ACCELERATION OF COLLAGEN SYNTHESIS IN BOVINE CHONDROCYTES, TENOCYTES AND LIGAMENT CELLS BY EXPOSURE TO MICRO LEVELS OF GLUCOSAMINE HCL AND LMW CHONDROITIN SULFATE

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Introduction
The Sys/DMOAD agents glucosamine (gln) and chondroitin sulfate (CS) have enjoyed wide popularity because of their efficacy in reducing/abrogating symptoms associated with joint disorders. In vivo animal studies and in vitro data suggest these agents retard the progression of cartilage lesions as well as up regulate matrix synthesis concomitant with down regulating of catabolic activity. Little data is available on whether they also effect collagen synthesis, a major component of cartilage and the dense connective tissues tendon and ligament. These studies investigate whether gln combined with CS stimulates collagen synthesis in three connective tissue types: articular chondrocytes, tenocytes and ligament cells. If the beneficial effect seen in articular joints is also manifest in tendon and ligament, then gln and CS may occupy a niche in the treatment of soft connective tissue trauma

Materials & Methods
All tissues were obtained from retired Holstein cows. Articular chondrocytes from the metacarpal joints, tenocytes from the extensor tendon adjacent to the metacarpal and ligament cells from tissue between the metacarpals were isolated by collagenase digestion. Cells were expanded by culture in DMEM/F-12 + 10% FCS, ascorbic acid and antibiotics and the second or third passage cells used after plating at high density into 12 or 48 well multicuture plates. All cells were acclimated to a metabolic steady state in specially prepared DMEM/F-12 + 10% FCS + ascorbic acid containing physiological levels of glucose (5 mM). Replicates (4-8) were exposed to varying dosages of Cosamin DS (CDS: a 5:4:1 mixture of gln HCl, LMW CS and manganese ascorbate, Nutramax Laboratories Inc.) and 10 uCi/ml tritiated proline for 24 hours. Collagen synthesis was indexed by two methods: uptake of label into collagenase (purified)-sensitive material and specific activity of hydroxyproline (hyp) after separation from proline by HPLC. The data was compared to control cultures as well as positive controls containing IGF-1. Significance was determined using the Student t-test and ANOVA.

Results
Twenty-four hour exposure of ligament cells, tenocytes and chondrocytes to CDS elicted a dose-dependent statistically significant stimulation of collagen synthesis. Maximum effect in ligament cells of 140% greater than controls was seen with 1-10 ug/ml CDS (equal to 0.5-5 ug/ml gln and 0.4-4 ug/ml CS (Figure 1). Chondrocytes and tendon cells responded in similar fashion but with less magnitude.

Discussion and Conclusion
Collagen synthesis by ligament cells, chondrocytes and tenocytes is significantly upregulated by exposure to a combination of gln and CS. These data are the first to demonstrate that 1) a combination of these agents increase collagen synthesis in chondrocytes equally as well as proteoglycan synthesis, and 2) that collagen synthesis and proteoglycan synthesis in ligament and tendon cells also respond to CDS. Surprisingly, very low levels, easily obtainable by the recommended oral dose, are required to elicit increases in anabolic activity. This is in keeping with the low intestinal absorption data on CS and the possible suggestion that both agents may act as biological response modifiers (1). The physiological effect of these responses under conditions of tissue trauma or injury remains to be demonstrated. However, similar effects seen on cartilage in animal models of OA suggest a protective function on preserving matrix integrity and lessening progression of lesion severity (2). Since tendon and ligament trauma constitute 30-50% of sports injuries, therapeutic application of gln/CS may represent an important extension of its use to include dense connective tissues.