INTRODUCTION:
In an effort to understand the mechanisms of disc degeneration and develop a method to prevent or retard disc degeneration, investigators have studied the effect of cytokine stimulation of disc cells. Cytokines such as BMP-2, BMP-7, and TGF-beta1 have been shown to stimulate proteoglycan and collagen type II synthesis. Our previous studies indicated that BMP-2 causes significant changes in the phenotype of the disc cells. Cells treated with BMP-2 for several days seem to be more chondrocytic and have a higher level of proteoglycan synthesis for a given concentration of BMP-2 as compared to cells not previously exposed to BMP-2. Possible cause for the difference in the cell phenotype may be (1) that BMP-2 treated cells have higher levels of endogenous production of other stimulatory molecules that act together with the exogenous BMP-2 or (2) that BMP-2 treated cells upregulate their sensitivity to BMP-2, perhaps by increasing their own receptor levels. Therefore, we tested the hypothesis that BMP-2 stimulation of disc cells that lead to upregulation of chondrocytic phenotype is accompanied by upregulation of the synthesis of other stimulatory cytokines (BMP-6, BMP-7, TGF-beta1) or the receptor for BMP-2 (BMPR-Ia, BMPR-Ib, BMPR-II).

METHODS:
Lumbar intervertebral discs from Sprague-Dawley rats were harvested immediately after euthanization. Anulus fibrosus tissue (including the transition zone) was carefully removed from the disc and the materials were digested with 0.2% pronase for 1 hour, followed by 0.025% collagenase for 4 hours. After enzymatic digestion, the suspension was filtered through 70 micron mesh. The filtered cells were seeded in the T-75 flasks with 10% fetal bovine serum (FBS) culture medium supplemented with penicillin at 100 units/ml, streptomycin at 100 mcg/ml, 2 mM L-glutamine, and 50 mcg/ml ascorbic acid.

When the cells reached 80% confluence, BMP-2 was added to the 0.01 % FBS media to reach final concentrations 200 ng/ml. Cultures without added BMP-2 acted as controls. Previous experiments indicated that BMP-2 at 200 ng/ml was capable of increasing proteoglycan production to 3 times that of controls. Three days later, the culture media were collected for the assay of sulfated-glycosaminoglycans (sGAG) by DMMB and collagen types I and II were determined by ELISA. Real time PCR was used to quantify gene expression levels of collagen types I and II, aggrecan, versican (G1 domain), BMP-6, BMP-7, TGF-beta1, BMP receptor types Ia, Ib, and BMP receptor type II. 18s rRNA used as internal control.

RESULTS:
BMP-2 increased sGAG production to 3.05 times that of control (P<0.01) (Figure 1). BMP-2 increased collagen type II production 2.04 times that of control (P<0.05) (Figure 2). Collagen type I production was not regulated by BMP-2 in a statistically significant manner (P>0.05). Consistent with the increase in proteoglycan levels, real-time PCR showed that BMP-2 increased aggrecan and collagen type II mRNA levels. BMP-2 increased aggrecan and collagen type II mRNA expression to 8.30 (P<0.05) and 4.61 (P<0.05) times that of control respectively. Collagen type I mRNA levels were not statistically significantly changed by BMP-2 (P>0.05). Versican mRNA expression in BMP-2 groups was significantly decreased to 0.54 times that of control (P<0.05). BMP-2 increased TGF-beta1 and BMP-7 mRNA levels to 2.32 and 2.45 times that of control (P<0.05). But there was no statistically significant change in BMP-6 mRNA level. BMP-2 did not change any of the BMP-receptor mRNA in a statistically significant manner (P>0.05) (Figure 5).

DISCUSSION:
As expected, BMP-2 at 200 ng/ml significantly increased the chondrocytic phenotype as measured by sGAG and collagen type II synthesis. The reduction in Versican mRNA is a novel finding. While versican is expressed within normal discs, it is expected to be less effective at increasing disc hydration and therefore the coordinated upregulation of aggrecan with down regulation of versican by BMP-2 may have function meaning in vivo. The finding that BMP-2 stimulation leads to upregulation of BMP-7 and TGF-beta1 suggests that part of the effect of BMP-2 on disc cells may involve the regulation of other chondrocytic cytokines.