Metformin's Effects on Chondrocyte Metabolic Activity

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INTRODUCTION: Metformin is widely prescribed for managing type 2 diabetes due to its efficacy in lowering serum glucose levels [1]. Beyond its hypoglycemic properties, metformin exhibits anti-tumor, anti-aging, and anti-inflammatory effects [2]. Since inflammation is a critical risk factor in developing osteoarthritis (OA) [3], metformin has recently attracted attention as a potential preventative treatment for OA. As an AMPK agonist, metformin has shown promising results in attenuating OA development in animal models [4]. AMPK is closely linked to cellular calcium signaling, a critical regulator of chondrocyte mechanobiology [5]. This study explores metformin's potential to inhibit cartilage degradation under inflammatory conditions and its effect on chondrocyte calcium signaling.

METHODS: Cartilage samples (3 mm diameter, 2 mm height) were harvested from bovine knee joints (1-2 months old) and cultured in chondrogenic media (Fig. 1a) [6]. *ECM Synthesis*: Cartilage samples were treated for 48 hrs with IL-1β (10 ng/ml) and a high concentration of metformin (10 mM) (n=10). Another group of samples was treated for 48 hrs with IL-1β (5 ng/ml) and metformin (100 μM) (n=6). New GAG and collagen synthesis was measured in the samples during the second 24 hrs of metformin treatment using a click chemistry method (Fig. 1b) [6]. *ECM Degradation*: Cartilage samples were treated with IL-1β (1 ng/ml) and metformin (10 mM) for a continuous 10-day culture (n=6). The longitudinal loss of nascent GAG from click-labeled cartilage was quantified by reading the fluorescence of the culture media every other day [6]. *Gene Expression*: Cartilage samples were treated with 1 ng/ml IL-1β and 100 μM metformin for 48 hrs (n=3 Ctrl & IL-1β; n=2 Met). RNA expression levels of ACAN, COL2, and ADAMTS4 were quantified with qRT-PCR, and the gene expression fold change was analyzed using the $2^{-\Delta\Delta C(T)}$ method after data was normalized to the reference gene β-Actin. *Spontaneous Calcium Signaling*: Cartilage samples were pre-treated with 100 μM metformin for 30 mins or 7 days (n=6 per group). Following metformin treatment, intracellular Ca^{2+} was labeled with Calbryte™ 520 AM, and samples were imaged on a Zeiss LSM880 confocal microscope for 30 minutes in 2-second intervals. Calcium videos were processed with a custom program to extract the spatiotemporal parameters of all spontaneous $[Ca^{2+}]_i$ peaks in chondrocytes.

RESULTS: *GAG and Collagen Synthesis*: IL-1β exposure inhibited the synthesis of both GAG and collagen as expected (Fig. 2). At a high concentration of 10 mM, metformin did not significantly affect the synthesis rates of GAG or collagen in either normal or IL-1β-treated cartilage, though there was a notable trend toward increased GAG synthesis and decreased collagen synthesis (Fig. 2a). At a lower dose of 100 μM, metformin exhibited no significant effects on GAG or collagen synthesis in normal or inflamed cartilage (Fig. 2b). *GAG Loss*: IL-1β exposure induced 3 times greater GAG loss compared to control. High-concentration metformin showed no rescue effects on IL-1β-induced GAG loss (Fig. 3). *Gene Expression*: IL-1β downregulated ACAN and COL2 gene expression. Metformin had no further beneficial impact on the expression of either. IL-1β increased ADAMTS4 expression 2-fold, which was reduced by metformin (Fig. 4). *Spontaneous Calcium Signaling*: Metformin did not affect the responsive rate of cell spontaneous $[Ca^{2+}]_i$ signaling after either a 30-minute or 7-day treatment. A 7-day treatment increased the number of multiple $[Ca^{2+}]_i$ peaks in responsive cells, an effect not seen in the 30-minute treatment. Pre-treatment with metformin for either time period decreased the $[Ca^{2+}]_i$ peak magnitudes significantly. Additionally, a 30-minute treatment increased the time between peaks, whereas a 7-day treatment increased the time for the $[Ca^{2+}]_i$ peaks to recover (Fig. 5a,b).

DISCUSSION: Metformin is typically administered orally for long-term management, reaching serum concentrations around 14 μM [7]. The 100 μM dose used in this study is more physiologically relevant than 10 mM, which is often employed for *in vitro* cellular studies due to metformin's slow action and potential application in intra-articular injection. This study showed that 10 mM metformin has detrimental effects on cartilage. Metformin acts through multiple pathways, reducing inflammation, increasing cellular autophagy, inhibiting chondrocyte apoptosis, and decreasing oxidative stress [8]. Future steps involve expanding our study to include these possible effects of metformin and verifying the results on human cartilage. **SIGNIFICANCE:** This study quantified the impact of metformin on chondrocyte metabolism and spontaneous calcium signaling.

