Supplemental Material

Tendon Cell Biology: Effect of Mechanical Loading

Mikołaj Stańczak[a,d] Bartłomiej Kacprzak[b] Piotr Gawda[c,d]

[a]AECC University College, Bournemouth, United Kingdom, [b]Orto Med Sport, Łódź, Poland, [c]Medical University of Lublin, Department of Sports Medicine, Lublin, Poland, [d]Interdisciplinary Scientific Group of Sports Medicine, Department of Sports Medicine, Medical University of Lublin, Lublin, Poland

Table 1. Table provides an in-depth analysis of tendon adaptations to mechanical load, highlighting differences in response to tension, compression, and shear forces, as well as changes during fetal and post-natal development. It outlines cellular and molecular responses, such as collagen synthesis and turnover, influenced by factors like growth hormones (e.g., IGF-1) and sex hormones, particularly estrogen. In adults, tendons show increased stiffness and cross-sectional area with training, but immobilization leads to significant losses in load capacity and stiffness. Gene expression patterns vary based on the tendon's loading environment, promoting region-specific adaptations that optimize tendon structure and function.

Section	Description	Type of Adaptation	Details of Adaptation	Key Mechanisms	Outcomes	References
Tendon Adaptation to Load	Tendons respond to various types of mechanical loads, including tension, compression, and shear.	Tension, Compression, Shear	- Tension: Aligns with load direction, promoting a dense Type I collagen matrix. - Compression: Forms a fibrocartilage phenotype with sparse, unaligned Type I collagen and increased proteoglycans. - Shear: Partially aligned collagen matrix and increased lubricating proteins (e.g., lubricin, hyaluronic acid).	Collagen alignment and proteoglycan modulation	Adaptations optimize tendon strength and durability in response to mechanical stresses.	[51], [74-77]
Fetal Development	Tendon tissue composition evolves in fetal stages, with changes in collagen density and cellularity.	Increased Collagen and Decreased Cellularity	- Collagen content rises, cellularity decreases, and lysyl oxidase-mediated crosslinking increases modulus. - Collagen organization differences in tensile vs. compressive tendon regions become evident late in fetal	Collagen synthesis, crosslink formation, and structural organization	Higher tensile strength and modulus prepare tendons for functional load-bearing.	[51], [65], [78]

			development. - Proteoglycans in compressed regions appear only in the post-natal phase.			
Cellular Response to Load	Tendon cells from tensile and compressive regions respond differently under mechanical load in vitro.	Proteoglycan Synthesis in Response to Cyclic Load	- Cells in compressive areas produce more proteoglycans during cyclic compression. - Specifically, a fivefold increase in aggrecan synthesis and larger decorin forms are produced in response to compression. - TGF-β and cyclic loading boost levels of large proteoglycans (e.g., biglycan).	TGF-β signaling and cyclic loading effects	Differential responses help optimize structure and function in region-specific manners.	[79], [80]
Adaptation to Load in Adults	Adult tendons adapt structurally and functionally to increased loading, enhancing stiffness, cross-sectional area (CSA), and mass.	Tendon Hypertrophy and Increased CSA	- In humans, 2-3 months of training increase tendon stiffness and CSA. - High-intensity exercise improves tendon properties more than low-intensity exercise. - Studies show intercollegiate runners and lifelong runners have larger Achilles tendon CSA than non-runners. - Animal	Direct mechanical loading promotes cellular activity and collagen synthesis in tendons.	Enhanced strength and durability, reducing injury risk under repetitive strain.	[81-89]

			models show running and swimming increase fibroblast density, tendon mass, and CSA.			
Collagen Turnover in Tendons	Collagen turnover rates in tendons vary, with debate on central versus outer tendon regions.	High or Low Turnover Depending on Region	- Core Turnover: 14C bomb pulse studies suggest low turnover after age 17 in central tendon regions. - Outer Turnover: Stable isotope methods indicate higher synthesis rates in the outer tendon regions, suggesting faster turnover outside the core.	Collagen synthesis and degradation; stable isotope tracking	Central core appears stable post-skeletal growth; outer regions more dynamic, responding to load.	[91-94]
Role of Growth Factors (IGF-1)	IGF-1 is critical for tendon hypertrophy and collagen synthesis, activated by loading.	Hypertrophic Growth Response	- IGF-1 levels rise within tendons in response to load and decrease with unloading. - IGF-1 signaling (via PI3K/Akt and ERK pathways) promotes protein synthesis and cell proliferation. - Knockout of IGF-1 receptors in tendon cells reduces hypertrophy under load.	IGF-1 signaling pathway, PI3K/Akt, ERK activation	Supports tendon growth and increases in mechanical properties in response to exercise.	[95-98]
Sex Differences and Estrogen's Role	Collagen synthesis varies in male and female tendons,	Sex-Dependent Collagen Synthesis and Structure	- Estrogen enhances collagen synthesis and incorporation	Estrogen effects on collagen synthesis,	Estrogen enhances collagen integration, but may	[102-105]

	influenced by estrogen levels.		in female tendons. - Post-menopausal women on ERT have more tendon collagen content. - Estrogen can affect tendon CSA, observed in active women using ERT or twin studies where one twin used ERT.	ERT influence	reduce Achilles CSA in females.	
Tendon Gene Expression	Gene expression profiles in tendons adapt to mechanical environments, influencing matrix composition.	Environment-Dependent Gene Expression	- Tensional loading promotes a tendon-specific cell phenotype. - Compressive loads induce fibrocartilage-like gene expression (e.g., aggrecan, decorin, biglycan). - Energy-storing tendons (Achilles, patellar) differ in gene expression from compression-exposed tendons (e.g., supraspinatus).	Epigenetic changes, gene expression modulation	Regional specialization of tendons allows optimization for tension vs. compression.	[49], [80], [106]
Adaptation to Unloading	Tendons lose mechanical strength and stiffness when immobilized, with long recovery times.	Loss in Load Capacity and Stiffness	- Full-body immobilization causes up to a 40% decrease in ACL load capacity and a 30% reduction in stiffness. - Single limb immobilization shows less reduction, suggesting a	Mechanical load removal impacts tissue composition, collagen content	Extended reloading is needed to restore mechanical properties but does not fully recover all structural elements.	[107]

			systemic signal mitigates stiffness loss.			