Metformin Alters Chondrocyte Matrix Metabolism Across Age and Disease States of Articular Cartilage

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INTRODUCTION: Metformin is a widely prescribed drug for managing type 2 diabetes and has been shown to have additional health benefits [1,2], attracting interest as a potential preventative treatment for osteoarthritis (OA) [3]. However, few studies have examined its direct impact on the cellular and metabolic activities of chondrocytes. This study explores metformin's effects on chondrocyte metabolism in adult human and calf articular cartilage.

METHODS: Cartilage samples (3 mm diameter, 2 mm height) were harvested from (i) calf knees (1-2 months old), which served as a model for young healthy tissue that is rarely available from human donors, (ii) human cadaver donor knees (2M, avg age = 38 yrs), and (iii) total knee replacement remnants (6F/4M, avg age = 65 yrs) and cultured in chondrogenic media (Fig. 1a-c) [4]. Cartilage samples were treated with metformin at a range of doses (10 μM-10 mM). Click chemistry-based assays quantified *in situ* chondrocyte proliferation, ECM synthesis, and ECM degradation [5]. *Chondrocyte Viability, Proliferation, and Mitochondrial Function*. Calf samples were treated with metformin for 7 days and assessed for *in situ* chondrocyte proliferation, cell viability using Live/Dead™ staining (n≥4), and mitochondrial function via fluorescent membrane polarity detection [6]. In addition, chondrocyte viability was assessed in healthy (n≥5) and OA (n=12) human cartilage samples treated with metformin for 10 days. *ECM Synthesis*. Cartilage samples were treated (i) with metformin alone for 7 (calf) or 10 days (healthy and OA human) (n=10), and (ii) with pro-inflammatory cytokine IL-1β (10 ng/ml) plus metformin for 2 days (n=10 calf). Synthesis rates of new glycosaminoglycan and collagen were measured at the end of treatment. *ECM Degradation*. Using the click chemistry assay, the longitudinal loss of GAG (n=10) and collagen (n=6) was tracked from calf cartilage samples exposed to IL-1β (10 ng/ml) with or without metformin, for 10 days and 28 days, respectively. *Spontaneous Calcium Signaling*. Calf samples were treated for 7 days with 100 μM metformin, then intracellular Ca^{2+} ($[Ca^{2+}]_i$) was fluorescently labeled with Calbryte™ 520 AM. Samples were imaged for 30 minutes in 2-second intervals on a confocal microscope. Videos were processed with a custom-developed program to extract the spatiotemporal parameters of the $[Ca^{2+}]_i$ peaks (≥ 149 chondrocytes per sample from n=6 samples were analyzed).

RESULTS: *Chondrocyte Viability*. A high 10 mM dose of metformin significantly reduced chondrocyte viability in both the top (p=0.002) and middle (p<0.001) zones of calf cartilage (Fig. 2a), whereas neither dose affected viability in healthy or OA human cartilage (Fig. 2b,c). *Spontaneous Calcium Signaling*. A 7-day treatment with 100 μM metformin did not alter the percentage of chondrocytes exhibiting spontaneous calcium peaks (Fig. 4a), but it affected several spatiotemporal parameters of the calcium peaks, including the number of multiple peaks (p=0.01), peak magnitude (p<0.001), and the time for peaks to recover (p=0.02) (Fig. 4b). *Chondrocyte Proliferation and Mitochondrial Function*. In calf cartilage, a 7-day treatment with 10 mM metformin nearly abolished chondrocyte proliferation (Fig. 5a), and reduced the number of cells with functional, polarized mitochondrial membranes (Fig. 5b). *ECM Synthesis*. (i) In all three tissue types, metformin had minimal effect on GAG synthesis (Fig. 3a). However, a high 10 mM dose of metformin reduced collagen synthesis in all three tissues by 30-50% (p<0.01). A low 10 μM dose reduced collagen synthesis by ~25% in healthy human cartilage (1.0±0.2 vs 0.6±0.1, p=0.005) (Fig. 3b). (ii) In calf cartilage, a two-day treatment with metformin at either dose had no significant effect on GAG or collagen synthesis. IL-1β significantly reduced GAG and collagen synthesis, which metformin was not able to reverse (Fig. 7a,b). *ECM Degradation*. IL-1β exposure induced ~5 times greater GAG loss compared to control (9±3% vs 46±12%, p<0.001). Metformin reduced GAG loss in a dose dependent manner, with the lowest tested 10 μM dose being the most effective (21±5%, p<0.001 vs IL-1β, p=0.06 vs ctrl) (Fig. 6a). Similarly, IL-1β caused greater collagen loss compared to control (4±1% vs 60±29%, p<0.001), which was significantly reduced by all metformin doses tested (p<0.001) (Fig. 6b).

