Introduction: Osteoarthritis is a degenerative disease affecting a large proportion of the population. Recently, there has been renewed interest in the use of nutraceuticals (such as glucosamine) for the treatment of symptomatic pain and pathology in arthritic joints. Meta-analysis has shown that the administration of high doses of oral glucosamine sulphates is effective in pain relief and improving joint function (1). Biochemically, an early change in the cartilage metabolism is a loss of the large aggregating proteoglycan, aggrecan. Functionally, this loss results in a decreased capacity for the tissue to sustain mechanical loading that leads to cartilage degeneration and a painful joint. Glucosamine (Glu) is increasingly used to treat patients suffering from osteoarthritis (2). Glucosamine hydrochloride (Glu HCl) has also been shown to be effective at reducing the release of aggrecan in explant cultures (3).

In this study we have used chemically modified glucosamine in order to decrease the concentration of glucosamine needed in vitro to elicit the reductions in catabolic events such as the loss of aggrecan resulting from treatment of cartilage explants with IL-1. We have monitored the effects of these modified compounds using the release of glycosaminoglycans from the tissue and the detection of aggrecan generated catabolites using a specific neocryptopine monoclonal antibody BC-3 (4). Model systems using cartilage explant cultures that mimic the degradative processes seen in osteoarthritis have been developed in which cytokine such as IL-1 are used to initiate the catabolic processes leading to cartilage degradation.

Methods: Cartilage explant cultures (bovine) were established using published methodologies (4). Explants were then incubated in either DMEM, DMEM supplemented with a chemically modified glucosamine (0.5-15mM, Glu 5) or DMEM supplemented with glucosamine hydrochloride (0.5-15mM, Glu 0) for 1 hour. IL-1 (10 ng/ml) was then added to half of the explant cultures in each experimental group. Cultures were maintained for 4 days in the experimental media after which media and explants were harvested for analysis. Glycosaminoglycan (GAG) concentrations of media samples and cartilage extracts were determined using the DMMB assay. RNA was extracted from cartilage explants and RT-PCR was performed using primers to cartilage matrix molecules, ADAMTS and MMPs. Western blot analysis was performed on the experimental media using Mab BC-3 to determine the presence of aggrecanase-generated aggrecan catabolites.

Results: Figure 1 shows the percentage GAG release from a number of experiments in which cartilage explants were incubated in the presence of glucosamine hydrochloride (Glu 0) or chemically modified glucosamine (Glu 5) in the presence and absence of IL-1. These results show that cultures treated with Glu 0 (0.5-15mM) showed no dose dependent inhibition of GAG release. However, ANOVA showed that at 15 mM Glu 0 did decrease the GAG release induced by IL-1 treatment (p=0.004). However, explant cultures preincubated with 0.5-15mM chemically-modified glucosamine showed a dose dependent decrease in IL-1 induced GAG release, showing significance values (p value) of 0.105 at 5mM, 0.013 at 10mM and <0.001 at 15 mM. The decreased release of GAG corresponded to a decrease in the detection of aggrecanase-generated aggrecan catabolites as assessed by Western blotting with Mab BC-3.

Discussion: At present, glucosamine salts are used as a nutraceutical treatment by patients suffering osteoarthritis and other degenerative joint diseases. However, the doses ingested by these patients are quite high (1.5 – 2.0 grams daily) and at present the molecular mechanisms underlying the beneficial effects of this treatment remain unclear. In this work, we prepared chemically modified glucosamine derivatives to further study the mechanisms of action of glucosamine on cartilage metabolism. The chemical modifications made to glucosamine (Glu 5) rendered the molecule more lipophobic thus facilitating its transport through the chondrocyte plasma membrane rather than competition with glucose (in the media) which utilizes classical membrane transport systems. Once inside the cell, Glu 5 is converted to glucosamine -6 phosphate by intracellular enzymes that remove the lipophilic modifications and thus present glucosamine -6 – phosphate to cellular regulatory systems. The results of this study have shown ‘proof – of – principle’ to using chemically-modified glucosamine as an effective and efficient method of getting glucosamine into chondrocytes at lower concentrations than glucosamine alone. The addition of these modified glucosamine derivatives had no effect on cell viability (results not shown) and showed a dose-dependent reduction in aggrecanase-induced proteoglycan degradation when cultures were exposed to IL-1. Collectively, this study has helped to increase our knowledge of molecular mechanisms underlying the use of glucosamine as a nutraceutical by sufferers of degenerative joint disease and in the future these chemically-modified glucosamine compounds will provide useful information on how intracellular glucosamine effects the intracellular signaling mechanisms regulating cartilage metabolism.