Adipokines Elicit Dose-Dependent Catabolic Responses in Cartilage and Meniscus Tissues

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INTRODUCTION: The causal association between obesity and osteoarthritis (OA) has long been hypothesized to be predominantly due to biomechanical overload. However, adipose tissue produces biologically active factors known as adipokines that have been implicated in numerous inflammatory disorders, including rheumatoid arthritis [1]. Elevated levels of the adipokines adiponectin, resistin, leptin, and visfatin in both the serum and synovial fluid are associated with increased progression or severity of arthritis [2]. In vitro studies have demonstrated that each of these adipokines can induce catabolic expression of MMPs and pro-inflammatory markers [3, 4]. However, the role of adipokines in OA is still poorly understood and little is known on how they affect different articular joint tissues. The aim of this study was to determine if cartilage or meniscal tissue explants had catabolic responses to adiponectin, leptin, resistin, or visfatin.

METHODS: The results presented are a compilation of two studies, the first involving adiponectin and resistin and the second involving leptin and visfatin. All methods were consistent between the two studies. Culture: Cartilage and meniscus tissue explants were aseptically harvested with a 4mm biopsy punch from the lateral femoral condyle and tibial aspect of the lateral meniscus of 4 immature bovine stifles each from different animals. Explants were trimmed to 2 mm thick, leaving the articular surfaces intact, and cultured in basal, serum-free medium consisting of high glucose DMEM with non-essential amino acids, gentamicin, HEPES buffer, ITS premix, ascorbate-2-phosphate, and proline. After a 24hr equilibration period in basal medium, explants were randomly allocated to treatment groups (n=6/treatment/tissue) and cultured in basal medium (controls) or basal medium supplemented with adipokines: 0.02, 0.2, or 2ug/mL of adiponectin or resistin in one study, or 0.02, 0.2, or 2ug/mL of leptin or visfatin in a second study. Samples were cultured for 9 days with media changes every 48 hours. On the last day, 20Ci/mL of [3H]-proline and 10μCi/mL of [35S]-sodium sulfate were added to the above media formulations to assess incorporation rates of protein and sulfated glycosaminoglycan (sGAG), respectively. Explants were cultured for 22 hours then washed in PBS with unlabeled proline and sulfate. Biochemistry: Explants were digested and assayed for radiolabel contents using a scintillation counter with radiolabeled media dilutions as standards. Conditioned media and explant digests were assayed for sGAG release and content, respectively, using the DMMB assay with chondroitin sulfate dilutions as standards. Analysis: Effects of dose for each combination of tissue and adipokine were analyzed with general linear models with significance at p<0.05 and Tukey’s test for pairwise comparisons. Data are presented as mean ± SEM.

RESULTS: Cartilage sGAG release was essentially unaffected by the tested adipokines at doses examined, with only the highest dose of resistin stimulating a significant increase of cumulative sGAG release compared to controls (Fig 1A). In sharp contrast, meniscus tissue was more sensitive to several adipokines, with elevated sGAG release (Fig 1B) following treatment with resistin (2 and 0.2ug/mL), leptin (2ug/mL), or visfatin (2ug/mL). Explant sGAG contents were generally consistent with release patterns. Cartilage explants treated with 2ug/mL resistin had lower sGAG content (Fig 2A) than explants treated with 0.02ug/mL resistin. Meniscus explants had a dose-dependent response to resistin, with both 0.2 and 2ug/mL treatments lowering sGAG contents compared to controls (Fig 2B). Protein (3H) incorporation did not significantly vary with dose for any adipokine in either tissue (data not shown), and sGAG (35S) incorporation rates did not significantly vary with dose for any adipokine in cartilage (Fig 3A). Meniscus sGAG incorporation (Fig 3B) was significantly inhibited by treatment with 0.2ug/mL of adiponectin or 2ug/mL of resistin.

DISCUSSION: Our results indicate that adipokines elicit catabolic responses in meniscal tissue at levels orders of magnitude lower than that necessary to elicit a response in cartilage and at levels found in OA synovial fluid. Among the adipokines in this study, resistin had the strongest effect on both tissues by stimulating the catabolic release of sGAG. In meniscus, 2ug/mL resistin depleted the tissue of sGAG to less than half of its original content and similarly reduced the rate of sGAG incorporation (reflecting catabolic and/or anti-anabolic effects). This finding is particularly relevant as elevated synovial fluid resistin levels following knee trauma exceed the concentrations used in this study [5]. In general, meniscal tissue appears to be much more catabolically responsive to adipokines than cartilage tissue. Combined with observations that meniscal tissue is more sensitive than cartilage to IL-1 induced degradation [6], these results suggest that meniscus is intrinsically susceptible to biologic degeneration and that depletion of sGAG in meniscus could be an early event in knee OA. Given that obesity evolves both increased adiposity and altered mechanical loading, future studies will explore possible synergistic interactions of adipokines and injury on cartilage and meniscus degradation.

Figure 1. Eight day cumulative sGAG release from cartilage (A) explants and meniscus (B) explants with adipokines. *indicates p<0.05

Figure 2. sGAG content of cartilage (A) explants and meniscus (B) explants treated with adipokines. *indicates p<0.05

Figure 3. sGAG incorporation (35-S) in cartilage (A) explants and meniscus (B) explants with adipokines. *indicates p<0.05

SIGNIFICANCE: Obesity is a growing epidemic with currently a third of U.S. adults considered obese. In addition, 50% of adults over 65 years have diagnosed arthritis. In light of new understanding regarding the systemic impact of adipose tissue, this study is significant as it provides insight into the connection between these two diseases and the pathology of OA, potentially leading to a new paradigm for early detection and treatment of OA.


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