

Application of a Transdermal Metformin Lotion Reduces Systemic and Local Tendon Inflammation in Mice

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DISCLOSURES: J. Zhang: None. D. Maloney: None. V. Pastukh: None. S. Hattori: None. JH-C. Wang: None.

INTRODUCTION: Tendinopathy is manifested as tendon inflammation and degeneration for which mechanical overloading is the major causative factor.

Inflammation is thought to be the hallmark of early stage tendinopathy. Prostaglandin E₂ (PGE₂), interleukin-1 β (IL-1 β), and high mobility group box1 (HMGB1) are potent mediators of inflammation and pain in tendinopathy. HMGB1, an upstream potent inflammatory mediator, has been identified in high levels in early-stage tendinopathy patients [1]. Metformin (Met), an FDA approved oral drug used for the treatment of type-2 diabetes, inhibits HMGB1 [2]. Our previous studies in an animal model of tendinopathy induced by mechanical overloading via intensive treadmill running (ITR) have shown that ITR induces HMGB1 release to tendon matrix, increases PGE₂ levels, and initiates tendinopathy development, and IP injection of Met can prevent ITR-induced tendinopathy [3,4]. However, Met given orally has systemic side effects on the body. Thus, we formulated a Met lotion as a novel transdermal drug to deliver Met directly into the tendon area to prevent tendinopathy development induced by ITR in mice.

METHODS: Met lotion at 3% and 6% concentrations were formulated in our lab and used for this study. A total of 30 mice were randomly divided into three equal groups and treated as follows: **Group 1:** ran on treadmill at 15 meters/min for 3 h/day, 5 days a week for 4 weeks (ITR); **Group 2:** 3% Met-lotion smeared on the skin surface of the hind legs before ITR daily for 4 weeks (ITR+3% Met-lotion); **Group 3:** 6% Met-lotion smeared on the skin surface of the hind legs before ITR daily for 4 weeks (ITR+6% Met-lotion). All mice were sacrificed after 4 weeks of ITR. Blood was immediately collected from each mouse for analyzing inflammatory markers HMGB1, PGE₂, IL-1 β , and HMGB1 using ELISA. Furthermore, Achilles tendon tissues were collected to conduct immunostaining for HMGB1 expression and perform histological analysis. **Statistical analysis** – The data underwent analysis using a two-tailed Student *t*-test, with five samples used in each group for every test. A *p*-value below 0.05 was regarded as indicative of significant differences between the two groups. This study was approved by IACUC.

RESULTS: Met lotion application decreased the high levels of PGE₂ and IL-1 β in the sera of mice after ITR for 4 weeks in a concentration-dependent manner (Fig. 1A, B). The immunostaining results from Triton-X-100 treated samples indicated that ITR induced HMGB1 release from cell nuclei to tendon matrix (red fluorescence in Fig. 2A, B), and many nuclei in ITR groups were negatively stained with HMGB1 (white arrows in Fig. 2A, B). However, Met lotion treatment inhibited HMGB1 release from the cell nuclei to tendon matrix. The nuclei of the most of cells in Met lotion treated groups were positively stained with HMGB1 (yellow arrows in Fig. 2C-F). Without Triton-X-100 treatment, very low levels of HMGB1 were positively stained in Met lotion treated tendons (Fig. 2I-L), however, high levels of HMGB1 were found in ITR groups (Fig. 2G-H). The inhibition effect of Met lotion was in a concentration-dependent manner as evidenced by the expression of HMGB1 in the tendon matrix of all samples is ITR > ITR+3% Met-lotion > ITR+6% Met-lotion. HMGB1 levels in serum agree with this finding (Fig. 2M). Histological analysis results showed that tendon cells in ITR tendon were round with cavities around cells (yellow arrows in Fig. 3C), while neither obvious structural change nor cartilage-like cells were found in the Achilles tendons of Met-lotion treated mice (Fig. 3E-L). The H&E staining results also indicated that ITR induced inflammation in paratenon tissues (red arrows in Fig. 3B, D). ITR also induced degenerative changes in tendon-bone junction areas as evidenced by small, thin and broken enthesis and many cartilage-like cells (yellow arrows in C) found in the tendons of the mice after 4 weeks ITR. However, Met lotion treatment inhibited inflammation as evidenced by the less inflammatory cells found in the tendon and paratenon tissues. Met lotion also decreased degenerative changes as evidenced by the elongated cells in the tendons treated with both concentrations of Met lotion (green arrows in Fig. 3G, K).

DISCUSSION: This study has shown that Met lotion as a transdermal application inhibited inflammation and degeneration, typical tendinopathy features, in an animal model of ITR-induced Achilles tendinopathy. Our study has also demonstrated that mechanical overloading induced typical inflammatory and degenerative changes of tendinopathy in mouse tendons characterized by HMGB1 release from the cell nuclei into tendon matrix accompanied with elevated levels of PGE₂ and IL-1 β and presence of chondrocyte-like cells. A topical application of Met lotion could inhibit HMGB1 release from cell nuclei, reduce PGE₂ and IL-1 β levels and reduce inflammation and degeneration in Achilles tendon. These results suggest that the transdermal application of Met is an effective yet safe and convenient method for preventing the development of tendinopathy.

