Introduction. Post-traumatic osteoarthritis (PTOA) is the condition characterized by chronic joint pain and stiffness and articular cartilage degeneration that develops after a joint injury. Extensive cartilage damage from high-energy impact loading often leads to PTOA. The prevention of the apoptosis and necrosis of chondrocytes may prevent or slow the progression of PTOA after injury. Previous work in our lab has shown that following impact injury, chondrocytes from bovine osteochondral explants release reactive oxygen species (ROS). Treatment with rotenone, an inhibitor of complex I of the mitochondrial electron transport chain, reduces both the production of ROS and cell death following impact injury, indicating a central role of mitochondria in the impact injury response of chondrocytes.

Normally, rotenone induces cell death because it inhibits aerobic energy production via the mitochondrial electron transport chain. The chondrocyte-sparing effect of rotenone after an impact injury indicates that these cells may not require energy production via mitochondria in order to survive. In chondrocytes, the mitochondria may not be the "power house" of the cell, but may actually serve as important signaling centers. We hypothesized that inhibition of energy production via the mitochondrial electron transport chain of chondrocytes would not significantly reduce tissue ATP content while the inhibition of glycolysis would significantly reduce tissue ATP content. To test this we treated osteochondral explants with oligomycin, an inhibitor mitochondrial ATPase and/or 2-fluoro-2-deoxy-d-glucose (2-FG), an inhibitor of glycolysis. Tissue ATP content and chondrocyte viability were measured to evaluate the effects of the drugs on energy metabolism.

Materials and Methods. Osteochondral explants (2.5 cm x 2.5 cm) were harvested from fresh bovine tibial plateaus and incubated in low glucose culture media (10% Fetal Bovine Serum, FBS), Low Glucose Dulbecco Modified Eagle Medium (DMEM), F12) under standard conditions (37°C, 5% CO₂) for at least 24 hours. Explants were then treated for 24 hours with 4 µg/mL oligomycin and/or 1 mM 2-FG.

ATP Content: The method for tissue ATP content was adapted from the method described by Long and Guthrie.1 Immediately after 6-mm cartilage plugs were harvested they were placed into a cocktail of phosphatase inhibitors (Sigma P5726). Sample masses were measured, and then the cartilage plugs were placed into a boiling water bath for 10 minutes to extract ATP. ATP levels in the supernatant were measured using the Sigma FLAA ATP Bioluminescent assay kit and the 1450 MicroBeta TriLux Microplate Scintillation and Luminescence Counter. Three explants were used for each dose group.

Viability: Confocal microscopy was used to image the fluorescent probes calcein AM and ethidium homodimer at 24 hours after treatment. Three explants were used for each dose group and 3-4 images were taken per explant.

Statistics: One-way ANOVA on Ranks and Tukey correction for multiple comparisons was used to compare treatment groups for ATP content and viability. A p-value of less than 0.05 was considered significant.

Results. The viability data obtained for each treatment group is shown in Figure 1.

The 24-hour chondrocyte viabilities for all treatments groups were not significantly different.

The ATP content of each treatment group is shown in Figure 2. The ATP content in each sample was calculated as concentration of ATP per gram of cartilage harvested.

Normal cartilage ATP content was 19.5 ± 9.8 µM/g, oligomycin-treated cartilage ATP content was 20.3 ± 6.2 µM/g, 2-FG-treated cartilage ATP content was 5.5 ± 3.2 µM/g, and the oligomycin and 2-FG-treated cartilage ATP content was 6.2 ± 1.2 µM/g. Analysis of the ATP content of each treatment group showed that 24-hour treatment with 2.4 µg/mL oligomycin did not yield significantly different ATP levels than those found in normal cartilage. 24-hour treatment with 2-FG and the combination treatment of oligomycin and 2-FG, however, did produce significantly lower amounts of ATP than those in both normal and oligomycin-treated cartilage (p<0.05).

Discussion. As expected, inhibition of the mitochondrial electron transport chain ATP production did not significantly reduce the ATP content of articular cartilage. However, the ATP content of articular cartilage was significantly reduced by glycolysis inhibition. Neither agent caused significant changes in chondrocyte viability, supporting the hypothesis that the reduction in ATP content was due to metabolic inhibition rather than to reduced cell density. These findings indicate that mitochondria play a minor role in articular chondrocyte energy production.

The role of the mitochondria in ROS production after impact injury and their apparent lack of contribution to the overall energy production in chondrocytes suggest that the primary functions of mitochondria are related to signaling and mechanotransduction. Further investigation of inhibitor effects and longer term experiments will be needed to better understand mitochondrial function in chondrocytes.

References. 1Long JA, Guthrie HD. Validation of a rapid, large-scale assay to quantify ATP concentration in spermatozoa. Theriogenology. 2006 May; 65(8):1620-30.

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Figure 1. Oligomycin and 2-FG-treated Viability at 24 hours. Results are based on 3 explants in each treatment group. Error bars indicated standard deviations.

Figure 2. Bovine Articular Cartilage ATP Content at 24 hours. The ATP concentration per gram of cartilage is shown for each treatment group. Results are based on 3 normal, 3 oligomycin-treated, 3 2-FG-treated explants and 3 oligomycin and 2-FG-treated explants. Error bars indicate standard deviations.