MECHANOBIOLOGY

Shear mechanosensing drives tendon adaptation

In tendon cells under shear stress, the induction of an influx of calcium by the mechanosensitive ion channel PIEZO1 upregulates collagen crosslinking, which increases tendon stiffness and potentially improves jumping performance.

Jeremy D. Eekhoff, Leanne E. Iannucci and Spencer P. Lake

Tendons, which transmit tensile forces between muscle and bone, function as mechanical springs by storing and releasing elastic strain energy to increase the efficiency of the locomotive system[1]. Within the hierarchically arranged microstructure of tendon, cells residing between collagen fibres experience shear loading when the fibres slide past one another as the tendon is strained[2,3]. Tendon cells sense and respond to this mechanical stimulus, enacting biological changes as a result of decreased or increased loading, and modulating the properties of the whole tissue[4–6]. However, the specific pathways through which mechanical signals in tendon are detected by the tenocytes and translated into chemical signals that initiate a biological response are unknown. Reporting in *Nature Biomedical Engineering*, Jess Snedeker and colleagues now show that, in tendon cells, the mechanosensitive ion channel PIEZO1 (which undergoes conformational changes in response to mechanical load and plays an important mechanobiological role in a wide range of tissues[7–9]) is responsible for mechanically induced calcium signalling[10]. The researchers propose a feedback mechanism by which tendon performance is improved through increased collagen crosslinking in response to PIEZO1 signalling.

Through simultaneous mechanical loading of rat-tail tendon explants and fluorescent calcium imaging of the tissue-resident tenocytes, Snedeker and co-authors observed that stretch-induced intracellular calcium signalling was dependent on the strain rate applied, and that the amount of strain required to elicit a response in at least half of the resident tenocytes was consistently near the upper limit of the expected physiological range. On the basis of these observations, the authors hypothesized that the mechanosensitive properties of tenocytes may stimulate the onset of adaptive tissue remodelling in vivo.

To test this premise, Snedeker and co-authors first used microfluidics to investigate the mechanosensitivity of the resident cell population to shear stress.

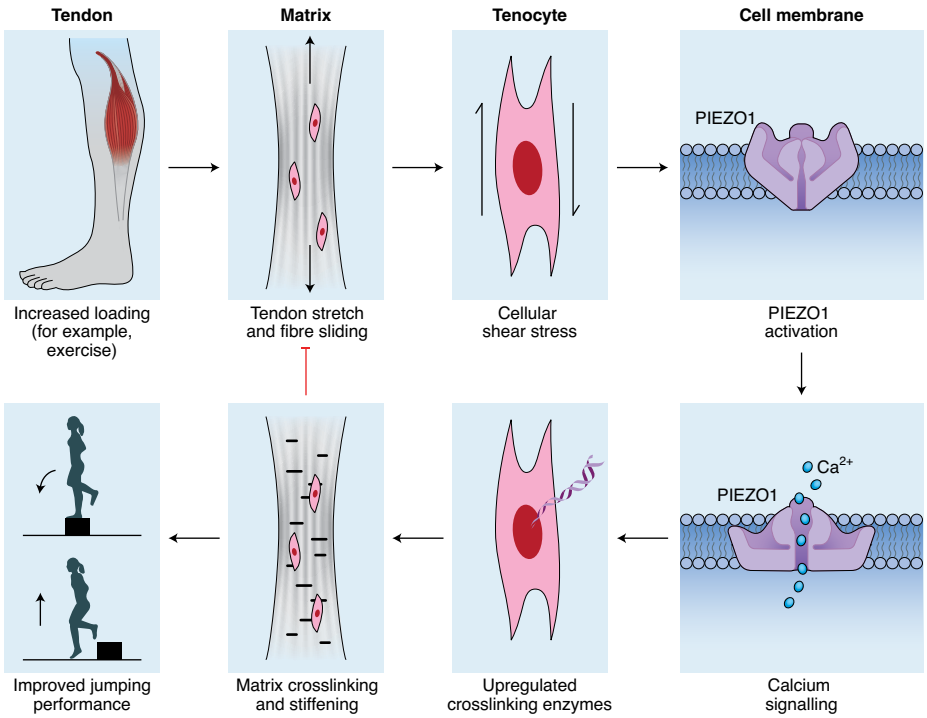

Fig. 1 | Shear mechanosensing in tenocytes regulates tendon adaptation. In the process of supporting load, tendons undergo stretch and fibre sliding, which causes shear stresses on resident tenocytes. Shear forces over a mechanical threshold result in PIEZO1 activation, calcium signalling and the downstream upregulation of crosslinking enzymes. The crosslinked and stiffened matrix may result in improved jumping performance. Additionally, tissue-level adaptation acts as a negative feedback mechanism by which subsequent loading results in less cellular shear stress and in a muted response. Schematic for 'Improved jumping performance' adapted with permission from ref. [10], Springer Nature Ltd.

