Metformin Modulates Early Collagen I and Promotes Osteogenic Maturation in Mouse BMSCs

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INTRODUCTION: Metformin, a widely used antidiabetic drug, has been reported to promote osteogenic differentiation of bone-marrow stromal cells (BMSCs) via AMPK-linked metabolic regulation [1–4]. However, its timing and context during osteogenic differentiation remain unclear. Here, we investigated whether metformin enhances early extracellular matrix (ECM) deposition and late osteogenic maturation in fibula-derived mouse BMSCs, and whether these effects depend on osteogenic versus baseline culture conditions. Type I collagen (COL1A1) was used as an early matrix marker and osteocalcin (OCN) as a late maturation marker, quantified per cell.

METHODS: BMSCs (P4–P5) were cultured in four conditions: standard medium (Normal), standard + 150 µg/mL metformin (Normal + Met), osteogenic medium (Osteo), and osteogenic + metformin (Osteo + Met). Standard medium was composed of 90% DMEM, 10% FBS, and 1% penicillin/streptomycin; the osteogenic formulation additionally contained ascorbic 2-phosphate, glycerol 2-phosphate, and dexamethasone. Metformin was replenished at media changes. Immunofluorescence was performed for COL1A1 (Day 7, Day 12) and OCN (Day 19), with DAPI nuclear counterstain. Images (20×, n=15 non-overlapping fields) were analyzed with fixed thresholds within each timepoint. The primary outcome was per-cell expression units, defined as thresholded red-channel positive area (image pixels) divided by the DAPI-labeled nuclei count per field. Data were analyzed by two-way ANOVA (factors: Medium and Metformin) with Tukey's HSD (α=0.05).

RESULTS: At Day 7, metformin increased COL1A1 expression in standard medium by +211.2 ($p<0.0001$) but did not add in osteogenic medium at this stage (**Fig.1**); two-way ANOVA showed a metformin main effect and a Metformin×Medium interaction (both $p<0.0001$). At Day 12, metformin significantly elevated COL1A1 in both conditions (+119.4 units in standard; +288.2 in osteogenic; both $p < 0.001$; **Fig. 2**); the interaction remained significant ($p=0.001$). At Day 19, metformin remained strongly positive for OCN (+263.0 units in standard; +76.7 in osteogenic; both $p < 0.01$; **Fig. 3**). Across all timepoints, two-way ANOVA showed robust main effects of metformin (all $p < 0.0001$) and significant Medium×Metformin interactions.

DISCUSSION: Using standard (non-osteogenic) medium as a baseline control, metformin produced a clear osteoinductive signal by 7 days, then delivered a pronounced additional gain in the osteogenic milieu by 12 days. This effect persisted into late differentiation at 19 days. Significant interactions at each stage support a time-dependent model in which metabolic modulation elevates early ECM output in non-inductive settings and subsequently augments osteogenic cues once differentiation is underway.

SIGNIFICANCE: Metformin acts as a practical adjunct to osteogenic supplementation, with the strongest incremental benefits after Day 7. These findings provide timing and context guidance for incorporating metabolic modulation into BMSC osteogenesis protocols.

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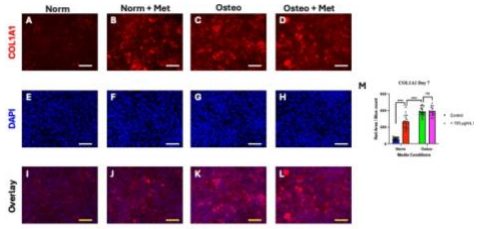

Fig. 1 COL1A1 expression at Day 7: early matrix deposition in fibula-derived mouse BMSCs. A–D: COL1A1 immunofluorescence (red) for mouse BMSCs cultured in normal medium **(A),** Met-containing normal medium (**B**), osteogenic medium **(C),** and Met-containing osteogenic medium (**D**). **E–H:** DAPI nuclei (blue) for the same fields. **I–L**: Overlays (COL1A1 + DAPI) for the corresponding fields. **M:** Quantification of COL1A1-positive area normalized to DAPI count (per-cell expression). Points represent individual fields; bars indicate mean ± SEM. Scale bar: 100 µm.

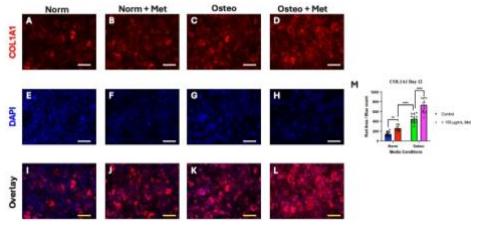

Fig. 2 COL1A1 expression at Day 12: transition-phase collagen in fibula-derived mouse BMSCs. A–D: COL1A1 immunofluorescence (red) for Normal medium (**A**), Normal + Met medium (**B**), Osteogenic medium (**C**), and Osteogenic + Met medium (**D**), imaged at constant exposure. **E–H:** DAPI nuclei (blue) for the same fields. **I–L:** Overlays (COL1A1 + DAPI) for the corresponding fields. **M:** Quantification of COL1A1-positive area per DAPI count. Points are individual fields; bars show mean ± SEM. Scale bar: 100 µm.

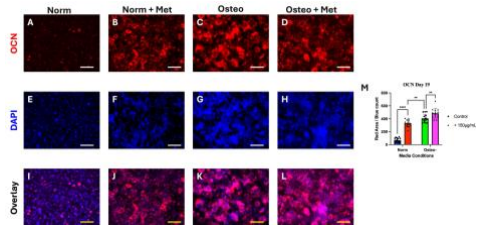

Fig. 3 OCN expression at Day 19: late osteogenic maturation in fibula-derived mouse BMSCs. A–D: OCN immunofluorescence (red) for Norm (**A**), Norm + Met (**B**), Osteo (**C**), and Osteo + Met (**D**), acquired at constant exposure. **E–H:** DAPI nuclei (blue) for the same fields. **I–L:** Overlays (OCN + DAPI) for the corresponding fields. **M:** Quantification of OCN-positive area normalized to DAPI count (per-cell expression). Points represent fields; bars indicate mean ± SEM. Scale bar: 100 µm.