Methods: Human cartilage explants were cultured in the presence or absence of the catabolic cytokines oncostatin M (OSM) and tumor necrosis factor alpha (TNFα). Fragments originating from aggrecanase and MMP-mediated cleavage of aggrecan and type II collagen and released into the supernatant was determined using a range of neo-epitope specific immunoassays; (1) sandwich ^{342}FFGVG-G2 ELISA, (2) competition NITEGE^{373}ELISA (3) sandwich G1-NITEGE^{373} ELISA (4) competition ^{374}ARGSV ELISA, and (5) sandwich ^{374}ARGSV-G2 ELISA all detecting aggrecan fragments, and (6) sandwich CTX-II ELISA, which is specific for the cross-linked neo-epitope EKGPDPxEKGPDP originating from the C-telopeptide of type II collagen.
Results: We found that quantitatively the accumulated release of aggrecanase- and MMP-derived aggrecan fragments was similar in bovine and human cultures of articular cartilage upon stimulation with catabolic cytoikines. However, in human cultures these fragments were continuously released during all phases of the 21 day culturing period, whereas in bovine cultures MMP-derived fragments were only observed in the late phase (day 16-21) and aggrecanase-derived fragments in the early and mid phase.
Conclusions: Our data confirm that major differences in bovine and human processing of articular cartilage exist in response to catabolic cytokines. This suggest that careful consideration should given to the application of this ex vivo model in drug screening programmes.

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SUPPRESSION OF MMP ACTIVITY IN BOVINE CARTILAGE EXPLANTS CULTURES HAS LITTLE IF ANY EFFECT ON THE RELEASE OF AGGRECAN FRAGMENTS CARRYING AGGRECANASE-DERIVED NEO-EPITOPES, WHEREAS CORRESPONDING MMP-DERIVED AGGRECAN AND COLLAGEN FRAGMENTS ARE COMPLETELY ABOLISHED FROM THE SUPERNATANT

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Purpose: Progressive loss of articular cartilage is a central hallmark in many joint disease, however, the relative importance of individual proteolytic pathways leading to cartilage erosion is at present unknown. In particular, the metabolic response induced by the inhibition of MMPs remains to be investigated in appropriate model systems, which also includes assessment of aggrecanase-derived matrix degradation. We therefore investigated the time-dependant release *ex vivo* of MMP- and aggrecanase-derived fragments of aggrecan and type II collagen into the supernatant of bovine cartilage explants cultures using neo-epitope specific immunoassays, and to associate the release of these fragments with the activity of proteolytic enzymes using inhibitors.
Methods: Bovine cartilage explants were cultured in the presence or absence of the catabolic cytokines oncostatin M (OSM) and tumor necrosis factor alpha (TNFα). In parallel, explants were co-cultured with protease inhibitors such as GM6001, TIMP1, TIMP2 and TIMP3. Fragments originating from aggrecanase and MMP-mediated cleavage of aggrecan and type II collagen and released into the supernatant was determined using a range of neo-epitope specific immunoassays; (1) sandwich ^{342}FFGVG-G2 ELISA, (2) competition NITEGE^{373}ELISA (3) sandwich G1-NITEGE^{373} ELISA (4) competition ^{374}ARGSV ELISA, and (5) sandwich ^{374}ARGSV-G2 ELISA all detecting aggrecan fragments, and (6) sandwich CTX-II ELISA, which is specific for the cross-linked neo-epitope EKGPDPxEKGPDP originating from the C-telopeptide of type II collagen.
Results: We found that (1) aggrecanase-derived aggrecan fragments are released in the early (day 2-7) and mid phase (day 9-14) into the supernatant from bovine explants cultures stimulated with catabolic cytokines, (2) the release of NITEGE^{373} neo-epitopes are delayed compared to the corresponding ^{374}ARGSV fragments, (3) the MMP inhibitor GM6001 did not reduce the release of aggrecanase-derived fragment, but induced a further delay in the release of these fragments, (4) no significant differences between aggrecan profile obtained with competitive assays vs corresponding sandwich assays could be detected, finally (5) the MMP-derived aggrecan and type II collagen fragments were released in the late phase (day 16-21) only.
Conclusions: Our data support the model, that aggrecanases and MMPs act independently in the processing of the aggrecan molecules, and furthermore that suppression of MMP-activity had little if any effect on the quantity of aggrecanase-derived fragments released from explants cultures.