Isolated primary tenocytes subjected to physiological levels of shear via fluid flow exhibited calcium responses that were dependent on the magnitude of the applied shear stress but not on the tenocytes' anatomical location. Through CRISPR–Cas9-mediated screening of genes associated with mechanosensitive ion channels with high expression in tendon, the authors then identified PIEZO1 as the sole gene that, when knocked down, resulted in a depletion of the shear-sensing calcium response in tenocytes (both for isolated tenocytes and for tenocytes in situ). By generating a tendon-specific PIEZO1 knockout mouse model, the authors then probed the effect of the reduction of PIEZO1 on tenocyte mechanotransduction. PIEZO1-conditional knockout mice exhibited impaired calcium-signalling responses to stretch, despite a decrease in PIEZO1 expression of only 40%. Also, tendons from the conditional knockout animals showed decreased stiffness but did not change in diameter. To further examine the potential role of PIEZO1 in regulating the mechanical properties of tendon, the authors stimulated tendon explants with the PIEZO1-agonist

Yoda1 for over two weeks; they found that the tissue displayed increased stiffness yet no changes in diameter. To verify the role of PIEZO1 in tissue remodelling in vivo, the authors measured the mechanical properties of tendon from PIEZO1 gain-of-function mice. Similarly, these tendons exhibited increased stiffness, by an amount comparable to the decrease of stiffness for the in situ models of PIEZO1 knockout and also to the stiffness increase for the Yoda1-stimulated tendon. Moreover, for PIEZO1 gain-of-function mice, stiffness increases seemed to be greater in load-bearing tendon (foot-flexor tendon) than in positional tendon (tail tendon), indicating that the downstream effects of PIEZO1 activation may be more robust in load-bearing conditions.

In rat tendon explants, Yoda1 stimulation or repeated loading over a mechanical threshold resulted in the upregulation of the crosslinking enzyme lysyl oxidase. This led Snedeker and co-authors to hypothesize that PIEZO1 activation modulates tissue stiffness through collagen crosslinking (Fig. 1). Through differential scanning calorimetry and crosslink autofluorescence imaging, tendons from PIEZO1 gain-of-function mice appeared to have a denser crosslink network than tendons from wild-type animals. The authors proposed that PIEZO1-induced collagen crosslinking in tendon functions as a 'mechanostat'; that is, on the basis of the input forces, the crosslink density changes to modulate shear stresses on resident cells and to control the mechanical properties at the tissue level.

Snedeker and co-authors also investigated whether PIEZO1-mediated tendon stiffening is related to athletic performance in humans. By taking advantage of the most well-known PIEZO1 gain-of-function mutation in humans to date, E756del, recruited individuals were grouped as either E756del-carriers (heterozygous or homozygous) or non-carrier controls, with similar demographics between groups. Although there were no differences in Achilles tendon morphology or in physical-activity levels between groups, E756del carriers overall performed better than non-carriers in a drop countermovement jump (a vertical jump on one leg after a downward drop from a predetermined height), compared to a static countermovement jump (a vertical jump on one leg from an upright standing position via a downward flexion of the knees and hips); the non-carriers overall performed similarly for these two jumping tests. The improved ability of E756del carriers in jumping manoeuvres that involve a high rate of energy storage may be related to the hypothesized increased crosslink-mediated stiffness of tendon resulting from the PIEZO1 gain-of-function mutation.

The identification of PIEZO1 as the driving factor of mechanically induced calcium signalling in tendon advances the understanding of tendon mechanobiology, and provides a specific mechanism for the long-recognized phenomenon of mechanosensation in resident tendon cells. Snedeker and colleagues' work adds to the growing body of literature describing the role of PIEZO1 in a wide range of tissues, and confirms previous reports of PIEZO1 activation by shear forces[8]. A simple mathematical model of shear stress on tenocytes informed by experimental data of interfibre sliding along with in vitro experiments of cells isolated from tendon in a microfluidic flow chamber demonstrated that calcium signalling is activated by shear stresses of similar magnitude to what are expected in vivo. Tissue-level mechanical changes (such as tendon stiffness) resulting from altered PIEZO1 signalling in both loss-of-function and gain-of-function models highlight the clinical relevance of this mechanosensitive ion channel.

In addition to demonstrating that PIEZO1 signalling influences the mechanical function of tendon, Snedeker and colleagues also provide evidence suggesting that the tissue-level changes from altered PIEZO1 signalling are a result of enzymatic collagen crosslinking. However, the indirect measurements of crosslinking using differential scanning calorimetry and tissue autofluorescence do not capture the full complexity of the collagen crosslink environment consisting of multiple divalent and trivalent crosslinks, whose profiles vary temporally owing to crosslink maturation[11,12]. Mixed reports on the effect of various forms of exercise on tendon properties, including changes in collagen crosslinking and cross-sectional area, reveal a more nuanced picture, where PIEZO1-induced crosslinking is likely to be one factor among many that influence how tendons adapt in response to mechanical loading[13–15]. Interestingly, the dependence of the strain threshold for PIEZO1 activation on strain rate may have implications for the different effects of endurance and resistance training on tendon mechanoadaptation.

Functional differences in jumping performance between carriers and non-carriers of the E756del mutation and the hypothesized implications for athletic ability are intriguing. It is, however, unclear how increased collagen crosslinking or stiffness would improve the efficiency of the storage and return of elastic strain energy in tendon. More research is needed to evaluate the potential link between PIEZO1-modulated tissue-level changes and the performance of physical tasks, including those dependent on high tendon loading such as jumping and sprinting. Additionally, although genetic variation has some impact on an individual's athletic potential, it is only one of many factors determining overall athletic ability.

Snedeker and colleagues speculate that the improved understanding of how tendon properties are regulated through PIEZO1 could help inform physical therapy to more efficiently exploit the anabolic cellular response to mechanical loading and to improve tendon healing. Also, the administration of a PIEZO1 agonist such as Yoda1 may mimic the effects of loading while reducing the need for a complicated and time-intensive physical therapy regimen. Regardless of the eventual therapeutic implications, a biologically motivated technique that leverages cellular mechanosensation could be uniquely beneficial for situations (such as the presence of concurrent injuries) where physical therapy is difficult or impossible. ❐

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Competing interests

The authors declare no competing interests.