found in the body of collagen fibres. However, in cartilage there were some areas similar to capsule but pigmentation was also present as large amorphous granules not associated with collagen fibres. Examination of the in vitro model clearly showed association of pigment with extracellular fibres of chondrocytes and osteosarcoma cells, as well as intracellular when cultured in HGA. Pigment was absent in control cultures.

Conclusions: We have detected distinctive patterns of ochronotic pigment deposition in the ECM of joint tissues in AKU. We have also produced and analysed an in vitro model of ochronosis that replicates pigment deposition in 7 days, whereas in vivo pigmentation takes many years to appear. This model should facilitate the analysis of the molecular mechanism of pigment deposition and help in the development of therapeutic strategies to prevent ochronosis and subsequent arthopathies. We have also shown that mineralisation of collagen in bone protects against ochronosis.

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**DYNAMIC COMPRESSION ALTERS NFκB ACTIVATION AND IκBα EXPRESSION IN IL-1β STIMULATED CHONDROCYTE/AGAROSE CONSTRUCTS**

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**Purpose:** Determine the effect of IL-1β and dynamic compression on NFκB activation and IκB-α gene expression in chondrocyte/agarose constructs

**Methods:** Chondrocyte/agarose constructs were cultured under free-swelling conditions or subjected to dynamic compression (15%, 1 Hz) for up to 360 min with IL-1β and/or PDTC (inhibits NFκB activation). Nuclear translocation of NFκB-p65 was analysed by immunofluorescence microscopy. Gene expression of IκB-α, iNOS, IL-1β and IL-4 were assessed by real-time qPCR coupled with molecular beacons. Statistical analysis for fluorescent intensity data was performed using the non-parametric Mann-Whitney U-test to compare data between treatment groups. For studies involving gene expression, we performed a logarithmic transformation of ratio values prior to analysis by a two-way ANOVA and the post hoc Bonferroni-corrected t-test and compared differences between unstrained and strained constructs for the different treatment groups.

**Results:** Nuclear translocation of NFκB-p65 was concomitant to an increase in nuclear fluorescence intensity which reached maximal values at 60 min with IL-1β (p<0.001). The application of dynamic compression or presence of the NFκB inhibitor, PDTC reduced nuclear fluorescence and NFκB nuclear translocation in cytokine treated constructs (p<0.001 and p<0.01, respectively, Fig. 1). IL-1β increased IκB-α expression (p<0.001) at 60 min and either induced iNOS (p<0.001) and IL-1β (p<0.01) or inhibited IL-4 (p<0.05) expression at 360 min. These time-dependent gene expression events were partially reversed by dynamic compression or the presence of PDTC (p<0.01, Fig. 2) in IL-1β treated constructs. However, co-stimulation by dynamic compression and PDTC favoured suppression (IκB-α, iNOS, IL-1β) or induction (IL-4) of gene expression (Fig. 2).

**Conclusions:** This study explored the potential of IL-1β and dynamic compression to influence NFκB activation and expression of IκB-α in chondrocyte/agarose constructs. IL-1β increased NFκB-
p65 nuclear activation and reached a threshold intensity causing transcription of its inhibitory protein, IκB-α. Once expressed, IκB-α shuttles active NFκB-p65 back into the cytoplasm to switch off transcription. However, continuous activation with IL-1β enabled changes in NFκB nuclear and cytoplasmic activity and led to the induction and inhibition of the inflammatory (IL-1β), inducible (iNOS) and anti-inflammatory (IL-4) genes at later time points. These time-dependent events could be inhibited by co-stimulation with dynamic compression and the NFκB inhibitor, suggesting that mechanical loading may impair IκB-α degradation and terminate transcription. In summary, this study demonstrates that NFκB is one of the key players in the mechanical and inflammatory pathways and its inhibition by a combined biophysical and therapeutic approach could be a strategy for attenuating the catabolic response in OA.

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JOINT MACROSCOPIC DAMAGES AND FUNCTIONAL IMPAIRMENT IN EXPERIMENTAL CANINE OSTEOARTHRITIS

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Purpose: Transection of the dog anterior cruciate ligament (ACL) is a well-known surgical procedure used to model structural damages of osteoarthritis (OA). In dogs, ACL disruption induces mechanical stresses on joint compartments unaccustomed to such loading solicitation. This phenomenon generates loss of tissue integrity, architecture, and therefore triggers the onset and progression of OA. Recently, interest has focused on the functional impairment of ACL dogs, highlighting a relationship between the gait disability and the severity of structural changes evaluated by magnetic resonance imaging (MRI). As macroscopic grades of OA lesions are often used to document changes in joint tissues, it would be relevant to investigate whether this latter system is powerful enough to detect a relationship with gait disability. The joint damages are therefore hypothesised to correlate with the evolution of limb impairment in ACL dogs.

Methods: Twenty-five dogs that underwent sectioning of the right ACL and had no treatment were retrospectively selected among previous studies. At baseline, week 4 and week 8, podobarometric gait analysis was performed to record peak vertical force (PVF) and ground contact area (GCA). At necropsy (week 8), macroscopic evaluation of the joint, including measurement of osteophytes and cartilage lesions on the condyles and plateaus, was conducted, along with synovial fluid volume determination. Gait analysis and macroscopic evaluation were done according to standardized operating procedures. Statistical analyses were carried out using Spearman correlation tests with α-threshold set at 0.05.

Results: ACL dogs had limb impairment as denoted by the decreasing PVF and GCA. At week 4, macroscopic evaluation of the joint, including measurement of osteophytes and cartilage lesions on the condyles and plateaus, was conducted, along with synovial fluid volume determination. Gait analysis and macroscopic evaluation were done according to standardized operating procedures. Statistical analyses were carried out using Spearman correlation tests with α-threshold set at 0.05.

Results: ACL dogs had limb impairment as denoted by the decreasing PVF and GCA. Four weeks after surgery, the limb impairment correlated with macroscopic findings and synovial fluid as assessed at week 8. Hence, dogs having higher GCA had higher fluid volume (p<0.04), higher medial condyle osteophytesis (p<0.03), and lower macroscopic lesions on lateral cartilage condyles (p<0.04). At week 8, higher GCA still correlated with

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