Overloading Healthy Articular Cartilage Induces Mitochondrial Dysfunction Reminiscent of Late Stage Osteoarthritis

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Introduction: Osteoarthritis (OA) is a debilitating degeneration of articular cartilage impacting the health of forty million people in the United States alone (1). This disease carries morbidity on the order of diabetes and cardiovascular disease when present in major joints such as the knee or hip (2). The strong correlation between OA and joint overuse suggests that excessive mechanical stress upon articular cartilage may be initiating persistent chondrocyte anabolic insufficiency that leads to joint deterioration (3). However, the mechanisms connecting joint overuse to tissue degradation and disease have not yet been elucidated. Our laboratory has identified a novel mechanotransduction pathway whereby acute, stress-dependent increases in electron transport chain (ETC) flux produce bursts of reactive oxygen species (ROS) which are critical to subsequent, antioxidant-inhibitable stimulation of ATP synthesis and extracellular matrix formation (4). Increases in mitochondrial ROS production with mechanical stress are linearly related such that increasing stress magnitudes increased ROS production and subsequently increased oxidative damage. These findings have significant bearing on OA research given that several mitochondrial defects have been identified in chondrocytes harvested from OA patients, in particular dysregulated redox functions, depressed mitochondrial membrane potential, and, most recently, disrupted mitochondrial respiration (5, 6). Therefore we hypothesized that mechanical overstimulation of healthy chondrocytes repeatedly ex vivo at stress magnitudes similar to those associated with development of OA would disrupt the mitochondrial mechanotransduction apparatus of chondrocytes in a manner consistent with the mitochondrial pathologies observed in chondrocytes from OA patients.

Methods: In order to test this hypothesis, we subjected bovine osteochondral explants to mechanical loads previously demonstrated to mimic normal, healthy physiological loading and injurious physiological overloading, 0.25 MPa and 1.0 MPa, respectively. These cyclic loads were sustained for 3 hours daily at a frequency of 0.5 Hz for either 1 or 7 days to investigate the singular or cumulative impacts of normal and excessive mechanical stress. Following loading, portions of the loaded tissue were digested in order to obtain chondrocytes that could be plated onto Bioscience Seahorse XF-96 Extracellular Flux Analyzer plates. This sophisticated instrument allows evaluation of several respiratory parameters of chondrocytes on a per-cell basis: basal rate of respiration, maximal rate of (uncoupled) respiration, spare respiratory capacity (the difference between basal and maximal respiration), and proton leakage (a measure which increases as mitochondrial membranes are damaged). Decreases in spare respiratory activity, a mitochondrion’s ability to respond to cellular demands such as stimulation after mechanical impact, and increases in proton leakage, a damage endpoint which increases as mitochondrial membranes are damaged often by oxidation, have both been identified in chondrocytes harvested from OA patients, in particular dysregulated redox functions, depressed mitochondrial membrane potential (7), and, most recently, disrupted mitochondrial respiration (5, 6). Therefore we hypothesized that mechanical overstimulation of healthy chondrocytes repeatedly ex vivo at stress magnitudes similar to those associated with development of OA would disrupt the mitochondrial mechanotransduction apparatus of chondrocytes in a manner consistent with the mitochondrial pathologies observed in chondrocytes from OA patients.

Results: In agreement with previously published results, single day 1 MPa loading caused significant increases in ROS production over 0.25 MPa loaded controls as measured by DHE oxidation in situ (data not shown). This increase in DHE oxidation was sustained throughout 7 days of repetitive 1.0 MPa loading. No significant differences in cell viability were observed in either group after the single day or 7 day regimes (data not shown). This sustained increase in oxidant production in overloaded explants coincided with a 50% reduction in basal respiration, maximal respiration, and spare respiratory capacity after 7 days relative to healthy loaded controls (Figure 1). Repeated overloading also caused a significant increase in proton leakage through the mitochondria, suggesting that damage to mitochondrial membranes is occurring (Figure 2). These results echo previous studies on chondrocytes harvested from OA patients; however, it is noteworthy that only those explants receiving 7 days of overloading demonstrated the fully depressed respiratory phenotype. Single day 1.0 MPa loaded explants demonstrate no significant changes in basal respiration, maximal respiration, spare respiratory capacity, and proton leakage. We also observed decreases in JC-1 red/green ratio similar to published results in OA chondrocytes consistent with depressed mitochondrial membrane potential, as would be expected in the presence of reduced respiration (data not shown).

Discussion: Given that 7 days of overloading was able to induce mitochondrial hallmarks of OA including increased levels of oxidant production, depressed spare respiratory capacity, increased proton leakage, and depressed mitochondrial membrane potential, we believe the changes observed following overloading may be analogous to an early event in OA pathogenesis. It
appears that dysfunctional mitochondrial function following one week of mechanical overloading may be a result of cumulative oxidative damage. Mitochondria are known to produce excess oxidants when functioning poorly; in this manner, once oxidative stress has begun, the mitochondria may produce excess superoxide even as their productive respiration declines. These results compliment previous studies demonstrating induction of this phenotype through disruption of manganese superoxide dismutase (MnSOD) expression (6) by demonstrating that genuine, in situ mechanical stress resulting in increased oxidation produces a similar net effect upon chondrocytes. Excessive loading as well as deficient antioxidant enzyme defenses appear capable of inducing a state of oxidative stress which compromises the metabolic machinery of chondrocytes. Because chondrocytes operate with a relative paucity of metabolites and dioxygen despite high metabolic demands upon them in maintaining cartilage, particularly along the articular surfaces of weight bearing joints, disruptions in cellular metabolism such as these may compromise chondrogenesis to a great enough extent that tissue function is eventually impacted. Studies are already underway to determine the impact that this mitochondrial phenotype has upon the chondrosynthetic activities of articular chondrocytes as well as to determine whether increases in antioxidant capacity can protect against this effect of overloading.

**Significance:** We believe the results presented here strongly support the hypothesis that overloading-induced oxidative damage to mitochondrial mechanotransduction mechanisms in chondrocytes may represent a potential pathway through which overloading directly contributes to pathogenesis in OA by compromising the metabolic response of chondrocytes to mechanical loading.

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Figure 1. A Week of Mechanical Overloading Depresses Mitochondrial Function of Articular Chondrocytes

Figure 2. A Week of Mechanical Overloading Increases Mitochondrial Proton Leak

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