Metformin Regulates Tendon Stem Cell Division: Mechanistic Insights and Therapeutic Implications for Tendinopathy

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INTRODUCTION - Tendinopathy is common and debilitating, and current therapies are largely palliative. We previously showed that metformin (Met), a type II diabetes drug, prevents tendinopathy, promotes scarless skin repair, improves tendon degeneration, and suppresses inflammation and senescence in aging mice [1-4]. However, the cellular mechanisms in tendon stem/progenitor cells (TSCs) remain unclear. This study investigated how Met regulates TSC division and function.

MATERIALS AND METHODS - Achilles tendon stem/progenitor cells (ATSCs) were isolated from adult C57BL/6J mice (n = 6; 7 months; 3 males, 3 females) using established methods. Confluent ATSCs were treated with Met (0–200 μg/mL) and/or lipopolysaccharide (LPS; 0–100 ng/mL) for 3 days. Outcomes were assessed by immunostaining, RT-PCR, and Western blot for AMPK activation, inflammatory markers, mitochondrial biogenesis, and stemness. All animal procedures were approved by the IACUC.

RESULTS - Metformin activated AMPK in a concentration-dependent manner, as shown by increased phosphorylation of AMPKα1 and β1, confirmed by Western blot. This activation was accompanied by upregulation of collagen I, bone sialoprotein (BSP), and mitochondrial DNA expression, indicating enhanced tendon matrix production and mitochondrial activity (**Fig. 1**).

Met also promoted stemness, increasing nucleostemin (NS) expression to ~99% in treated cells compared with 75% in untreated controls, and restoring NS to ~95% in LPS-challenged cells, where expression otherwise dropped to ~17%. In parallel, Met inhibited nuclear release of HMGB1, maintaining ~82% nuclear retention versus 30% in LPS-treated cells, and suppressed inflammation by keeping CD68 expression below 10% at baseline and reducing LPS-induced expression to ~42% compared with ~95% in LPS alone (**Fig. 2**).

Further, Met enhanced mitochondrial biogenesis, as reflected by increased expression of NDUFB8 and TFAM. Morphology studies revealed that Met favored symmetric division of ATSCs, which preserved stemness, activated AMPK, upregulated TFAM, and retained HMGB1 in the nucleus. In contrast, asymmetric division was associated with loss of stemness, diminished AMPK activity, HMGB1 cytoplasmic release, and increased CD68 expression (**Fig. 3**).

DISCUSSION - Metformin exerted multiple beneficial effects on tendon stem cells by simultaneously activating AMPK, promoting tendon matrix gene expression, enhancing mitochondrial biogenesis, and suppressing inflammatory pathways. These actions converge on a central mechanism: the regulation of ATSC division fate. By favoring symmetric division, Met preserved stemness, maintained AMPK signaling, and sustained mitochondrial transcriptional activity, all of which support tendon homeostasis and regenerative capacity. In contrast, asymmetric division was linked to loss of stemness, diminished AMPK activity, HMGB1 release, and CD68 upregulation, features consistent with inflammatory and degenerative processes in tendinopathy. These findings suggest that Met not only corrects inflammatory and degenerative changes in ATSCs but also actively biases cell fate decisions toward regeneration. Importantly, this provides a mechanistic explanation for our previous in vivo observations that Met improves tendon healing and prevents tendinopathy progression. Thus, the therapeutic potential of Met extends beyond its systemic metabolic effects to include a direct, stem-cell–mediated action on tendon biology.

SIGNIFICANCE - By biasing tendon stem cell fate toward symmetric division, metformin sustains stemness and reduces inflammation, supporting its potential as a disease-modifying therapy for tendinopathy.