DISCUSSION: Metformin is typically administered orally for long-term management, reaching serum concentrations of around 14 μM [7]. In this study, the 10 μM dose was the most effective at preventing GAG loss, but it also inhibited collagen synthesis in healthy human cartilage. In contrast, the high 10 mM dose, commonly used for *in vitro* studies due to metformin's slow cellular action, failed to reduce GAG loss and had adverse effects, including reduced chondrocyte viability in calf cartilage and suppressed collagen synthesis in all three tissue types. Metformin altered spontaneous calcium signaling dynamics, inhibited chondrocyte proliferation, and impaired mitochondrial function. These results suggest that metformin directly modulates chondrocyte behavior, potentially through effects on the AMPK signaling pathway [3]. **CLINICAL RELEVANCE:** Physiological doses of metformin may help protect cartilage in OA patients, but its use in younger individuals, as reflected in the calf cartilage model, or those with otherwise healthy cartilage should be approached with caution due to dose-dependent effects on chondrocyte metabolism.

REFERENCES: [1] Bailey+ 2024. [2] Wang+2017. [3] Li+ 2020. [4] Zhou+ 2015. [5] Porter+ 2022. [6] Delco+ 2019. [7] Hess+ 2018.

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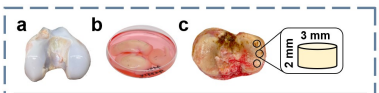

Fig. 1 Cartilage sample harvest and anesthetic exposure. Samples were harvested from **(a)** calf knees (1-2 months old), **(b)** human cadaver donor knees (2M, avg age = 38 yrs) and **(c)** total knee replacement remnants (6F/4M, avg age = 65 yrs).

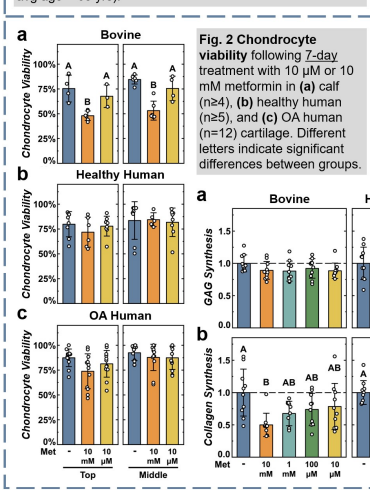

Fig. 2 Chondrocyte viability following 7-day treatment with 10 μM or 10 mM metformin in **(a)** calf (n≥4), **(b)** healthy human (n≥5), and **(c)** OA human (n=12) cartilage. Different letters indicate significant differences between groups.

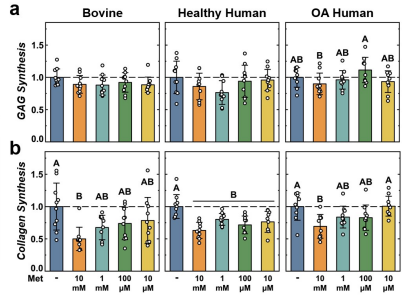

Fig. 3 ECM synthesis following 7-day treatment with metformin (10 μM-10 mM) in calf, healthy human, and OA human cartilage (n=10). Nascent **(a) GAG** and **(b) collagen** was measured in cartilage samples using a click chemistry method. Different letters indicate significant differences between groups.

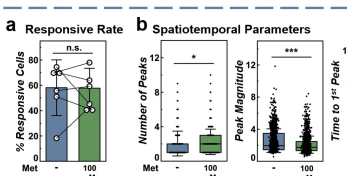

Fig. 4 Spontaneous calcium signaling of chondrocytes in calf cartilage**. (a)** Chondrocyte responsive rate and **(b)** spatiotemporal characteristics of spontaneous $[Ca^{2+}]_i$ peaks in middle zone cartilage. Samples were treated with metformin (100 μM) for 7 days prior to calcium signaling experiments (number of chondrocytes per sample ≥ 149 from n=6 cartilage samples) (*: p<0.05, ***: p<0.001, n.s.: not significant).

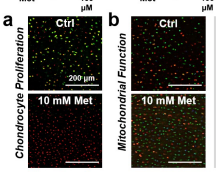

Fig. 5 Chondrocyte (a) proliferation (green) and **(b) mitochondrial function** (red) inhibited by 7-day treatment with 10 mM metformin in calf cartilage.

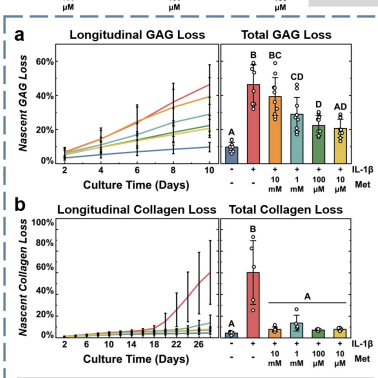

Fig. 6 ECM Degradation. The longitudinal and total loss of **(a) GAG** (n=10) and **(b) collagen** (n=6) from calf cartilage measured using click chemistry. Cartilage samples were exposed to IL-1β (10 ng/ml) and treated with metformin (10 μM-10 mM) for 10 or 28 days. Difference letters indicate significant differences (p<0.05).

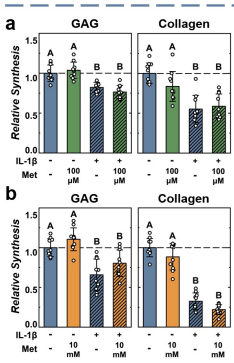

Fig. 7 ECM synthesis following 48-hr exposure to IL-1β (10 ng/ml) and treatment with **(a)** 100 μM or **(b)** 10 mM metformin in calf cartilage (n=10). Different letters indicate significant differences.