Phosphofructokinase-mediated Glycolysis Tunes Extracellular Matrix Remodelling in Human Tendinopathy and In Vitro Models of Tendon Fibrosis

Maja Wolleb[1,2], Amro A. Hussien[1,2], Greta Moschini[1,2,3], Maja Bollhalder[1,2], Barbara Niederoest[1,2], Katrien De Bock[3], Jess G. Snedeker[1,2]

[1]Institute for Biomechanics, ETH Zurich, Zurich, Switzerland. [2]University Hospital Balgrist, University of Zurich, Zurich, Switzerland. [3]Laboratory of Exercise and Health, Department Health Sciences and Technology, ETH Zurich

maja.wolleb@hest.ethz.ch

INTRODUCTION: Extracellular matrix (ECM) homeostasis is a crucial element of tendon health, and the maintenance of functional tissue relies heavily on tensional homeostasis[1]. It is widely appreciated that tendon cells respond to the altered mechanical cues by initiating processes that functionally adapt the ECM. Although we begin to understand the molecular mechanisms underlying tension-mediated ECM remodeling, we lack a general picture of the sequence of events that leads to the disruption of ECM homeostasis in tendon. Here, we investigated how cellular contractility relates to glycolysis and tendon ECM homeostasis. We hypothesized that matrix tension, cytoskeletal reinforcement, and glycolysis converge to regulate ECM turnover. First, we investigated the transcriptomic and metabolic profile of human tendinopathic biopsies. This showed a substantial metabolic switch along with activation of catabolic ECM processes. With an in vitro 2D mechano-variant model, we then mechanistically connected the impact of tenocyte metabolism on the matrix by tuning the rate-limiting glycolytic enzyme PFKP. Additionally, we probed the role the mechanosensitive transcriptional regulator YAP/TAZ in this relationship.

METHODS: All procedures involving human tissues were ethically approved by the approprate oversight boards. Informed consent was obtained from all human donors. *Clinical tendinopathy:* Human tendon samples were collected from patients undergoing surgical reconstruction of the anterior cruciate ligament or rotator cuff tears. *RNAseq:* Illumina libraries were prepared with the TruSeq Stranded Total RNA Sample Preparation kit. *Bioinformatic analyses* were carried out using *gProfiler and EnrichR*. *Targeted Metabolomics:* Human tendons were cryo-milled in liquid nitrogen. Metabolites were extracted from pulverized tissues in methanol-based extraction buffer at -80 ℃ overnight. Samples were loaded into a Dionex UltiMate 3000 LC System equipped with a C-18 column coupled to a Q-Exactive Orbitrap mass spectrometer. Data were analyzed by integrating the peak areas. *Mechanovariant substrates:* PDMS substrates at desired Young's Moduli were prepared and coated with collagen type I as previously described [2]. *Cell culture:* All *in vitro* experiments were performed in DMEM low glucose (1 g/L), unless stated otherwise. *RT-qPCR:* All genes were normalized to *B2m* and *Anxa5* reference genes. *Fusion-protein overexpression*: mScarlet-tagged PFKP glycolytic enzyme was stably overexpressed using a lentiviral transduction protocol. *Glucose metabolic flux*: Glucose metabolism was assessed using ^{14}C-labeled glucose tracers. *Metabolic labeling of newly synthesized protein:* Nascent protein deposition was labeled by incorporation of methionine analog, L-azidohomoalanine (AHA), and visualized with fluorescent cyclooctynes via copper-free cycloaddition. *Functional ECM degradation:* Tenocytes catabolic activity was assessed by measuring the proteolytic digestion of highly-quenched fluorescein-labeled gelatin (DQ-gelatin™) coated PDMS. *Statistics:* Data analysis was performed using two-way ANOVA or t-tests. All data are visualized as Mean ± SEM.

