Relevance to Musculoskeletal Conditions:
It is thought that hip subluxation and consequent abnormal stress concentrations lead to hip arthrosis in certain conditions, specifically dysplasia, labral injury and impingement. An ability to correlate histopathologic changes in articular cartilage with patterns of surface contact in loaded joints could confirm this hypothesis in a large animal model.

Introduction:
Articular cartilage of the knee will deform under load to improve joint congruity and contact area. This process has been demonstrated by microscopy of rabbit knee joints frozen under load and prepared for histology by freeze-substitution fixation. Due to small size, and limited relevance to human disease, the rabbit hip is not an ideal animal model of dysplasia or labral injury. The possibility that freeze-fixation could be successfully applied to the loaded sheep hip joint was tested in this study.

Materials and Methods:
Following institutional animal care guidelines, whole hip joints from 20 adult white alpine sheep were flash frozen under load and prepared for histology by freeze-substitution fixation. This process has been demonstrated by microscopy of rabbit knee joints frozen under load and prepared for histology by freeze-substitution fixation. Due to small size, and limited relevance to human disease, the rabbit hip is not an ideal animal model of dysplasia or labral injury. The possibility that freeze-fixation could be successfully applied to the loaded sheep hip joint was tested in this study.

Results:
On LM and SEM sections through the apex of the femoral head, (Fig. 1), the articular surfaces were tightly apposed and congruent in 41 hips. In 11, areas of joint surface were separated by fluid, and in 4, surface-contact was not detectable and the head center was minimally eccentric. LM: In the areas of femoro-acetabular contact, cartilage fixation was comparable to that achieved by conventional techniques. Cell and nuclear contours were maintained, staining of collagen fibers and proteoglycan was homogeneous and voids typical of ice crystal formation were absent. Ice crystal damage was apparent only in the articular cartilage not in contact with an opposing surface. In these regions, the radial collagen fibers were separated by clear voids, primarily in the vertical planes holding cell clusters, (chondrons). Bone fixation was always excellent, with osteocytes visible in lacunae and no apparent crystal damage. Marrow fat cells were surprisingly well preserved in subchondral bone underlying areas of joint contact. Mild degenerative changes, including surface fibrillation, cell cloning and duplication of the tidemark, were apparent in some hips and easily distinguished from ice crystal artefact.

SEM: As with LM, cells and collagen fibrils showed excellent preservation in the areas of surface contact, although small voids in chondrocytes were commonly seen at higher magnifications. In the unloaded areas, larger voids were obvious, especially in the cell planes. Some separation of collagen fibrils within large fibers was also apparent in these non-loaded regions, but the longitudinal integrity of the fibers was never disrupted. The presence of degenerative changes, specifically superficial fibrillation, did not influence fixation quality, but collagen fiber alignment in loaded regions was distinctly different in fibrillated specimens. Typically, collagen fibers of the radial zone in fibrillated regions were bent into a horizontal direction, whereas they remained vertical in intact regions.

Discussion:
Whole mount preparations of intact joints allow one to correlate mechanical conditions – specifically joint contact area and congruity – to microanatomy and pathology. Loading, as used here, should place the joint into a functional configuration and additionally prevents separation of the surfaces during preparation. Provided that any shrinkage artefact can be controlled and quantified, this method can be applied to measurement of cell and cartilage dimensions in loaded joints. The principal concern with any freezing method is artefact from ice crystal formation, which was anticipated here, due to large specimen volume and consequently slower rates of cooling and fixation penetration. However, the freeze-fixation technique, applied here to specimens up to 10cm thick, generally preserved critical features of cartilage structure in a manner satisfactory for LM and SEM study. In some specimens the fixation quality was unsatisfactory but only in the unloaded areas. The reason for this is unclear. Higher free water concentration or freedom to expand in non-loaded areas are possible explanations. All but four joints were preserved in a closely articulated state, thus joint relationships were clearly evident in most preparations. In the four (8%) which did not conform, it is possible that dissection violated their integrity to an extent that they could not remain sealed as the load was applied. It is also possible that anatomic features rendered them slightly unstable in these conditions, since subtle variations were common. Orthogonal sections were valuable here because the direction of head subluxation was inconsistent. Many similar technical issues could be elucidated and controlled with further experience.

References:

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