

cartilage damage, three sections (apart from 150 $\mu$ m respectively) were stained by Safranin O and 400 $\mu$ m in width of articular cartilage between anterior and posterior edge of medial meniscus was contoured into 3 areas according to the dyeability: Grade I; intact cartilage, Grade II; mildly denatured cartilage with reduced Safranin O staining, and Grade III; severely denatured cartilage with no Safranin O staining. Each area was measured using Zeiss Axio Vision Image Analysis system. To analyze the structural alteration of knee joint after treadmill exercise and OVX, right knee joints were fixed in 70% ethanol and subjected for  $\mu$ CT analyses. Animal care and experimental procedures were in accordance with the guidelines of the ethics committee of our university. Kruskal-Wallis test followed by Tukey-Kramer methods was used for statistical analysis.

**Results:**  $\mu$ CT analyses did not reveal the apparent alteration in bone structure and osteophyte formation between OVX and SHAM group regardless of treadmill exercise. However we observe significant loss in trabecular bone volume in the OVX group. Image analyses of the articular cartilage indicated that % area of Grade III was significantly higher in OVX+RUN group (72.1%) if compared to that of SHAM+CAGE (10.6%,  $p < 0.05$ ). Loss of proteoglycan in articular cartilage was also observed in both OVX+CAGE and SHAM+RUN groups (Grade III; 24.2% and 20.1% respectively) although the difference was not statistically significant. Immunohistochemical staining for type II collagen further support these data, since we observe marked decrease in type II collagen amount in OVX+RUN group. Hematoxylin and Eosin staining showed that OVX enhanced cellularity of synovial membrane after forced running suggesting that depletion of estrogen might enhance synovitis in the damaged joint.

**Conclusions:** In this study, we showed that the combination of OVX and forced running greatly accelerated articular cartilage denaturation in mice. In contrast, the effects of OVX or forced running respectively were not so apparent on articular cartilage maintenance. These data strongly support the evidence that OA is a multifactorial disease and our data strongly indicate the great contribution of hormonal regulation on articular cartilage homeostasis in adults. Crosstalk between hormonal regulation and mechanotransduction on articular cartilage homeostasis is still unclear, our data suggest that estrogen depletion enhances inflammatory response after articular cartilage damage. Since prevalence of OA greatly increased after menopause, our experimental system will be of great use for analyzing OA progression in aged women.

#### 111 INCREASED SENSITIVITY TO NERVE GROWTH FACTOR IN THE MONOSODIUM-iodoacetate MODEL OF OSTEOARTHRITIS

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**Purpose:** Osteoarthritis (OA) is often characterised by episodes of increased pain with associated synovitis. Nerve growth factor (NGF) levels are increased in the OA joint, and during inflammation. Pain flares may be due to either increased release, or to increased sensitivity to NGF. It was hypothesised that OA leads to a sustained and enhanced increase in NGF-induced pain behaviour. The Aim of this study was to find out whether pain behaviour induced by intra-articular injection of NGF is increased in the MIA model of OA and to explore possible mechanisms behind this enhanced response to NGF.

**Methods:** OA was induced in the left knee joint on day 0 in male Sprague Dawley rats ( $n=8$  animals/group) weighing approximately 200g, by intra-articular injection of 1mg of MIA in 50 $\mu$ l saline. Saline injected animals were used as non-arthritis controls. Intra-articular injection of NGF (10 $\mu$ g/50 $\mu$ l) or saline control was given in the left knee joint when OA pathology had fully established (day 20). In order to explore whether inflammation during the development of OA may contribute to any increased sensitivity to NGF, indomethacin (2mg/kg, daily, orally) or saline control was given from before induction of OA (day -1) to day 18. To avoid confounding by analgesic actions of indomethacin, animals underwent a 2 day washout period before NGF injection. Joint tissues were harvested from parallel groups of animals at day 20, corresponding to the time of NGF injection in order to explore mediation of indomethacin effect through altered joint structure. Synovial inflammation was measured as the macrophage fractional area (% synovium occupied by ED1+ve cells), thickness of the synovial lining and joint swelling. Chondropathy, channels breaching the

osteochondral junction (OCJ) and osteophytes were scored as measures of structural damage. Pain behaviour was measured as hind-limb weight-bearing asymmetry. Data are reported as the mean [95% confidence interval].

