Topical or Injectable Metformin Ameliorates Age-Related Degeneration in Mouse Achilles Tendon

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INTRODUCTION

Aging of tendons is marked by tenocyte senescence, proteoglycan accumulation, and cellular dedifferentiation—hallmarks of degenerative tendinopathy. In aged murine Achilles tendons, metformin (Met) delivered intraperitoneally (IP) has been shown to reduce inflammatory and senescence markers (dsHMGB1, CD68, SA-β-gal, p53, p16), decrease proteoglycan deposition, and improve tendon microstructure [1]. More broadly, Met is recognized for its systemic anti-aging effects, including modulation of nutrient-sensing pathways, reduction of oxidative stress and inflammation, and mimicking caloric restriction [2]. Given that age-related alterations in tenocyte morphology and extracellular matrix composition compromise tendon integrity [3], we hypothesized that both systemic (IP) and local (topical lotion) Metformin administration would preserve tenocyte identity and function, thereby exerting anti-aging effects in tendon.

METHODS

Six-month-old wildtype mice (6/group, 3 males and 3 females) were treated over 12 months with one of four regimens: (1) Met IP injection (160mg/kg/day), (2) Met lotion (160mg/kg/day), (3) Control lotion, or (4) Untreated control (Control). At 18 months of age, Achilles tendons were harvested and examined using hematoxylin-eosin (H&E) and Safranin O fast green (SO&FG) staining. The tendon degeneration was determined by cell morphology changes. Tenocyte nuclei were semi-quantitatively classified into five categories according to nuclear length-to-width (L/W) ratio: Normal tenocyte (>3.5), Reversible=can be back to tenocyte (3.5–2.5), Oval=is turning to chondrocyte (2.5–1.5), and Round=chondrocyte (<1.5) Nuclei not fitting these criteria were assigned to an Unclassified group (**Fig. 1**). Proteoglycan expression in nuclei was further assessed via SO&FG staining to identify chondrocyte or non-tenogenic differentiation. We analyzed 3 images from every slide located at distal, middle, and proximal part of Achilles tendon in 2 slides per sample and average the results cell/image. Statistics calculated by Mann-Whitney.

RESULTS

Safranin O staining revealed proteoglycan presence only in all non-normal/reversible nuclei, indicating differentiation toward chondrocyte phenotypes during aging (**Fig. 2**). Changed tendon cells with proteoglycan expression prevail in control (**Fig. 2G**) and control lotion (**Fig. 2H**) groups compared to Met IP (**Fig. 2E**) and Met lotion (**Fig. 2F**) groups. Met-IP and Met lotion application showed a trend to increase proportions of normal nuclei and decreased reversible, oval, round, and unclassified nuclei versus controls (**Fig. 3**). Control lotion (**Fig. 3D**) and untreated groups (**Fig. 3C**) exhibited declines in normal/reversible nuclei and rises in abnormal forms.

DISCUSSION

Systemic and local metformin preserved tenocyte morphology and prevented age-associated proteoglycan-positive chondrogenic differentiation. Importantly, our study was limited by a relatively small sample size per group, which may have reduced statistical power. Larger cohorts will be required to confirm the efficacy of topical metformin and determine whether its effects can reach statistical significance.

SIGNIFICANCE

This study demonstrates that metformin, particularly through IP administration, mitigates age-related cellular and structural changes in tendons. Topical delivery also showed promising trends, supporting further investigation as a non-invasive therapeutic strategy.

REFERENCES

1. Zhang J, et al. J Orthop Res. 2023. 2. Sunjaya AP, Sunjaya AF. Diabetes Metab. 2021. 3. Kwan KYC, et al. Int J Mol Sci. 2023.

ACKNOWLEDGEMENTS

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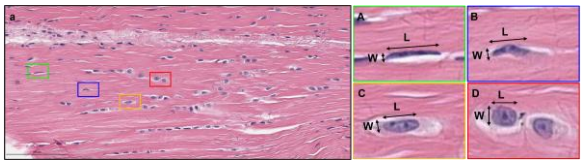

Fig. 1 Classification of tendon cell types by nuclear morphology. Achilles tendons from 18-month-old mice (20× magnification) contained distinct cell populations (**a**). Cells were quantified based on nuclear length-to-width (L/W) ratio: normal tenocytes (L/W > 3.5; **A**), reversibly changed tenocytes (L/W 3.5–2.5; **B**), oval-shaped cells (L/W 2.5–1.5; **C**), and round-shaped cells (L/W < 1.5; **D**).

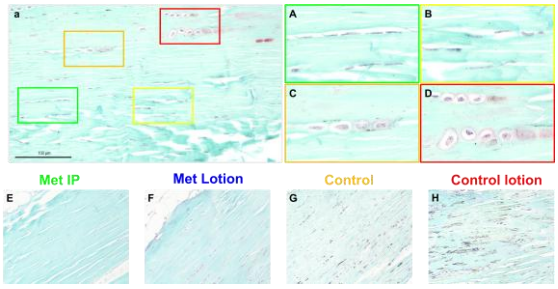

Fig. 2. Met IP and Met lotion application decreases proteoglycan expression in aging tendon.
Mouse Achilles tendon 18-month-old (**a**) contain normal tenocytes and changed tenocytes, as well as chondrocytes. Normal tenocytes with normal nuclei shape (**A**) and reversibly changed tenocytes with curved or thickened nuclei (**B**) do not express proteoglycan deposition, whereas severely changed or chondrogenic-differentiated cells with oval- (**C**), and round-shaped (**D**) nuclei expressed proteoglycan deposition (purple-violet color). Achilles tendon presented by mostly normal tenocytes in Met IP (**E**), much less cells expressed proteoglycan were detected in Met lotion-treated tendons (**F**), compared to Control (**G**), and control lotion-treated mice (**H**) in 40× magnification.

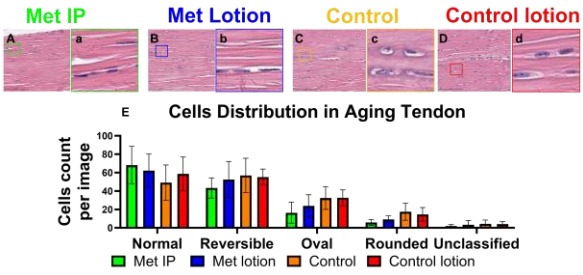

Fig. 3. Met IP and Met lotion application lead to proportional increase of normal tenocytes in Achilles tendon vs tenocytes with reversible changes, severely changed tenocytes and chondrogenic differentiated cells, compared to control and control lotion groups. Metformin increases normal tenocytes and reduces abnormal cell types in aging Achilles tendon. Met IP (**Aa**) and Met lotion (**Bb**) groups showed higher proportions of normal tenocytes and fewer reversibly changed, oval, round, and unclassified cells compared to Control (**Cc**) and Control lotion (**Dd**). Cell distribution quantification is shown in the chart (**E**). $n = 6$ (2 slides/sample; 3 images/slide).