Cellular Energetics Are Altered During Embryonic Tendon Healing

Christoph D. Hart[1], Nishanth E. Sunny[2], Catherine K. Kuo[1,3]
[1]Fischell Department of Bioengineering, University of Maryland, College Park, MD; [2]Department of Animal and Avian Sciences, University of Maryland, College Park, MD, [3]Department of Orthopaedics, University of Maryland School of Medicine, Baltimore, MD
ckk@umd.edu

DISCLOSURES: Christoph Hart (N), Nishanth Sunny (N), Catherine Kuo (N)

INTRODUCTION: Tendon injuries are a significant source of pain and disability, and approaches to regenerate adult tendon after injury remain elusive. Our goal is to develop strategies to promote adult tendon regeneration based on embryonic healing. Embryonic and fetal tendons have greater healing capacity than adult tendons[1,2], but the mechanisms responsible for the improved healing of embryonic and fetal tendons over adult tendons remain unknown. In adult tendons, injury increases energy metabolism during healing[3], experimentally induced inhibition of lactate synthesis leads to improved mechanical properties[4], and inhibition of glucose use leads to improved fiber alignment[5]. Collectively, these studies suggest potential roles for energy metabolism in tendon healing. To date, no studies have investigated energy metabolism of healing embryonic tendons as a potential contributor to their improved healing capacity compared to adult tendons. Here, we begin to investigate the protein synthesis and mitochondrial energy metabolism of healing embryonic tendons by analyzing levels of amino acids and the metabolic intermediates of the tricarboxylic acid (TCA) cycle. We found that embryonic tendons experience altered metabolism after injury compared to their uninjured counterparts.

METHODS: *Injury*: Fertilized white leghorn chicken eggs sourced from the University of Maryland were used for experimentation with approval from the University of Maryland's Institutional Animal Care and Use Committee. At day 16 (D16) of incubation, a window was created in the eggshell to access the embryo's calcaneal tendon. As previously described[2], a transection in the tendon midsubstance spanning the middle 25% of the tendon width was performed on one calcaneal tendon (**Inj**) per embryo. The contralateral tendon served as a non-injured control (**Ctrl**). After injury, the window was covered with tape and the tendon was allowed to heal at 37.5°C in a non-rocking incubator until harvest. *Tissue harvest and metabolomic analysis*: Our previous studies showed extracellular matrix (ECM) was present in the injury site 48h after injury[2]. To capture healing timepoints during ECM formation, embryos were sacrificed via decapitation 12h and 24h after injury. Using a previously established tendon marking protocol[6], tendons were dissected, flash frozen, and homogenized in a lysis buffer for the extraction of intracellular metabolites. Gas chromatography-mass spectrometry (GC-MS) was used to measure levels of 18 metabolites including essential and non-essential amino acids and intermediates of the TCA cycle. Protein content was measured using bicinchoninic acid (BCA) assay. At least N=5 biological replicates, consisting of at least 3 pooled tendons each, were analyzed for each experimental group (Inj and Ctrl). *Statistics:* Paired Student's t-test was used to compare injured and control samples.

RESULTS: All essential and non-essential amino acids analyzed were significantly different between Inj and Ctrl tendons at 24h or both 12h and 24h of healing (**Table 1**). Three of the six TCA cycle intermediates, citrate, lactate, and malate, were significantly different between Inj and Ctrl tendons (**Table 1**). Protein content was higher in Inj tendons at both timepoints compared to Ctrl tendons (**Fig. 1a**). All amino acids and TCA metabolites that were significantly different between Inj and Ctrl tendons were higher in Inj tendons than Ctrl tendons (**Fig. 1b, 1c**).

DISCUSSION: Our data suggest that protein synthesis and mitochondrial energy metabolism are increased in healing embryonic tendons compared to uninjured tendons. As reported in previous studies, adult human tendons have greater levels of glutamate, lactate, and pyruvate during healing than uninjured tendons[7]. In adult mice, lactate, alanine, citrate, glutamate, and aspartate increase after injury[4]. In our study, all metabolites except pyruvate increased in injured embryonic tendons compared to uninjured controls. Future studies will compare protein synthesis and mitochondrial energy metabolism of healing adult and embryonic tendons.

SIGNIFICANCE: This is the first investigation into the cellular energetics of embryonic tendon healing. Findings from this study could be used to inform future development of therapeutic strategies to promote regenerative adult tendon healing.

REFERENCES: [1] Favata et al. 2006, *Journal of Orthopaedic Research* 24(11); [2] Nguyen et al. 2023, *Scientific Reports* 13(1); [3] Ackerman et al. 2021, *Current Rheumatology Reports* 23(3); [4] Zhang et al. 2018, *The American Journal of Sports Medicine* 46(9); [5] Izumi et al. 2022, *Journal of Orthopaedic Research* 40(6); [6] Navarro 2022, *Journal of Biomechanics* 133; [7] Valkering et al. 2017, *Knee Surgery, Sports Traumatology, Arthroscopy* 25(1807-1816)

Comparisons of metabolite levels			
Metabolite group	Metabolite	P-values	
		12h Inj vs Ctrl	24h Inj vs Ctrl
Essential amino acids	Isoleucine	**<0.001**	**<0.001**
	Leucine	**<0.001**	**<0.001**
	Phenylalanine	**<0.001**	**<0.001**
	Threonine	**<0.001**	**0.004**
	Tyrosine	**0.002**	**0.003**
	Valine	**<0.001**	**<0.001**
Nonessential amino acids	Alanine	**0.003**	**0.013**
	Aspartate	**0.000**	**0.001**
	Glutamate	**0.007**	**0.002**
	Glycine	0.166	**0.017**
	Proline	**0.004**	**0.026**
	Serine	**0.003**	**0.018**
TCA intermediates	Citrate	**<0.001**	**0.003**
	Fumarate	0.281	0.431
	Lactate	**0.015**	**0.004**
	Malate	**0.011**	**0.023**
	Pyruvate	0.403	0.191
	Succinate	0.066	0.997

Table 1: P-values for each analyzed metabolite reveal differences in aspartate, fumarate, and glycine. P<0.05 was considered statistically significant.

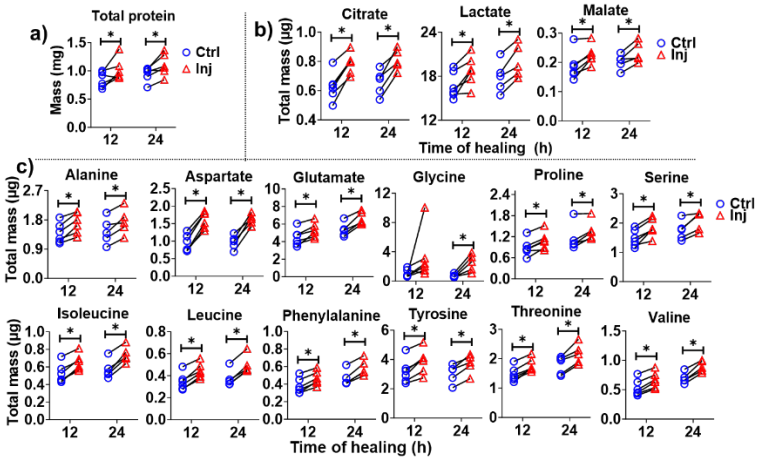

Figure 1: Total protein content (**a**), TCA intermediate masses (**b**), and amino acid masses (**c**) reflect that injury of embryonic tendons affects protein synthesis and energy metabolism. * P<0.05 was considered statistically significant.