Injurious Loading Alters PEP and NADH: A Metabolic Analysis of Articular Chondrocytes

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DISCLOSURES: Dr. June owns stock in Beartooth Biotech. Drs. June and Brahmachary own stock in OpenBioWorks. Neither company was involved in this study.

INTRODUCTION: Traumatic joint injuries increase risk for the development of osteoarthritis (OA). Macroscopic tissue changes due to traumatic injury occur in human cartilage explants with high compression and high strain rates [1]. However, the effects of traumatic injury on the metabolism of articular chondrocytes remain unclear. Studying the metabolic pathways of chondrocytes subjected to injurious loading provides a direct assessment of the physiological state of cartilage [2]. Therefore, this study's objective is to quantify the differences in the metabolic profiles of bovine articular chondrocytes between injurious and physiological compression. A key input for metabolism is oxygen, and this study quantified changes in oxygen consumption between physiological and injurious *in vitro* compression.

METHODS: Sample Preparation: To induce *in vitro* injury, human OA and bovine articular chondrocytes were encapsulated in 4.5% W/V type VIIA low-gelling temperature agarose using established methods (Figure 1A) [3]. In all, 180 gels from n = 10 ten bovine donors and n = 10 OA donors were created. Gels from each donor were divided into three loading groups: injury, physiological, and control. Loading: Hydrogels were submerged in 5mL of PBS (Phosphate Buffered Saline) and incubated with a CO2 saturation of 5%, O2 saturation of 20%, n = 7 (normoxia) or 5% n = 3 (hypoxia) and temperature of 37°C. The hydrogels were subsequently loaded, either injurious, physiological or control (Figure 1B). O2 Testing: Hydrogels from both loading conditions were reintroduced to culture conditions (either normoxia, 20% O_2 or hypoxia 5% O_2) 0, 1, 4, or 24 hours prior to oxygen testing. After loading, hydrogels were bisected, and half of the gel was flash frozen using liquid nitrogen for metabolomics while the other half was immediately probed for oxygen saturation (Figure 1C.1). Statistical Design: Samples were randomly assigned to each group and timepoint to mitigate errors throughout the loading process. Each donor was replicated across all groups.

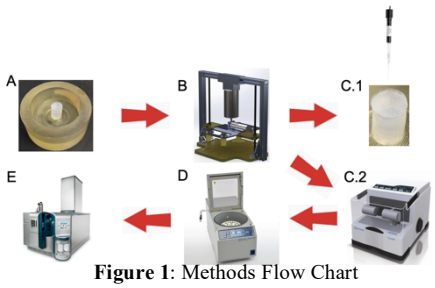

Figure 1: Methods Flow Chart

Metabolite extraction: After flash freezing, samples were pulverized and kept at –80°C for 12 hours. Samples were then homogenized using a temperature controlled CryoMill (RETSCH) for a total of 12 minutes, and subsequently dissolved in 70:30 methanol:acetone with 1% Formic for 12 hours (Figure 1C.2). The buffer was removed from the hydrogel supernatant using a vacuum concentrator at 45°C, until only sediment remained and then returned to –80°C (Figure 1D). Mass Spectrometry (MS): The samples were reconstituted in 10µL of 50:50 acetonitrile:HPLC water and vortexed for 1 minute. The samples were then transferred to MS vials and processed by the MS facility (Figure 1E).

RESULTS: Hydrogels from bovine donors in hypoxia (n = 3) showed a metabolic response through oxygen consumption as early as 4 hours after injury, whereas hydrogels from human OA donors (n = 3) did not show changes in oxygen consumption after injury in hypoxia (Figure 2A, 2B). Hydrogels from bovine donors in normoxia (n = 7) consumed oxygen as early as 1 hour post injury, whereas hydrogels from human OA donors (n = 7) did not show a metabolic response after injury or physiological loading (Figure 2C, 2D). 24hrs after injury bovine hydrogels had elevated levels of phosphoenolpyruvate (PEP) when compared to 24 hours after physiological loading. NADH levels were significantly elevated in OA cells directly after and 4 hours post physiological loading.

