Adipokine Dysregulation Alters Meniscus Fibrochondrocyte Mechanoresponse to Microenvironmental Cues

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INTRODUCTION: Meniscal injuries impact >1million patients annually[1] and often require surgical repair, given that adult menisci have little to no regenerative capacity.[2-3] Metabolic syndrome (MetS) and obesity are frequently comorbid with musculoskeletal complications, including tendinopathy, meniscal tears, and osteoarthritis.[4] Previous studies suggest that aberrant inflammatory signals arising from adipose tissue (*e.g.,* adipokine dysfunction) elicit cartilage and meniscus degeneration *in vitro*[5] and *in vivo*.[6-7] Furthermore, elevated serum and synovial fluid adipokines (including leptin, resistin, visfatin, and adiponectin) are associated with increased OA severity.[8-9] Although these findings suggest AD may alter cellular microenvironments throughout the joint, precisely how these cues influence the mechano-regulatory behavior of meniscus fibrochondrocytes (MFCs) is currently unknown. The objective of this study was to determine whether adipokines impact MFC mechanosensing and contractility *in vitro*.

METHODS: Juvenile bovine menisci were segmented into inner and outer body regions, and the superficial region was removed. Meniscal segments then were minced to ~1mm^3 and cultured in basal medium (DMEM with 5% fetal bovine serum and 1% penicillin/streptomycin/fungizone) for 7-10d to allow for cell egress. MFCs were expanded and used between passage 1-3. For all experiments, cells from a minimum of three donors were assayed in triplicate. MFC mechanoresponse was assayed in 2D by seeding isolated cells on fibronectin-coated polyacrylamide (PA) hydrogels (E=5, 15, or 55kPa) or glass slides. Cells were equilibrated for 24h in basal medium followed by 24h of culture in basal medium (controls) or basal medium supplemented with adipokines (leptin, visfatin, or resistin) at dosages of 10, 100, or 1000ng/mL. Cells then were fixed, permeabilized, and stained for paxillin, αSMA, and actin (phalloidin) and counterstained with DAPI. Samples were imaged at 20X using a Zeiss Axio Scan Z1 slide scanner. Cell morphology was quantified in CellProfiler, and focal adhesion (FA) properties were quantified using FAAS.[10] A 2D migration assay was performed using ibidi gap culture-inserts. MFCs were seeded at an initial density of 50,000 cells/cm^2 and allowed to equilibrate for 24h to establish confluent monolayers. Culture inserts then were removed to generate a ~500μm cell-free gap, and fresh basal media (controls) or adipokine treatments (100ng/mL in basal media) were introduced. Phase contrast images were taken after 8h and gap closure was determined using ImageJ. Cell-mediated matrix contraction was assessed by measuring the contraction MFC-embedded collagen I gels (1×10^4 cells/gel) cultured in basal media or 100ng/mL adipokine treatment. Differences among treatment groups were assessed using unpaired t-tests or one-way ANOVA as appropriate with significance set at multiple comparison-corrected α=0.05.

RESULTS: Brief (24h) adipokine exposure increased MFC spreading and promoted the formation of cellular protrusions on soft (5kPa) gels. As shown in **Fig. 1a**, control cells exhibited rounded morphology on soft gels, whereas treated cells formed elongated processes, resulting in a dose-dependent increase in cell area (p<0.01, **Fig. 1b**) and decrease in solidity (p<0.01, **Fig. 1c**). Similar dose-dependent morphological changes were observed on stiffer gels (15 or 55kPa) and glass for both leptin- and visfatin-treated cells (data not shown). On glass, leptin or visfatin treatment increased the number of FA per cell, average FA area, and total adhesion area per cell (p<0.01, **Fig. 1d-e**). Since dynamic assembly and disassembly of focal complexes is required for cell migration,[11] we next examined whether adipokine exposure influenced MFC motility using a gap closure assay (**Fig. 2a**). On average, untreated cells closed ~60% of the gap after 8h, whereas leptin- or visfatin-treated cells closed only 33% and 29% of the gap, respectively (p<0.05, **Fig. 2b**). Both treatments reduced the contraction of MFC-embedded collagen gels (**Fig. 2c**). On average, leptin- and visfatin- treated gels contracting to 37% and 50% of their initial area, respectively, compared to 14% for controls (p<0.005, **Fig. 2d**)