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BIOMECHANICAL PROPERTIES OF THE BONE AND CARTILAGE COMPARTMENTS IN A RAT KNEE OA MODEL

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Purpose: Osteoarthritis (OA) is a disease that affects the entire articular joint, with both changes to the bone and cartilage compartments.
Optimal biomechanical properties are pivotal for maintenance of joint integrity and function. Altered biomechanical properties of bone and cartilage are observed in OA disease and might add to worsening of disease.
We investigated biomechanical properties of the bone and cartilage compartments in healthy and affected rat knees from an OA model with combined traumatic knee surgery and increased bone turnover.
Methods: Two groups each of 10 6-months old rats were subjected to a combined ovariectomy and partial medial meniscectomy (OVX+MNX) or sham surgery (SHAM).
Animals were sacrificed 8 weeks after surgery, and one tibia was processed for biomechanical indentation testing. The tibias were cast in Acrylfix-mould with the shaft fixated in the mould and the tibial plateau protruding.
Indentation test was performed centrally on the medial part of the tibial plateau (Figure 1). An indenter area of 0.78 mm^2 and a fixed

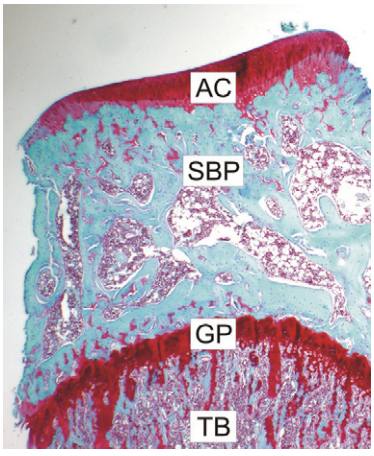

Figure 1. AC: Articular cartilage; SBP: Subchondral bone plate; GP: Growth plate; TB: Trabecular bone.

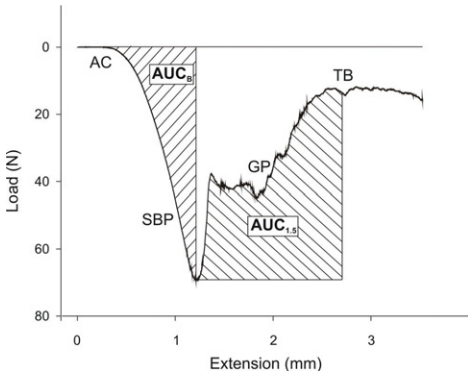

Figure 2. Extension/load curve. AUC_B and $AUC_{1.5}$ are indicated. AC, SBP, GP and TB indicate compartments likely to affect the measurements, as shown in Figure 1.

indenter speed at 0.1 mm/min was used, and an extension/load curve for each specimen was created (Figure 2).
Maximum load (N) was recorded at the point of first break.
Stiffness (N/mm) was calculated as the tangent to the extension/load curve in the elastic part of the curve, before break was observed.
Area under the curve was calculated from start to the point of initial break (AUC_B), and from initial break plus 1.5mm further ($AUC_{1.5}$).
Results: Maximum load (N) at initial break was 130±23 N for sham-animals, and 116±28 N in the MNX + OVX-group (Mean±SD).
Stiffness (N/mm) in the elastic part of the curve before break was calculated to 189±69 N/mm for sham-animals and 153±57 for MNX + OVX-animals (Mean±SD).
AUC_B (N·mm) in the sham group was calculated to 49±18, while it in the affected MNX + OVX group was 58±27 (Mean±SD).
$AUC_{1.5}$ showed a 49% significant ($p<0.05$) increase in MNX + OVX-animals versus sham-animals, with areas of 103±25 and 69±26, respectively (Mean±SD).
Conclusions: The significant increase in $AUC_{1.5}$ in the MNX+OVX group indicates that deeper trabecular bone structures were affected by the combined meniscectomy and ovariectomy. The increase in $AUC_{1.5}$ represents a decrease in bone strength, likely the result of increased bone resorption due to loss of estrogen from ovariectomy, or from OA disease induced by meniscectomy.
Maximum load at initial break and AUC_B were not markedly altered between groups, indicating that the upper part of the subchondral plate was little affected by intervention.

