INTRODUCTION:
Delayed gadolinium enhanced magnetic resonance imaging of cartilage (dGEMRIC) and contrast enhanced computed tomography (CECT) have been proposed for diagnostics of proteoglycan (PG) loss in osteoarthritis (OA). In these methods negatively charged anionic contrast agents are assumed to distribute into the cartilage inversely proportional to spatial distribution of fixed charge density (FCD). [3,4] However, it has been reported in vitro that diffusion of clinical contrast agents may reach equilibrium only after 8-9 hours. [3] Furthermore, it has been suggested that diffusion rate may be significantly dependent on steric hindrance and thereby on structural integrity of cartilage matrix.

Osteoarthritic degeneration may be initiated by mechanical overloading of articular cartilage [1]. For effective treatment and prevention of the progression of tissue degradation it would be important to detect the injury immediately after an acute trauma. Previous studies indicate that injury after mechanical impact increases the permeability of articular cartilage, which probably accelerates the diffusion of contrast agents in articular cartilage [2]. Thus, it might be possible to detect acute cartilage injury using the CECT by measuring contrast agent diffusion into articular cartilage.

The aim of this study is to investigate the potential of the CECT to diagnose acute injury of articular cartilage and compare the use of two anionic contrast agents.

METHODS:
Intact bovine patellae (n=9) were prepared within 6 hours from slaughtering. Four osteochondral plugs (average thickness=1.75±0.17mm, d=6.0mm) were detached from each patella. Two of the plugs served as intact reference samples and the other two were injured using a custom made drop tower (figure 1). Subsequently, the sample sides were carefully covered with paraffin to allow the contrast agent penetration only through articular surface. The contrast agent diffusion in samples was imaged for 25 hours in 30ml bath of phosphate buffered saline (PBS including 5mM EDTA and 2.7mM KCl) containing 10mM anionic ioxaglate (q=-1, 1269g/mol, Hexabrix™, Mallinckrodt, St. Louis, MO, USA) or 60mM anionic iodine (dissociated NaI, q=-1, 126.9g/mol, Sigma-Aldrich, St. Louis, MO, USA). Contrast agent concentrations were chosen to produce similar x-ray absorption and isotonic osmolarity.

The contrast agent distribution in articular cartilage was measured with the CECT after 1h, 3h, 5h, 64.3±4.4, 71.2±3.8, 76.3±3.3, 81.9±2.8, 81.7±2.7.

Table 1. Normalized contrast agent concentrations (% of the immersion solution concentration, meansSD) in articular cartilage during measurements. With both contrast agents the contrast agent penetration was greater in the mechanically injured samples than in the reference samples (p<0.05, at all time points). At all time points the difference between injured and reference samples is greater with ioxaglate than with iodine (p<0.05).

RESULTS:
With both contrast agents the contrast agent penetration was greater in the mechanically injured samples than in the reference samples (p=0.05, at all time points, figure 2, table 1). Even though the injury could be detected using both ioxaglate and iodine the detection was more sensitive with ioxaglate as the difference in partition of ioxaglate between the intact and injured tissue was significantly greater (p<0.05, at all time points). The largest difference in ioxaglate concentrations between the intact and injured samples was detected in deep cartilage and between mid-cartilage and articular surface. With iodine the largest difference was detected in mid-cartilage. Interestingly, the depth dependent difference between the intact and injured tissue in the partition of ioxaglate was almost constant at all time points whereas with iodine some time dependent changes were revealed (figure 2).

DISCUSSION:
The diffusion rate and equilibrium content of both contrast agents were significantly increased in the injured samples. This suggests that the CECT could be used to detect impact injuries in articular cartilage immediately after the trauma. With ioxaglate the difference between the injured samples and the reference samples was significantly greater compared to iodine. Ioxaglate is a much larger molecule than iodine which probably decreases its penetration rate into cartilage. Possibly, for this reason, penetration of ioxaglate into the injured cartilage improves relatively more than the penetration of iodine. Importantly, the difference between the intact and injured tissue in partition of ioxaglate was significant (p<0.05) already in the time point of 1h. This suggests that in clinical practice the injuries could be detected without reaching the diffusion equilibrium.