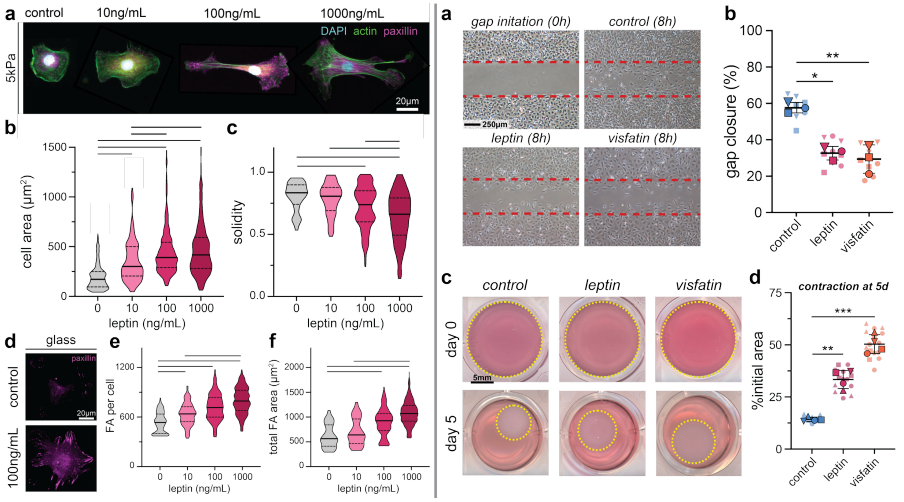

Fig. 1. (a) Immunofluorescence images of outer MFCs seeded on 5kPa gels. **(b-c)** Leptin treatment enhances 2D MFC mechanoactivation in a dose-dependent manner. Treated cells exhibited increased spreading **(b)** and decreased solidity **(c)**. **(d)** Paxillin staining of outer MFCs seeded on glass. **(e-f)** Dose-dependent increases in FA number per cell and total FA area were observed in leptin-treated cells. Data as median±IQR. Bars indicate significant differences (p<0.01). **Fig. 2. (a)** Phase contrast images of gap closure assay. **(b)** Acute leptin or visfatin treatment (100ng/mL introduced at time of gap initiation) impaired cell migration, as indicated by decreased gap closure after 8h. **(c)** Images of MFC-laden collagen gels. **(d)** Quantification of gel area after 5d in culture. Leptin or visfatin treatment (100ng/mL replenished every 48h) slowed gel contraction. Data as mean±std; different shapes correspond to independent donors (*p<0.05, **p<0.005, ***p<0.001).

DISCUSSION: While clinical associations between MetS and musculoskeletal disorders are well-documented, the molecular and mechanobiological mechanisms underpinning these observations remain poorly defined. Our results indicate that adipokine exposure at concentrations commensurate with those found in OA synovial fluid[12] alters MFC morphology, migration, and contractility *in vitro*. In 2D culture, acute adipokine exposure produced striking differences in cell spreading and elongation on both soft and stiff PA gels. Notably, the area of treated cells on 5kPa gels was similar to that of control cells on 55kPa gels, suggesting that these adipokines alter cells' mechanoactivation threshold and cause them to interpret soft microenvironments as though they were stiffer. Adipokine treatment also slowed 2D cell migration and 3D collagen gel contraction. Given that adipokine exposure increased focal adhesion size and density, this may indicate that the dynamic turnover of cellular adhesions to the microenvironment is compromised with adipokine exposure. Future studies will include time-course quantification of focal adhesion assembly and disassembly to understand how adipokine exposure influences adhesive contact dynamics as well as bulk RNAseq analysis to delineate the specific downstream pathways impacted by adipokine treatment in soft vs. stiff microenvironments.

SIGNIFICANCE: Obesity and MetS impact over one-third of U.S. adults and are frequent comorbidities of musculoskeletal disorders. Together with the growing evidence for systemic drivers of joint disease, including inflammation and adipose-joint crosstalk, this study suggests a synergy between metabolic and mechanosensitive mechanisms that may lead to new paradigms for treatment and prevention of joint injury and degeneration.

REFERENCES: [1] Khetia+ *Sports Med Arthrosc,* 2007. [2] King *Clin Orthop Relat Res*, 1990. [3] Ionescu+ *Tissue Eng Part A*, 2011. [4] Englund+ *Arthritis Rheum*, 2009. [5] Nishimuta+ *Conn Tiss Res*, 2017. [6] Collins+ *PNAS*, 2021. [7] Collins+ *Sci Adv*, 2025. [8] Calvet+ *Arthritis Res Ther*, 2016. [9] Orellana+ *Cartilage*, 2021. [10] Berginski & Gomez *F100 Res*, 2013. [11] Mason+ *eLife*, 2024[12] Duan+ *Rheumatol Int*, 2012.

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