INTRODUCTION:
Interstitial solute transport is essential for cellular activities in avascular articular cartilage. Increased solute transport via fluid convection has been proposed as a mechanism by which dynamic loading increases cell metabolism in cartilage explants; this may have application in mechanical conditioning of engineered cartilage constructs. However, optimal loading conditions have not been determined for specific solutes. The goal of this study was to determine effects of radially unconfined dynamic compression over a range of frequencies and amplitudes on desorption of a D-glucose analog from cartilage explant disks. Results may aid in understanding of cartilage physiology and targeted delivery of bioactive substances.

METHODS:
Cartilage disks from adult bovine distal femurs, dissected to 4 mm diameter x 1 mm thickness, were bathed overnight in PBS containing 7 mM of a 340 Da fluorescent glucose analog, 2-NBDG (Molecular Probes). Explants were then mounted in radially unconfined axial compression in a well-mixed PBS desorption bath. Compression was applied with a precision displacement actuator in series with a 100 N load cell and a non-porous platen, to ensure radially directed interstitial fluid flow and solute desorption (Fig. 1). On each of three days, a 30-minute compression protocol was applied (Fig. 2). Day 1: 0% static compression; day 2: dynamic compression (Table 1); day 3: 0% static compression (control). Compression amplitudes were applied with respect to cut thickness in a triangular waveform (Fig. 3). Dynamic compression amplitude and frequency were chosen to vary the extent of interstitial fluid flow. Dynamic compression peak-to-peak amplitudes varied from physiological strains up to 50% and frequencies varied from near ambulatory rates down to the intrinsic rate of stress relaxation in cartilage (gel diffusion rate). Bath solute concentration was measured using a fluorescence plate reader. Explants were re-equilibrated overnight in solute baths between compression protocols. Desorption bath concentration was normalized to values measured at 0% static compression on day 1. Normalized values were compared with unity using a single-group Student’s t-test. Differences among dynamic compression protocols were analyzed using 2-way ANOVA. Statistical significance was determined at p<0.05.

RESULTS:
There were no differences in desorption under static compression between days 1 and 3, indicating no changes in solute desorption due to explant degradation (Figs. 4 and 5). Dynamic compression at 50% strain amplitude relative to cut thickness did not significantly increase glucose desorption from cartilage explant disks relative to 0% static compression for any loading frequencies. Desorption increased significantly as period decreased and tended to be greatest during 10 and 20% compression amplitudes. After 30 minutes, the greatest desorption was at 10 and 20% amplitudes and 10 s periods, with increases of 30±11% and 37±8%, respectively (Fig. 6). At this time point, increases were also significant for 5% amplitude with 100 s period (Fig. 6), while at earlier time points, 5% amplitude with 10 s period and 20% amplitude with 100 and 1000 s periods resulted in significant increases as well (not shown). Dynamic compression at 5% and 50% amplitude with a 1000 s period tended to decrease solute desorption (Fig. 6). There were no significant time-related differences in desorption for any of the compression protocols.

DISCUSSION & CONCLUSIONS:
Dynamic compression resulted in increased desorption of a 340 Da analog of the bioactive solute glucose from cartilage explants. Dynamic compression protocols stimulating the greatest increase in solute desorption were similar to those seen to stimulate chondrocyte metabolism in vitro. Since solute transport can influence pericellular concentrations of bioactive solutes, findings support a role for alterations in solute transport in mediating the cartilage biological response to dynamic compression. Methods and results may also assist in identifying loading regimes that can augment or limit solute transport for tissue engineering applications.

REFERENCES:

ACKNOWLEDGEMENTS:
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Table 1. List of dynamic compression amplitudes applied to cartilage explant disks on day 2.

<table>
<thead>
<tr>
<th>Compression Amplitude</th>
<th>Period (s)</th>
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<tbody>
<tr>
<td>5%</td>
<td>10, 100, 1000</td>
</tr>
<tr>
<td>10%</td>
<td>10, 100, 1000</td>
</tr>
<tr>
<td>20%</td>
<td>10, 100, 1000</td>
</tr>
<tr>
<td>50%</td>
<td>10, 100, 1000</td>
</tr>
</tbody>
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Figure 1: Diagram of experimental apparatus
Figure 2: Schematic of experimental protocol
Figure 3: Example of applied compression amplitudes with 100s period.
Figure 4. Example of measured solute fluorescence intensity during desorption. Star: p<0.05 vs day 1: 0% static.
Figure 5. Example of dynamic and control solute desorption normalized to day 1. Star: p<0.05 vs day 1: 0% static.
Figure 6. Percentage increase in desorption due to dynamic compression at t=30 minutes. Star: p<0.05 vs day 1: 0% static.