RESULTS: Transcriptomic analysis of diseased human tendons shows a significant enrichment of processes related to glycolysis and fibrosis-associated ECM remodeling, with upregulation of glycolytic (e.g. *PFKP*, *HK1*) and matrisome-related (*COL1A1*, *COL3A1*, *MMP13*) genes **(Fig. 1A-B).** Targeted metabolomics further confirmed these findings and revealed significant abundance of glycolysis intermediaries (e.g. GAP, DHAP) and L-lactic acid **(Fig 1C).** We previously showed that loss of ECM tension and subsequent actin cytoskeletal contractility correlated with proteolytic (e.g. MMP9) activity and glycolytic switch in tendon *ex vivo* models [3]. To mechanistically uncouple the contribution of cytoskeletal tension, we moved to a 2D mechano-variant PDMS model with tunable elasticity **(Fig. 2A)**. Interestingly, we found a dose-response increase in the enzymatic activity of Phosphofructokinase (PFK) (p=0.0276) and glucose flux (p=0.0222) on the conditions that potentiated F-actin and pMLCII processes (i.e. 180 kPa) **(Fig. 2B).** Increasing the available glucose substrate (an approach commonly employed to provoke glycolysis) mediated a catabolic phenotype in tendon cells with upregulation of MMP9 and MMP13 and functional degradation of ECM in high glucose compared to low glucose cultures **(Fig. 2C-F).** Next, we tuned glycolysis using a genetic model by overexpressing the rate-limiting enzyme PFKP **(Fig. 2G).** Tenocytes overexpressing PFKP showed an increase in *MMP13* transcripts **(Fig. 2H)** and significant functional degradation of fluorescent DQ-gelatin substrates **(Fig. 2I)**. Inhibition of YAP/TAZ activity by Verteporfin resulted in a significantly decreased PFK activity **(Fig. 2J)** and PFKP-mScarlet intensity **(Fig. 2K)**.

DISCUSSION: This work interlinks contractile cytoskeletal reinforcement with an associated PFKP mediated regulation of glycolysis, and with consequent tendon ECM turnover. Our data support the hypothesis that mechanically potentiated glycolysis can regulate matrix turnover. We revealed this relation by tuning PFKP regulated metabolic activity of tenocytes and characterizing how this interacts with cytoskeletal reinforcement (increased contractility). We revealed mechanical-metabolic crosstalk to involve the mechanosensitive transcriptional regulator YAP/TAZ, which appears to act as a contextual potentiator of matrix synthesis and turnover [4]. We consider this work to provide an important piece of the puzzle in connecting matrix adaptation and cellular metabolism with important consequences of this bilateral interaction on tendon matrix homeostasis and tendon disease.

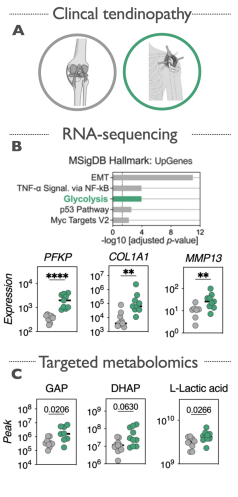

Figure 1: (A) Sample collection and analysis of (B) transcriptional and (C) metabolic processes (n=6) in tendinopathy.

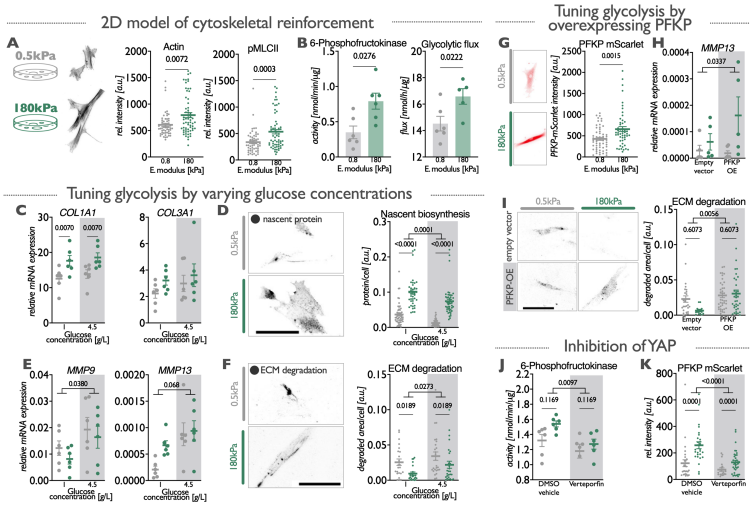

Figure 2: (A) In vitro model of tenocyte cytoskeletal reinforcement (180kPa) and (B) increase of PFK activity, glucose flux and (C) increase in anabolic gene expression and (D) protein synthesis. ECM catabolism is decreased (E) on the transcriptional level and (F) functional ECM degradation but elevated in high glucose conditions. (G) PFKP-mScarlet intensity on stiff substrates as a function of glycolytic activity. (H) Upregulation of catabolic genes and (I) functional ECM degradation of PFKP-OE tenocytes. (J) YAP inhibition inhibits PFK activity and (K) PFKP-mScarlet intensity. Scale bar = 100µm.

CLINICAL RELEVANCE: Deciphering the relationship between mechanical tension, metabolic reprogramming, and extracellular matrix homeostasis holds the possibility to disrupt disease progress in chronic tendon disorders.

REFERENCES: (1) Millar et. Al, *Nature Reviews Disease Primers*, 2021. (2) Hussien et. Al, *Adv. Healthc. Mater*, 2023. (3) Wunderli et. Al, *Matrix Biology*, 2019. (4) Jones et. Al, *PNAS*, 2021.