**Results:** Pain behaviour was increased following induction of OA. Pain behaviour was reduced during the period of indomethacin treatment in OA animals to saline injected control levels by day 18 whereas vehicle-treated arthritic controls still showed an increase in pain behaviour. Enhanced and sustained pain-response was observed following intra-articular injection of NGF in OA animals (31.4 [24.6–38.2] g compared with that observed in non-arthritis controls (7.1 [4.3–9.8] g,  $p < 0.001$ ). Pre-treatment with indomethacin significantly inhibited the subsequent pain behavioural response to intra-articular NGF injection in OA knees (17.3 [12.1–22.5] g) compared to vehicle treated arthritic controls (31.4 [24.6–38.2] g,  $p < 0.001$ ). Synovial inflammation, chondropathy, channels breaching the OCJ and osteophyte scores at day 20 (before NGF injection) were not affected by indomethacin treatment.

**Conclusions:** NGF-induced pain behaviour is increased and sustained in the MIA model of OA. Pre-treatment with indomethacin reduced this enhanced response. This effect of indomethacin was not associated with any reduction in joint damage nor any significant sustained reduction in inflammation. Although synovitis may contribute to the development of this enhanced sensitivity to NGF, involvement of other factors such as possible effects of indomethacin on pain processing at the level of the spinal cord, deserve further investigation.

#### 112 INHIBITION OF TRANSFORMING GROWTH FACTOR ALPHA SIGNALING SLOWS PROGRESSION OF OSTEOARTHRITIS IN A DMM MODEL

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**Purpose:** No cures currently exist for the degenerative joint disease osteoarthritis (OA). Furthermore, the complex and multi-variable nature of OA continues to challenge the development of effective therapies. In an attempt to identify potential targets for disease modifying osteoarthritis drugs (DMOADs), our lab recently established a surgical model of OA to study gene expression changes in degenerating cartilage. Transforming growth factor alpha (TGF $\alpha$ ) gene expression was upregulated in our model and further in vitro studies showed that TGF $\alpha$  suppressed chondrocyte expression of anabolic factors aggrecan and type II collagen and increased expression of the catabolic factor matrix metalloproteinase 13 (MMP13). We thus identified TGF $\alpha$  as a novel therapeutic OA target. The purpose of this project is to examine the role of TGF $\alpha$  in the development of OA in vivo. We hypothesize that inhibition of TGF $\alpha$  signaling will delay disease progression in surgical OA models.

**Methods:** Ten week old male Tgfa null mice and control littermates received either destabilization of medial meniscus (DMM) or sham surgery. At 7 and 14 weeks post-surgery knee joint histopathology was assessed using the OARSI scoring method and tissues were immunostained for disease markers such as MMP13 and type II collagen neopeptides. In addition, we performed DMM and sham surgeries on six month old mice to create a more severe surgical model and assessed OA histopathology after 7 weeks. Lastly, we observed the development of spontaneous OA in eighteen month old Tgfa and control mice in a variety of joints.

**Results:** DMM surgery produced mild and moderate OA in ten week old mice after 7 and 14 weeks. Tgfa null mice had lower OARSI scores and expressed less MMP13 and type II collagen neopeptides than their control littermates. When DMM was performed on six month old animals, severe OA was observed. In older mice however, there was no protection in the Tgfa KO group compared to controls. Eighteen month old mice developed mild spontaneous OA, but again there was no protection in the Tgfa KO group when compared to controls.

**Conclusions:** TGF $\alpha$  signaling plays an important role in osteoarthritis progression in vivo in mild surgical models. However, in severe OA models and in spontaneous OA development, lack of TGF $\alpha$  alone does not appear to be sufficient to prevent or delay disease progression. TGF $\alpha$  should be investigated further as a potential target for DMOAD development, especially in the acute post-injury stages.