knees and for this study each measurement was the average of three separate measurements measured over 1 second. On day 15 the animals were euthanized and the right knees dissected out, disarticulated and the tibial plateau photographed. The images were then divided into 4 regions, medial anterior and posterior, lateral anterior and posterior and each region scored for pathology by 5 blinded observers using a 0 to 6 scale. The total score for each joint was then calculated. Both the pain and pathology results were analyzed using a student’s t-test to compare the results from the two experimental groups.

**Results:** Pain - On each day that pain was measured the difference in weight bearing between the MIA and saline injected knees was significantly less in the rats dosed with Anakinara compared to those dosed with vehicle (p<0.001). This difference in weight bearing resulted in approximately a 50% reduction in the pain response in Anakinara dosed rats compared to those dosed with vehicle.

**Gross Pathology:** In contrast to the pain results Anakinara had no effect in reducing the cartilage degradation seen in the MIA model. The average joint score for both groups was 2.69.

**Conclusions:** The IL-1RA antagonist was able to significantly reduce the pain measured in the MIA model but had no effect on the cartilage degradation seen in this model.

**P70**

**GLUCOSAMINE AND ITS N-ACETYL-PHENYLALANINE DERIVATIVE STIMULATE CARTILAGE REPAIR AND AFFECT GENE EXPRESSION IN RABBIT EXPERIMENTAL OSTEOARTHRITIS**

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**Purpose:** The aim of this study was to evaluate the effects of Glucosamine (GlcN) and its N-acetyl phenylalanine derivative (NAPA), in rabbits with experimental Osteoarthritis (OA).

**Methods:** Experimental OA was induced by two intra-articular injections of vitamin A (Vit A) in rabbit knees. A severe OA without exposure of subchondral bone was obtained 35 days after the first injection. Then, rabbit OA left knees were intra-articularly injected with 2.5 mM GlcN or 2.5 mM NAPA, right knees were left untreated as OA control. Animals intra-articularly injected with normal saline solution were used as healthy control. Rabbits were sacrificed 70 days following first Vit A administration for histological assessment (May Grunwald-Giemsa, Hematoxylin-Eosin and Alcian Blue staining) and molecular analysis (Real Time-PCR) of the knee joints. Cathepsin B enzymatic activity was analyzed in rabbit primary chondrocytes.

**Results:** Histological sections obtained from articular cartilage showed a major cellularity, a better tissue organization and a major presence of matrix components in NAPA-treated samples and to a lesser extent in GlcN-treated samples compared to cartilage sections from OA knees. Molecular analysis of mRNA expression levels of several genes coding for matrix components and remodeling enzymes in OA samples showed a lower expression compared to healthy tissues. Whereas GlcN and NAPA-treated samples showed a restored expression levels compared to OA cartilage, fairly close to values found in healthy animals.

Previously, we reported an inhibitory effect of GlcN and NAPA on Cathepsin B enzymatic activity in vitro assay. In order to analyze the effects of GlcN and NAPA on Cathepsin B in vivo, we isolated rabbit primary chondrocytes from healthy cartilage. First passage culture chondrocytes were left untreated, treated with TNFα, with TNFα plus GlcN and with TNFα plus NAPA. Cathepsin B enzymatic activity resulted inhibited by NAPA and to a lesser extent by GlcN.

**Conclusions:** The double effect exerted by GlcN and NAPA, improved expression level of genes involved in cartilage homeostasis and inhibitory effect on Cathepsin B enzymatic activity, may explain the cartilage repair observed by histological analysis of OA joints treated with GlcN and NAPA.

**P71**

**MENISCAL TRANSECTION IN GUINEA PIGS AS A MODEL FOR THE EARLY STAGES OF OSTEOARTHRITIS**

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**Purpose:** Failure to develop therapies for osteoarthritis patients suggests that the degenerative changes in the joint are probably no longer sensitive to treatment. It is therefore important that early diagnosis of osteoarthritis becomes available when treatment can still halt or even reverse the joint destruction. Our goal was to develop a guinea pig model with only mild forms of osteoarthritis that could serve as a model for early human osteoarthritis to discover early osteoarthritis biomarkers and to test new treatments that are aimed at intervention early in the disease process.

