INTRODUCTION. Glucosamine (Glcn), a basic structural unit of cartilage proteoglycans, and glucosamine sulfate (GSh) have been widely used to treat osteoarthritis (OA) (1) almost all over the world, with the idea that Glcn would increase cartilage glycosaminoglycan (GAG) synthesis. However, recent clinical trials have not uniformly shown that Glcn or GSh would be more effective than placebo in the treatment of OA (2-5). Also, it is not evident that the low level of Glcn achieved in body fluids (about 10 µM in serum) after oral administration of GS (6) would be sufficient to affect GAG synthesis (7). In this study, reversed-phase high-performance liquid chromatography-electrospray ionization mass spectrometry (RP-HPLC/ESI-MS) was used to study whether the intracellular levels of UDP-glucuronic acid (UDP-GlcA) and UDP-hexosamines (UDP-Hex), the essential precursors of GAG synthesis, would change by exogenously added Glcn or GS. Due to the same molecular mass, RP-HPLC/ESI-MS measurements used in this study could not distinguish between UDP-N-Acetylglucosamine (UDP-GlcNAc) and UDP-N-acetylgalactosamine (UDP-GalNAc), therefore, the UDP-Hex refers to the pool of the UDP-GlcNAc and UDP-GalNAc.

METHODS. Bovine primary chondrocytes from femoral condyles were treated with different concentrations of glucose, Glcn or GS either in high- or low-glucose DMEM for 6, 10, 20, 30, 60 and 120 min after the cells were cultivated in 6-well-plate for 2 or 8 days. The intracellular levels of UDP-glucose (UDP-Glc), UDP-GlcA and UDP-Hex were measured with RP-HPLC/ESI-MS after water-acetonitrile extraction, and the values were normalized against DNA contents of the samples. The expression of aggrecan mRNA and GAG synthesis were quantitated using real time qRT-PCR and 35S-sulfate incorporation. Statistically significant differences (p<0.05) between the control and the treated groups were analyzed with nonparametric two-related-samples tests.

RESULTS. Effects of Glcn, GS and glucose on UDP-hexosamine levels. To define the optimum time to measure the intracellular UDP-sugar levels, chondrocytes were treated with 1 mM Glcn or GS in low-glucose DMEM. It was observed that UDP-Hex content increased within 10 min of the treatment (n=3, data not shown), however, the highest level was reached at 30 min after addition of 1 mM GS (Fig.1A). The ratio of UDP-Hex/UDP-Glcn appeared to increase following the time course of the treatment (n=2, data not shown). No change in UDP-Hex was found either by treatment of 1 mM glucose in low-glucose DMEM or 1 mM GS in high-glucose DMEM (n=3, data not shown). In the following experiments, low-glucose DMEM was used. When treated with 10 µM of GS for 30 or 60 min, the intracellular contents of UDP-Hex, UDP-Glc and UDP-GlcA remained at the control level (Fig.1B). However, the ratio of UDP-GlcA/UDP-Glc appeared to slightly decrease (data not shown).

After 2-day-culture period of chondrocytes, an addition of 1 mM concentration of GS for 30 min increased the content of UDP-Hex, while the content of UDP-GlcA and UDP-Glc decreased (Fig. 2A). However, addition of 10 µM concentration of GS did not have effect on UDP-sugar levels (Fig. 2A). After 8-day-culture period, the ratio of UDP-Hex /UDP-Glc, and especially UDP-GlcA/UDP-Glc were higher than in 2-day-cultures (Fig. 2B). The content of UDP-Glc increased during 30-min-long treatment in 1 mM concentration of GS (Fig. 2B). Similar to 2-day-culture period, 10 mM concentration of GS did not have any marked effect on the UDP-sugar levels of chondrocytes (Fig. 2B).

DISCUSSION. D-GlcN is naturally made from glucosamine-6-phosphate through fructose-6-phosphate by the enzyme glutamine:fructose-6-phosphate amidotransferase, for use as a precursor for chondroitin sulfate (CS) via formation of UDP-GlcA and UDP-GalNAc. The mechanism of Glcn on OA was initially suggested to be stimulation of cartilage GAG synthesis. It has been shown that serum Glcn concentration reached 1.9-11.5 µM after ingestion of 1500 mg of GS (6). Therefore, we investigated whether exogenous, physiologically available level of Glcn and GS could potentiate CS synthesis in chondrocytes by increasing the intracellular UDP-GlcA and UDP-Hex levels. No changes in the intracellular UDP-Hex levels were detected after the cells were treated with 10 µM concentration of GS in low-glucose DMEM for 30 or 60 min. Even though the levels of UDP-Hex increased in 1 mM of GS treatment, the level of UDP-GlcA appeared to decrease. Furthermore, 1 mM concentration of GS is much too high to be reached in the extracellular and intracellular fluids after commonly prescribed oral dose. Our finding that low GS concentration does not stimulate CS via increase in UDP-Hex and UDP-GlcA is in line with previous studies with mouse and human chondrocytes (8,9). It has been estimated that the chondrocytes have enough capability to form sufficient level of Glcn for CS synthesis from endogenous glucose (8-10). It was determined that only 9% of galactosamine (GalN) derived from exogenous Glcn was involved in the synthesis of CS in human chondrocytes, while the rest of GalN used in CS synthesis was formed from endogenous glucose (9). Interestingly, the intracellular levels of UDP-Hex and UDP-GlcA were higher after 8-day-culture period than after 2-day-culture time, and it seems likely that the level of UDP-GlcA could be the rate-limiting factor of GAG synthesis in newly isolated chondrocytes. Low GS concentration did not affect the UDP-sugar levels in 8-day-old cultures.

GS did not significantly affect aggrecan mRNA expression and GAG synthesis, which further confirmed our previous findings (7). Our results indicates that exogenous, physiologically relevant level of GS does not affect the UDP-hexosamine levels in bovine primary chondrocytes.


**Effects of glucosamine sulfate on intracellular UDP-hexosamine and UDP-glucuronic acid levels in bovine primary chondrocyte**

*Qu C-J; *Jauhiainen M; *Auriola S; *Helminen HJ, +*Lammi MJ
+University of Kuopio, Kuopio, Finland

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