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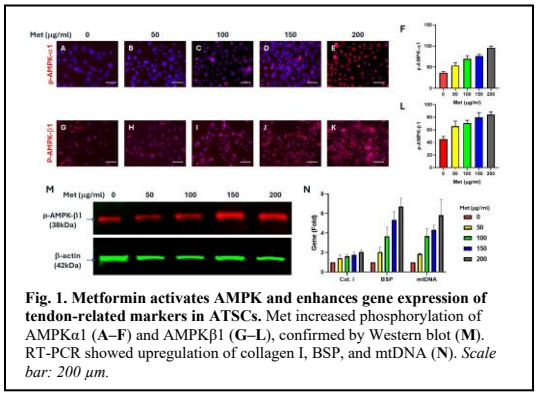

Fig. 1. Metformin activates AMPK and enhances gene expression of tendon-related markers in ATSCs. Met increased phosphorylation of AMPKα1 (**A–F**) and AMPKβ1 (**G–L**), confirmed by Western blot (**M**). RT-PCR showed upregulation of collagen I, BSP, and mtDNA (**N**). *Scale bar: 200 μm.*

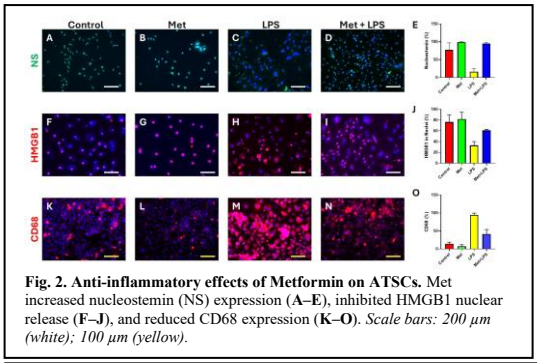

Fig. 2. Anti-inflammatory effects of Metformin on ATSCs. Met increased nucleostemin (NS) expression (**A–E**), inhibited HMGB1 nuclear release (**F–J**), and reduced CD68 expression (**K–O**). *Scale bars: 200 μm (white); 100 μm (yellow).*

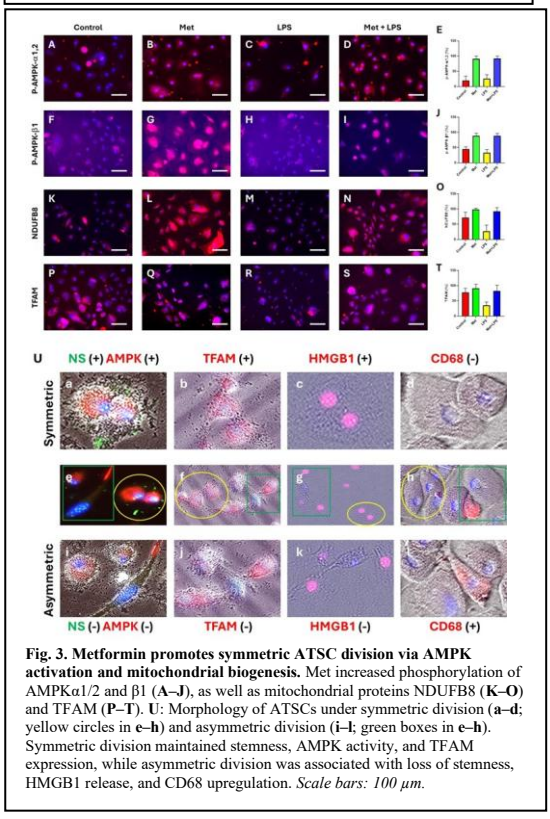

Fig. 3. Metformin promotes symmetric ATSC division via AMPK activation and mitochondrial biogenesis. Met increased phosphorylation of AMPKα1/2 and β1 (**A–J**), as well as mitochondrial proteins NDUFB8 (**K–O**) and TFAM (**P–T**). **U**: Morphology of ATSCs under symmetric division (**a–d**; yellow circles in **e–h**) and asymmetric division (**i–l**; green boxes in **e–h**). Symmetric division maintained stemness, AMPK activity, and TFAM expression, while asymmetric division was associated with loss of stemness, HMGB1 release, and CD68 upregulation. *Scale bars: 100 μm.*