**Methods:** Osteoarthritis was induced by bilateral transection of the medial meniscus in Dunkin-Hartley guinea pigs using a surgical procedure that was minimally invasive to avoid cartilage damage due to inflammation and/or intra-articular bleeding. At 4, 8, and 12 weeks after surgery the severity of osteoarthritis was macroscopically and histologically assessed and serum and urinary biomarkers were measured. The effect of daily treatment with 0.15 mg/kg Risedronate, 20 mg/kg Pioglitazone, 5 mg/kg Anakinra, and 0.5 mg/kg Galardin was evaluated in a 12-week study design.

**Results:** Four weeks after meniscal transection small lesions in the cartilage were macroscopically observed at the medial side of the joint, starting at the femoral head and the central part of the tibial plateau. Clear, but mild progression of osteoarthritis was detectable over time. However, 12 weeks after surgery the lesions were still restricted to the medial side of the joint and did not reach into the subchondral bone. Mild signs of osteoarthritis, but significantly less than in the meniscal transection group, were observed in the control and sham groups as well due to the susceptibility of the strain to the spontaneous development of osteoarthritis. Increased cartilage destruction in the meniscal transection group was also histologically observed. Of the urinary biomarkers tested, only CTX-II was significantly increased in the meniscal transection group, but only at week 8. No increase in urinary HP/LP ratio (a measure for the relative turnover of cartilage over bone metabolism) nor in serum COMP levels were observed in the meniscal transection group within 12 weeks after surgery.

Strategies for treatment were chosen such that different processes in osteoarthritis development were targeted: bone destruction (Risedronate), inflammation (Pioglitazone and Anakinra), and cartilage destruction (Galardin). Unfortunately, none of these treatments showed beneficial effects on the macroscopic score in the meniscal transection model.

**Conclusions:** Mild joint destruction was observed in the first 12 weeks after bilateral meniscal transection. However, these early degenerative changes in the cartilage could not be detected by classic biomarkers. Probably, the cartilage destruction is still too mild to be measured in the systemic circulation. Further research into new biomarkers is needed to detect and monitor the early stages of osteoarthritis. Proteomics and metabolomics hold promise for the future to find such biomarkers. Previously, an un-
nary metabolic fingerprint has been identified that distinguishes healthy guinea pigs from ones that spontaneously developed osteoarthritis. The meniscal transection guinea pig model of early osteoarthritis might prove to be a useful tool to search for early osteoarthritis biomarkers. Furthermore, this model is suitable to test newly developed therapies aimed at treating osteoarthritis in an early stage, for instance by stimulating anabolic processes rather than preventing joint destruction. The ineffectiveness of the compounds tested further underscores the urgent need for a new generation of drugs.

**P72**

**DIFFERENTIAL DISTRIBUTION PATTERN OF SYMPATHETIC NERVE FIBRES AND SUBSTANCE P DURING FRACTURE HEALING IN MICE**

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**Purpose:** Fracture repair constitutes a sequential event following bone injury and recapitulates the steps of endochondral ossification observed during embryonic skeletal development and growth. Because of the different phases of fracture healing (inflammation, cartilage formation and remodelling) the fracture callus provides an excellent tool for analysis of cartilage and bone formation in adults. Neurotransmitter containing nerve fibres of sympathetic and sensory origin are known to innervate bone and fracture callus, however, little is known about their role in fracture healing and their influence on callus maturation and bone formation. The current research intends to understand the role of the peripheral nervous system for organization and differentiation of the growth plate stain positive for SP and NK1. Additionally, specific chondrocytes within the hypertrophic zone of the growth plate stain positive for SP and its receptor neurokinin 1 (NK1) with differential intensity peaks during the healing process. TH-positive nerves penetrate the callus in early stages of the healing process, while at later time points when a cartilaginous matrix has been formed they retract towards the callus periphery. Notably, substance P (SP) -positive nerve fibres seem not to penetrate the cartilage tissue as well as the newly formed woven bone whereas chondrocytes originated from callus tissue express SP and its receptor neurokinin 1 (NK1) with differential intensity peaks during the healing process. Additionally, specific chondrocytes within the hypertrophic zone of the growth plate stain positive for SP and NK1.

