Introduction:
Ascorbic acid (vitamin C) acts at both transcriptional and post-transcriptional levels to enhance the synthesis of collagen and aggrecan in an articular cartilage explant system (1). While the importance of ascorbic acid to the regulation of chondrocyte gene expression has been established, the mechanism by which this essential nutrient is transported into chondrocytes has not yet been investigated. Two distinct types of ascorbate transporters have been described thus far, the glucose transporters (GLUTs) and the sodium-dependent vitamin C transporters (SVCTs). We have demonstrated that chondrocytes express SVCT2 (1) and others have reported that chondrocytes express GLUT1 and GLUT3 (2). The reduced form of ascorbic acid (AA) is transported by the SVCTs, which are stereoselective for L-ascorbic acid (L-Asc) over D-isoascorbic acid (D-Asc) and the oxidized form of ascorbic acid, dehydroascorbate (DHA) (3). However, DHA but not AA is capable of being transported by the GLUT family (4, 5). We have characterized ascorbic acid transport in human chondrosarcoma cells and primary human chondrocytes to discern the physiologically relevant pathways of ascorbic acid transport in cartilage.

Methods:
Cell Culture: The human chondrosarcoma cell line 105KC was kindly provided by Joel Block and cultured as previously described (6), supplemented with ascorbyl-2-phosphate (A2P). Primary human chondrocytes were isolated from surgical waste tissues, resulting from knee replacement surgery.

RT-PCR: RNA was extracted from cells in monolayer, using the Trizol reagent (Gibco BRL) followed by the Qiagen RNeasy kit (Qiagen, Valencia, CA). The purity and yield of the total RNA was determined by spectrophotometry. Superscript II Reverse Transcriptase (Gibco BRL) and random hexamer primers were used to generate cDNA from total RNA. Intron spanning primers were designed for GLUT1, GLUT3, SVCT1, and SVCT2, corresponding to the human sequences available in Genbank. Standard PCR procedures and AmpliTaq Gold DNA polymerase (Roche) were utilized to amplify the target sequences.

Ascorbic Acid Transport Assay: Ascorbic acid transport of 189μM L-[14C]-ascorbic acid (L-14C-Asc, NEN) was measured using a modified version of the uptake assay published by Wilson and Dixon (7). Ascorbic acid uptake was terminated by washing the cells four times in ice-cold PBS. The cells were lysed with 10% SDS and scintillation counting was performed on the cell lysate. In experiments with sodium free transport buffer, isosmotic concentrations of LiCl were substituted for NaCl. To generate 14C-DHA, L-14C-Asc (in 0.4mM DL-homocysteine) was oxidized using 1 unit ascorbic acid oxidase (Sigma 1mM ascorbic acid for 30 minutes at room temperature).

Statistical Analysis: All statistical computations were performed using GraphPad Prism (GraphPad, San Diego, CA). Descriptive statistics were calculated for subgroups of interest. Pairwise comparisons were performed using paired t-test and ANOVA.

Results:
The 105KC cells and the primary human chondrocytes both expressed transcripts for SVCT2, GLUT1, and GLUT3. In addition, expression of SVCT1 was also detected in 105KC cells but not the primary chondrocytes. Transport of the reduced form of AA into both cell types was enhanced by sodium (a representative experiment for 105KC cells is shown in Figure 1). Transport in the presence of sodium increased linearly over time, whereas AA uptake in the absence of sodium plateaued after 20 minutes (p = 0.0255 for paired t-test). The transport of AA was independent of glucose. In contrast, the uptake of DHA was suppressed 40% by 10mM glucose (data not shown, p < 0.01). Transport of DHA into the 105KC cells was significantly more robust than transport of AA, with 90% more DHA than AA transported into the cells (p < 0.001). Competition experiments with excess reduced and unlabeled AA forms revealed that only the L-form of ascorbic acid was able to significantly suppress the uptake of L-14C-Asc (Figure 2A, p < 0.01). However, all of the excess unlabeled and oxidized forms of ascorbic acid (L-Asc, D-Asc, sodium L-ascorbate, and A2P) were able to compete with 14C-DHA transport (Figure 2B, p < 0.05).

Discussion:
Chondrocytes display both a sodium dependent mechanism of vitamin C uptake and a glucose inhibitable mechanism of vitamin C transport. The dependence of AA uptake on external sodium in both the 105KC cells and primary human chondrocytes suggests that the transport in chondrocytes is mediated by the SVCTs. The 40% suppression of DHA uptake in the presence of 10mM glucose suggests that GLUT1 may be the predominant transporter of DHA in the 105KC cells, as the IC50 for GLUT1 is 10mM D-glucose (5). Additionally, the majority of the vitamin C transported into these cells was in the form of DHA. This observation may be a result of the upregulation of GLUT1 and GLUT3 expression, which has been demonstrated previously in human carcinomas (5). The stereoselective results of the competitive uptake assay with reduced ascorbic acid forms were consistent with the specificity of uptake reported previously for the SVCTs (3). We provide here the first evidence demonstrating that the transport machinery for DHA, presumably the GLUTs, is not stereoselective. Work is ongoing to discern the relative importance of the SVCTs and the GLUTs for physiological transport in human chondrocytes.

References:

Figure 1: Ascorbic acid uptake into 105KC cells is dependent on sodium. The results are expressed as total 14C cpm. Each point represents mean total 14C cpm +/- the standard deviation for each time point.

Figure 2: Stereoselectivity of ascorbic acid and DHA transport into 105KC cells. (A) L-14C-Asc (reduced form) was incubated with 10.6-fold excess competitors, unlabeled and reduced ascorbic acid forms. (B) 14C-DHA (oxidized form) was incubated with 10.6-fold excess competitors, unlabeled and oxidized ascorbic acid forms. Bars represent the % Transport (based on the samples without competitors set to 100%)

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