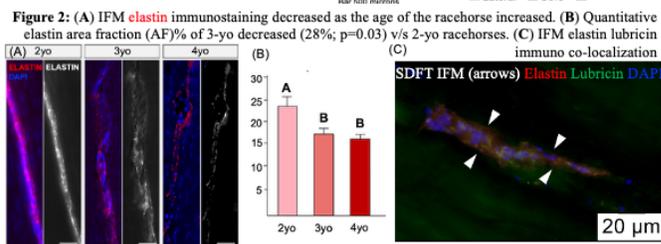
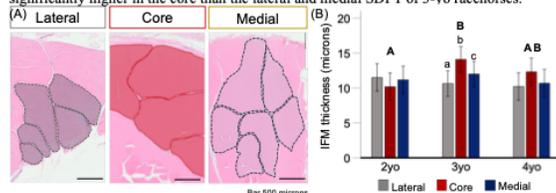
**DISCUSSION:** Athletic training elicits adaptation in the structure, function and biochemical composition of equine SDFT, particularly between the 2- (race training initiated) and 3-yo (peak racing age) age groups. Specifically, IFM thickness increased as reflected in increased IFM tensile yield strain property; even though, in contrast to our hypothesis, total tendon elastin and lubricin contents and IFM elastin AF% decreased. Greater than 90% of elastin and lubricin localized to the IFM. Elastin degradation increased with athletic training and is evident as increased desmosine concentration per unit elastin. Our results suggest that athletic training alters the tendon proteoglycan, elastin and lubricin contents, whereas, interestingly, the collagen content was not significantly impacted. Cyclical tensile tests may help interpret the disconnect where yield strain of IFM increased even though IFM elastin AF% decreased with age and athletic training. While there was no histological evidence of injury in the evaluated SDFTs, differing times (2-24 hrs) of tissue harvest prior to storage and injuries that led to racehorse death/euthanasia are limitations that can affect the results.

**SIGNIFICANCE:** Equine SDFT and the human Achilles tendon bear structure-function analogy, succumb to frequent clinical injury, and are highly prone to reinjury due to fibrotic healing despite lengthy rehabilitation. Our results demonstrating SDFT hierarchical structure-function and elastin-lubricin adaptive remodeling in response to athletic training sheds light on cumulative microdamage mechanisms culminating in clinical tendinitis/tendinopathy.

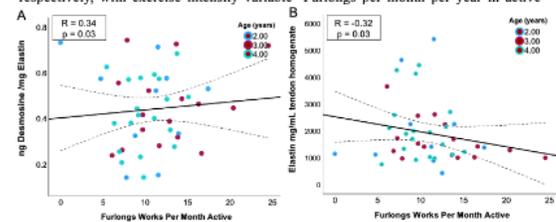
**REFERENCES:** 1. Kannus+ 2005 2. Thorpe+ 2015 3. Godinoh+ 2017

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**Figure 1:** (A) Fascicle cross-sectional area (CSA) was significantly higher in the core than the lateral and medial SDFT. (B) Interfascicular matrix (IFM) thickness was significantly higher in the core than the lateral and medial SDFT of 3-yo racehorses.



**Figure 2:** (A) IFM elastin immunostaining decreased as the age of the racehorse increased. (B) Quantitative elastin area fraction (AF%) of 3-yo decreased (28%; p=0.03) v/s 2-yo racehorses. (C) IFM elastin lubricin immuno co-localization



**Table 1:** Mean ± SD yield strain (mm/mm), yield stress (GPa), pre-yield modulus (GPa), failure strain (mm/mm), failure stress (GPa), and failure modulus (GPa) of intact ('Control') and 5U/mL elastin-depleted ('Elastase') IFM fascicles.

		Yield Strain mm/mm	Yield Stress GPa	Pre-Yield Modulus GPa	Failure Strain mm/mm	Failure Stress GPa	Failure Modulus GPa
IFM	Control (n= 94)	0.12 ± 0.004	0.28 ± 0.02	2.89± 0.19	0.17 ± 0.007	0.33 ± 0.03	1.07 ± 0.01
	Elastase (n= 14)	0.07 ± 0.01	0.17 ± 0.06	2.62 ± 0.54	0.1 ± 0.02	0.18 ± 0.08	-0.30 ± 0.27
	p-value	<0.001	0.093	0.643	<0.001	0.060	<0.001
Fascicle	Control (n= 96)	0.14 ± 0.004	178.3 ± 18.24	1563.4 ± 158.32	0.2 ± 0.007	216.5 ± 22.02	647.7 ± 70.38
	Elastase (n= 19)	0.11 ± 0.10	234.1 ± 143.8	2368.2 ± 280.4	0.15 ± 0.16	254.4 ± 52.9	402 ± 169.1
	p-value	0.006	0.242	0.053	0.003	0.510	0.183