GRAVITATIONAL EFFECTS ON GLUCOSE DIFFUSION INTO ARTICULAR CARTILAGE TISSUE.
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INTRODUCTION
Articular cartilage reduces friction and absorbs impact during joint movement. Cartilage tissue consists of chondrocytes suspended in an extracellular matrix (ECM) composed of type II collagen, glycosaminoglycans, proteoglycan aggregates and water. Chondrocytes require glucose for metabolic activity and for proteoglycan synthesis (Mobasher et al., 2002). Cartilage is avascular. Thus, chondrocytes must obtain nutrients and oxygen by diffusion through the ECM. Articular cartilage is critical for astronaut mobility during and after flight. Bone and muscle mass are reduced following spaceflight, but little has been published on articular cartilage health and maintenance in microgravity.

This project’s aim was to assess the effects of altered gravitational loads on glucose diffusion through articular cartilage. The experiments were conducted on the NASA C-9 aircraft as part of NASA’s Reduced Gravity Student Flight Opportunities Program. The goals were to: 1) develop a flight-certified apparatus to house the experiments, 2) modify a model system (Marshall et al., 2006) to quantify glucose diffusion into cartilage in microgravity (MG), and hypergravity (HG) environments in a 20-second period, and 3) measure glucose diffusion into cartilage when subjected to MG or HG and compare them to standard 1x gravity In-Flight Controls (IFC).

METHODS
Bovine articular (tibial plateau) cartilage plugs, with underlying bone attached were collected using a drill at a slaughterhouse 2 weeks before the experiments. The plugs were cultured at 37°C in high humidity in DMEM, changed every 3-4 days.

The device developed to conduct the experiments is the Cartilage Syringe Assembly (CSA). It consists of 3 syringes connected by a luer-lock valve platform (Figure 1). Each CSA was enclosed in a heat-sealed plastic bag to prevent fluid leaks into the aircraft cabin. One syringe contained a plug inserted in a rubber stopper to allow cartilage surface exposure to the overlying fluid. Before the experiment, this syringe contained saline (PBS). At the start of each experiment, fluorescent glucose (2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose) was pushed from another syringe into that containing the cartilage, yielding a final glucose concentration of 50µM. After 20 seconds, the glucose solution was removed and a mineral oil solution was delivered into the tissue-containing syringe. The mineral oil (plus storage on ice packs) reduced glucose diffusion out of the tissue. Oil red O dye was used to monitor mineral oil diffusion into the tissue during storage. At the conclusion of each of 2 flights, the cartilage and solutions were frozen (-20°C) for later analysis.

CSAs were assembled the day before each flight and refrigerated. The next morning they were placed in stowage containers at room temperature. A CSA was removed from the stowage container before a MG (N=27) or HG (N=7) episode or before the IFC (N=17). IFC studies were conducted during level-flight turns at 1x gravity.

To assess glucose diffusion into the cartilage during each experiment, the bone was removed and the cartilage placed in PBS. The PBS was removed after a 5-minute incubation, and fresh PBS was placed on the tissue. This was repeated 20 times to extract the glucose from the cartilage. The fluorescent glucose in each cartilage sample was measured using a Tecan Cytofluor. The tissue wet and dry weights were also recorded.

RESULTS AND DISCUSSION
Before the C-9 flights, studies were conducted to determine if a detectable amount of fluorescent glucose could diffuse into the cartilage in the CSA within 20 seconds. The average experimental time in MG on the C-9 aircraft was estimated at 20 seconds. Preliminary experiments demonstrated that measurable glucose diffusion into the cartilage was feasible within this time frame (Figure 2).

The cartilage samples from the C-9 experiments were weighed and the amount of fluorescent glucose was measured. The weight of each tissue sample showed no significant difference in the dry or wet weights between groups (figure 3). This indicates that tissue size was similar for the experiments in each condition.
The amount of glucose, normalized to dry weight, that diffused into cartilage in MG and HG was significantly less than the amount that diffused into the cartilage at 1xG (IFC) (figure 4). However, there was no significant difference in glucose diffusion in MG and HG.

The hypergravity environment may act like static compression, which significantly decreases glucose diffusion into cartilage (Quinn et al., 2001). A portion of the fluid would be forced out of the tissue and the spaces within the ECM lattice would be reduced. This would reduce cartilage fluid and physically restrict glucose diffusion into the tissue.

The amount of diffusion was also reduced in MG. This may reflect decreased fluid circulation between the tissue and glucose solution. Buoyancy-driven convection is reduced in microgravity (Smith et al., 2004) which could reduce glucose diffusion into the cartilage. Thus, although the ECM architecture might not change during exposure to MG (as expected in HG), the fluid flow between cartilage and the glucose solution may have decreased, reducing glucose diffusion.

In summary, a device was developed, tested and used to measure glucose diffusion into cartilage in MG and HG. Reduced diffusion was seen in both MG and HG compared to IFC, but the mechanisms underlying the reduction may differ between the two altered gravity conditions. Chondrocyte nutrition is critical for cartilage maintenance and function. Cartilage deterioration during extended spaceflight could lead to mobility problems during flight and after return to Earth.

REFERENCES


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