REFERENCES: [1] Nasri+ 2014. [2] Wang+ 2017. [3] Sokolove+ 2013. [4] Li+ 2020. [5] Lv+ 2017. [6] Porter+ 2022. [7] Hess+ 2018. [8] Song+ 2022.

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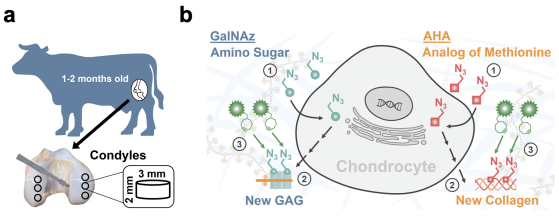

Fig. 1 Experiment design and methods. (a) Cartilage samples were harvested from the femoral condyles of juvenile bovine calf knee joints (1-2 months old). (b) ECM synthesis and degradation were measured using a copper-free click chemistry-based method. Azide-modified macromolecule building blocks were incorporated by chondrocytes into their respective ECM component. A fluorescent dye was "clicked" onto the azide-tagged macromolecules and fluorescently detected.

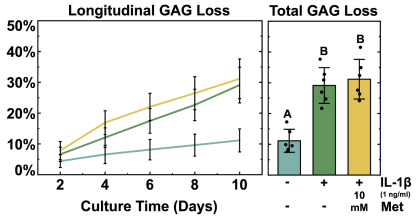

Fig. 3 ECM degradation. The longitudinal and total loss of GAG from cartilage samples, indicative of cartilage degradation, measured using the click chemistry method (n=6). Cartilage samples were exposed to 1 ng/ml IL-1β and treated with 10 mM metformin for **10 days**. Different letters indicate significant differences.

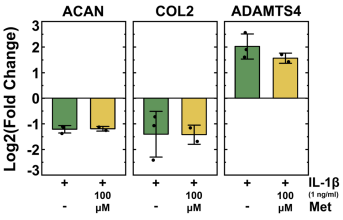

Fig. 4 Gene expression. RT-qPCR Log2 fold change relative to control of genes commonly associated with ECM synthesis and degradation in cartilage. Cartilage samples were exposed to 1 ng/ml IL-1β and treated with 100 μM metformin for **2 days** (n=3 Ctrl & IL-1β, n=2 Met).

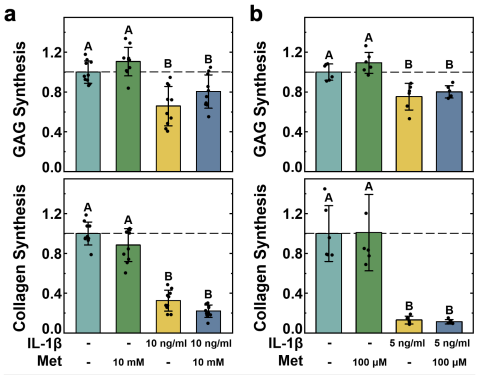

Fig. 2 ECM synthesis. Synthesis of new GAG and collagen by chondrocytes measured using the click chemistry method. (a) Synthesis during **48 hr** treatment with high dose metformin (10 mM) (n=10). (b) Synthesis during **48 hr** treatment with low dose metformin (100 μM) (n=6). All synthesis normalized to ctrl group. Different letters indicate significant differences.

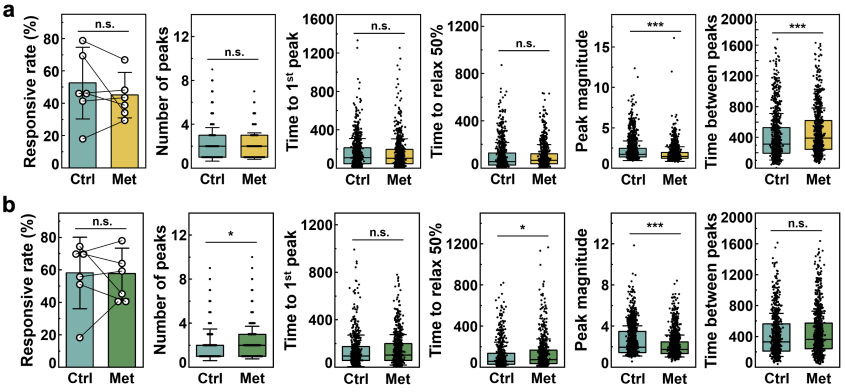

Fig. 5 Chondrocyte calcium signaling. Responsive rate and spatiotemporal characteristics of spontaneous $[Ca^{2+}]_i$ peaks in metformin treated cartilage. Cartilage sample were treated with 100 μM metformin for either (a) **30 minutes** or (b) **7 days** prior to calcium signaling experiments (total number of chondrocytes ≥ 149 from n=6 cartilage samples) (*: $p < 0.05$, ***: $p < 0.001$, n.s.: not significant).