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GAIT ALTERATIONS IN RATS FOLLOWING APPLICATION OF AN ALTERED LOADING DEVICE

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Purpose: Experimental interventions to alter compressive forces in joints of animal models used in the study of cartilage degeneration may also effect the load borne in the experimental limb [1,2]. In this study the mean peak vertical forces of each hind limb of adult rats were determined following application of a varus loading device used to alter loading in the knee.
Methods: Twelve, SD rats greater than 9 months of age (~650 g) were randomly assigned to Control, Sham, or Loaded groups. A varus loading device (VLD), previously developed to study the effects of altered loading on articular cartilage of the rabbit knee, was adapted for application to the rat knee (Fig. 1A) [3]. Animals in the Sham and Loaded groups underwent surgery to apply

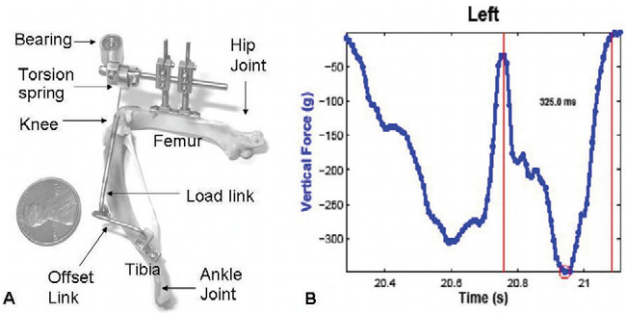

Figure 1. A) VLD applied to rat femur and tibia, B) Sample vertical force data for a left limb. The initial portion of the curve shows the front paw strike followed by the hind limb paw strike. The minimum vertical force is identified by a circle.

transcutaneous bone plates to the lateral aspect of the left tibia and femur. These animals were fit with a VLD which was engaged 12hrs/day for 12 weeks. Engaging the VLD resulted in a controlled overload to the medial compartment of the knee equal to 50-80% body wt (BW)-Loaded group or 0% BW-Sham.
Peak vertical reaction forces during spontaneous locomotion were recorded and data averaged for each leg of each group (Fig 1B). The testing apparatus consisted of a Plexiglass chute 114 (L) × 23 (W), 40 (height) cm, a 6 DOF load cell (20E12A-I25, JR3 Inc.) placed at the center of the chute (force plate 6.35 × 10 cm, data sampled at 120 Hz), and a video camera. Video recordings captured at 60 fps were used to confirm hindfoot contact with the force plate and measure the speed at which the animal crossed the force plate. Animals were acclimated to the gait chute and testing protocol through multiple weekly training sessions for 2-3 weeks prior to testing. Data were collected from 10 passes across the force plate for each each limb (experimental and contralateral) of each animal during spontaneous walking. Data were subsequently screened to ensure a consistent walking speed >100 mm/s and that the hind foot landed solely on the force plate. Analyses of Variance were used to compare means among Groups (Control, Sham, and Loaded) and Leg (Experimental, Contralateral).
Results: The mean peak vertical force was reduced in Sham and Loaded groups of the experimental limb in comparison to Control ($p<0.01$, $p<0.01$; respectively; Fig. 2). In addition, the mean speed was reduced in the experimental leg of Sham and Loaded

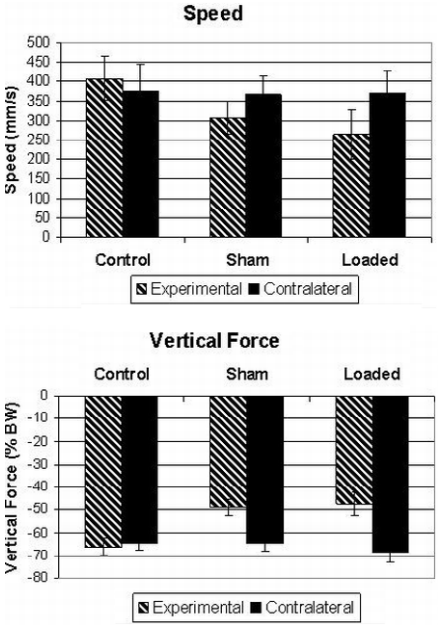

Figure 2. Group and Leg specific Means ± 2 SEM.