DISCUSSION: The decreased oxygen saturation of bovine samples over time suggests an increase in oxygen consumption from the chondrocytes, this altered oxygen consumption demonstrates the chondrocyte metabolic response to injury conditions in both normoxia and hypoxia. The lack of response to injury of human OA cells suggests an impaired metabolic response in hypoxia. In normoxia there was no response to loading at all. 24 hours post-injury loading bovine cells showed higher levels of PEP when compared to physiological loading, suggesting a potential metabolic bottlenecking, or redirecting of glycolytic flux. The increased levels of NADH in OA cells when compared to bovine cells suggests a potential redox imbalance in the ETC, yet NADH measurements must be interpreted with caution due to the potential for chemical artifacts.

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding how mechanotranduction and metabolic pathways are affected by different loading conditions in *in vitro* cartilage experiments is necessary to understand cartilage health, particularly in the context of post traumatic OA. This knowledge is crucial for developing targeted therapeutics or preventative measures to maintain or restore cartilage function for people with post-traumatic OA.

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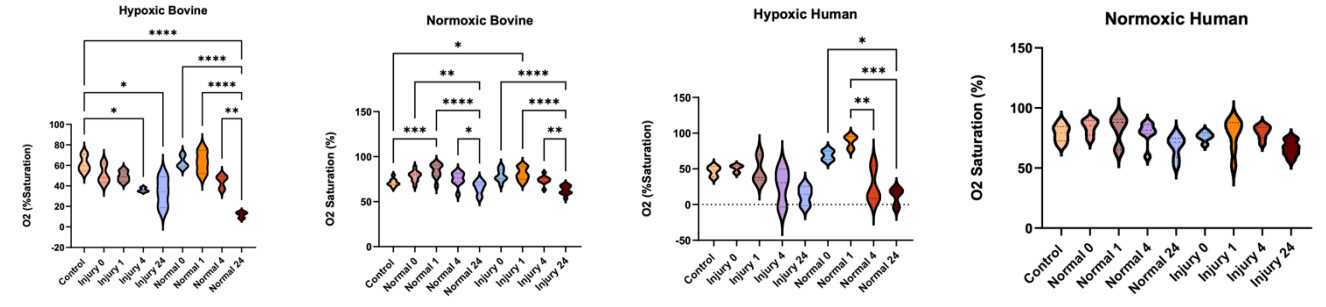

Figure 2: Oxygen Saturation Data. **A**: Bovine cells in hypoxia. **B**: Bovine Cells in normoxia. **C**: Human cells in hypoxia **D**: human cells in normoxia.

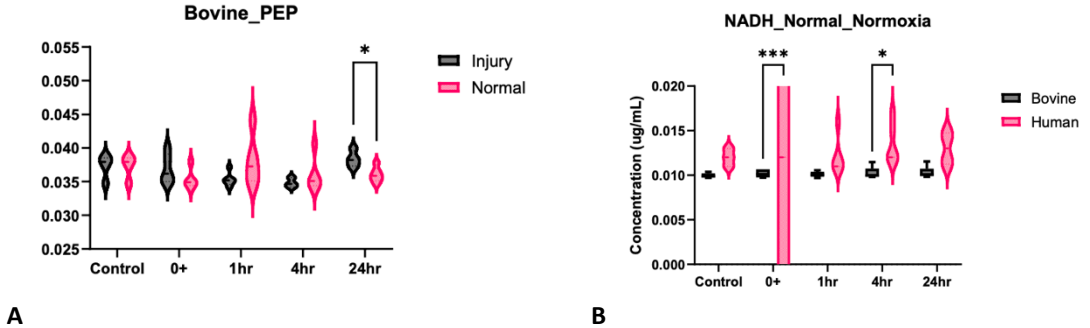

Figure 3: Metabolite Data. A: PEP measured from Bovine subjected to injury and normal loading. B. NADH measured from bovine and human subjected to normal loading.