**Results:** Our fracture model demonstrated characteristic stage-specific localisations of tyrosine-hydroxylase (TH) -positive fibres during the healing progress. TH-positive nerves penetrate the callus in early stages of the healing process, while at later time points when a cartilaginous matrix has been formed they retract towards the callus periphery. Notably, substance P (SP) -positive nerve fibres seem not to penetrate the cartilage tissue as well as the newly formed woven bone whereas chondrocytes originated from callus tissue express SP and its receptor neurokinin 1 (NK1) with differential intensity peaks during the healing process. Additionally, specific chondrocytes within the hypertrophic zone of the growth plate stain positive for SP and NK1.

**Conclusions:** Specific localisations of tyrosine-hydroxylase (TH) -positive fibres during cartilage differentiation and endochondral ossification in adults.

**P73**

**MATURATION-DEPENDENT CHANGE IN ACOUSTIC STIFFNESS OF RABBIT ARTICULAR CARTILAGE FACED WITH EACH OTHER**

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**Purpose:** The purpose of the study was to investigate maturation-dependent changes of acoustic (ultrasound) stiffness and other ultrasound features of articular cartilage in healthy rabbit patello-femoral joint.

**Methods:** Five groups of rabbits of various ages (3 weeks, 8 weeks, 6 months, 1 year, 2.5 years) consisting of five rabbits per group were examined. Signal intensity (index of stiffness), signal duration (index of surface irregularity) and interval between signals (index of thickness) of the ultrasound reflection from articular cartilage were examined at two sites: center of patellar groove and center of patella. Correlations in these indices between the two sites were examined statistically. The two sites were also observed macroscopically and microscopically with a light microscope.

**Results:** At the two sites, signal intensity was least in 3-week-old specimens. The signal intensity significantly increased at 6 months of age and significantly decreased at the age of 2.5 years. At the two sites, the signal durations were least at the ages of 8 weeks or 6 months and the intervals between signals were least at the ages of 6 months. The signal intensity of the two sites was significantly correlated with each other (p=0.002, r=0.424). There was no correlation in signal durations of the two sites. The interval between signals of the two sites was significantly correlated with each other (p=0.002, r=0.422). Cartilage surfaces of all specimens were smooth and no degenerative changes were macroscopically or microscopically evident.

**Conclusions:** Change in the signal intensity and the interval between signals was maturation dependent. The maturation-dependent change in patellar groove and patella was correlated with each other.

**P74**

**DEVELOPMENT OF A NOVEL METHOD FOR THE MECHANICAL EVALUATION OF ARTICULAR CARTILAGE IN RABBIT**

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**Purpose:** Validate an indentation method to characterize the mechanical behaviour of articular cartilage in rabbit’s femoral condyles.

**Methods:** 12 samples (six with 16 weeks old and 6 with 19 weeks old) were obtained and kept at -70°C. Everyone was defrosted at 4°C and immersed in phosphate buffer (pH 7.2), and then they were fixed in cylindrical container using polymethyl-methacrylate to do the assay. The samples received compression loads, following the method described by Jin and Lewis (2004). A 0.15 millimeters (mm) depth deformation in 0.15 seconds (s) was made and it was supported during 1200s. Two indenter tips (1 and 2mm diameter) were used to do the assay. The highest and equilibrium load (Pmax an Peq) were gotten from this method directly. The theoretical model employed to characterize articular