SIGNIFICANCE/CLINICAL RELEVANCE: Our findings offer the initial evidence that the transdermal application of Met lotion could be utilized as an anti-inflammatory drug to prevent tendinopathy development, without the typical side effects associated with oral ingestion or metformin injection.

ACKNOWLEDGEMENTS: This work was supported in part by a DOD Award (W81XWH-22-9-0016-014) and Pittsburgh Foundation Grants (AD2022-130408; AD2023-134256).

REFERENCES: 1) Akbar M, et al., RMD Open: 2017;3:e000456. 2) Horiuchi, T, et al., J Biol Chem: 2017; 292(20): 8436-8446; 3) Zhao, G, et al., PLoS One: 2019; 14(9):e0222369; 4) Zhang J, et al., Foot & Ankle Int: 2020;41(12)1455-1465.

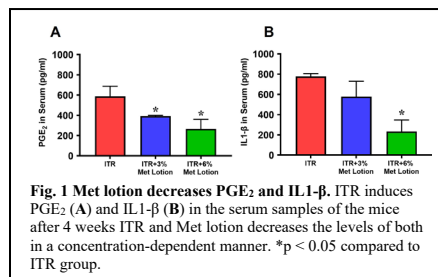


Fig. 1 Met lotion decreases PGE₂ and IL-1 β . ITR induces PGE₂ (A) and IL-1 β (B) in the serum samples of the mice after 4 weeks ITR and Met lotion decreases the levels of both in a concentration-dependent manner. **p* < 0.05 compared to ITR group.

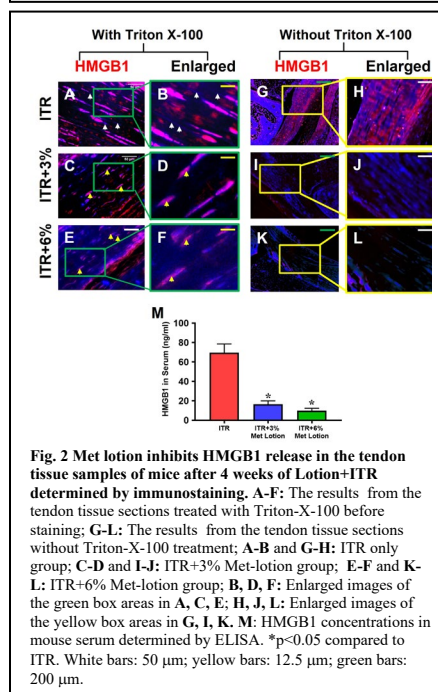


Fig. 2 Met lotion inhibits HMGB1 release in the tendon tissue samples of mice after 4 weeks of Lotion+ITR determined by immunostaining. A-F: The results from the tendon tissue sections treated with Triton-X-100 before staining; G-L: The results from the tendon tissue sections without Triton-X-100 treatment; A-B and G-H: ITR only group; C-D and I-J: ITR+3% Met-lotion group; E-F and K-L: ITR+6% Met-lotion group; B, D, F: Enlarged images of the green box areas in A, C, E; H, J, L: Enlarged images of the yellow box areas in G, I, K, M: HMGB1 concentrations in mouse serum determined by ELISA. **p* < 0.05 compared to ITR. White bars: 50 μ m; yellow bars: 12.5 μ m; green bars: 200 μ m.

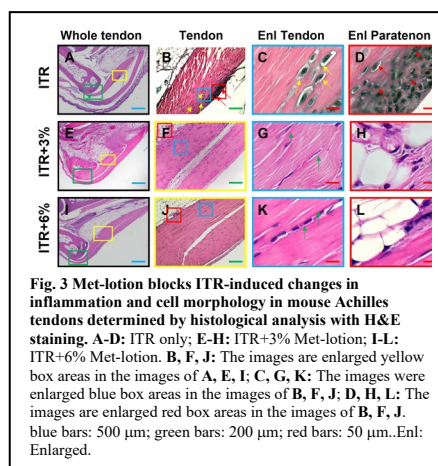


Fig. 3 Met-lotion blocks ITR-induced changes in inflammation and cell morphology in mouse Achilles tendons determined by histological analysis with H&E staining. A-D: ITR only; E-H: ITR+3% Met-lotion; I-L: ITR+6% Met-lotion. B, F, J: The images are enlarged yellow box areas in the images of A, E, I; C, G, K: The images are enlarged blue box areas in the images of B, F, J; D, H, L: The images are enlarged red box areas in the images of B, F, J. blue bars: 500 μ m; green bars: 200 μ m; red bars: 50 μ m. Enl: Enlarged.