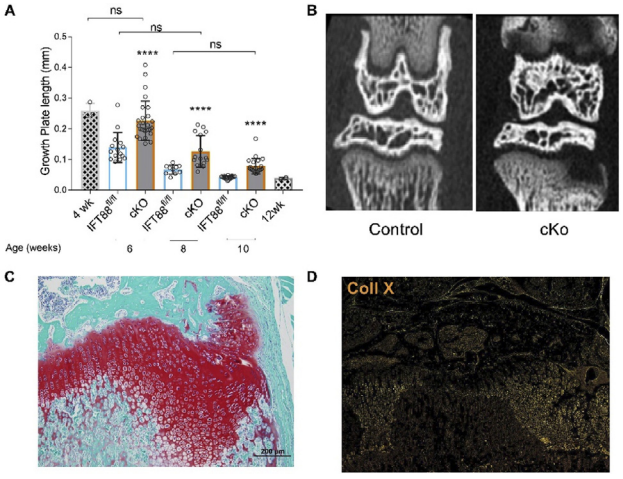
Results: By μCT analyses, GP length in wild-type mice reduces from ~260 μm to 130 μm between 4 and 10 weeks of age. Deletion of IFT88 at 4, 6 or 8 weeks of age resulted in longer GPs in cKO mice (solid bars) at every timepoint compared with control mice (open bars, Fig 1A). Two weeks after tamoxifen, cKO GP lengths were not statistically significantly different to controls at time of treatment, indicating inhibition of growth plate closure. No changes were observed in the epiphyseal plate but there were increases in trabecular bone density immediately below the GP in cKO mice. Interestingly, some cKO mice exhibited extremely long GP at the edges of the tibia, which appeared as large holes by μCT (Fig 1B), whilst the centre of the GP appeared less affected. Histological sections of GP confirmed longer growth plates with increases in both proliferative but, most notably, hypertrophic chondrocyte populations. The large, often bi-lateral holes, observed by μCT were largely filled with disorganised hypertrophic chondrocytes (Fig 1C), which IHC analysis indicates were expressing type X collagen (Coll X) (Fig 1D). Intriguingly, double (femoral and sciatic) neurectomy, at 8 weeks of age, also resulted in large, often bilateral, disorganised populations of hypertrophic chondrocytes, but only in the contralateral (loaded) limb.

Conclusions: In contrast to premature closure of the GP observed at earlier timepoints in development, deletion of IFT88 in the adolescent GP results in longer growth plates and inhibition of GP closure due to the failure of ossification. Histological analyses suggest deletion of IFT88 does not impair Hh-PTHrP stimulated proliferation or hypertrophic differentiation, but interferes with the ossification process at the chondro-osseous junction. This phenotype is most pronounced in the lateral edges of the GP, possibly supporting a role for IFT88 in GP mechanotransduction. The role of mechanics in influencing GP ossification is further supported by a similar phenotype seen in the contralateral limb of double neurectomised mice. Quite how mechanics and IFT88 interact to control GP closure is currently unclear.



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KNEE JOINT DISTRACTION-INDUCED SHIFT FROM CATABOLIC TO ANABOLIC STATE OCCURS AFTER THE DISTRACTION PERIOD

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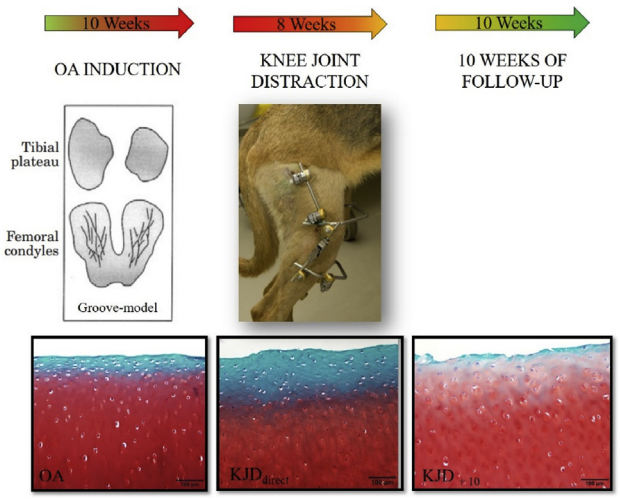
Purpose: Knee joint distraction (KJD) is a validated joint-preserving treatment strategy for severe osteoarthritis (OA) that provides long-term clinical and structural improvement. Data from both human trials and animal models indicate clear cartilage regeneration from 6 months and onwards post-KJD. However, recent work showed that **during** distraction, the balance between catabolic and anabolic indicators is directed towards catabolism, as indicated by collagen type 2 markers, proteoglycan (PG) turnover and a catabolic transcription profile [unpublished data]. The focus of the present study was to investigate cartilage changes directly and 10 weeks **after** joint distraction in order to elucidate the shift from a catabolic to an anabolic cartilage state.

Methods: Knee OA was induced bilaterally in 8 dogs according to the groove model. After 10 weeks of OA induction, all 8 animals were treated with knee joint distraction on the right side, employing the left knee as an OA control. After 8 weeks of distraction, 4 dogs were euthanized directly (KJD_{direct}), and after 10 weeks of follow-up the 4 remaining dogs (KJD_{+10}). Macroscopic and microscopic cartilage degeneration was assessed using the OARSI canine scoring system. RT-qPCR was used to determine relative expression of aggrecan (*ACAN*), collagen type II *(COL2α1)*, cartilage oligomeric matrix protein (*COMP)* and matrix metalloproteinase-3 (*MMP3)* in the cartilage. PG content was determined by the Alcian Blue assay and the synthesis of PGs was determined using $^{35}SO_4^{2-}$ as a tracer.

Results: Directly after KJD, macroscopic cartilage damage of the right tibial plateau was higher as compared to the left OA control (OARSI score KJD_{direct} : 1.7±0.2 vs 0.6±0.3; $p < 0.001$). 10 weeks post-KJD this difference persisted (OARSI score KJD_{+10}: 1.4±0.6 vs 0.6±0.3; $p = 0.05$). Microscopically, an increase in the total OARSI score was seen after 10 weeks post-KJD. This was mainly due to an increase of chondrocyte clusters at 10 weeks of follow-up, resulting in an increase in the sub score chondrocyte pathology. Remarkedly the sub score intensity of proteoglycan staining decreased directly after KJD (indicating a loss of PGs) but increased after 10 weeks of follow-up (see also figure), suggesting a mixed response depending on the item scored. Cartilage gene expression analysis showed downregulation of *COL2α1* (-1.3 ± 0.3), *ACAN* (-4.4 ± 1.0, $p < 0.01$) and *COMP* (-1.7 ± 0.5) directly after knee joint distraction compared to the left OA control suggesting enhanced catabolic activity during KJD. In contrast, after 10 weeks of follow-up the expression of *COL2α1* and *COMP* were increased in the right distracted knee as compared to the left OA control (2.6 ± 1.1 and 2.5 ± 1.2 respectively) as well as compared to the situation directly after KJD (3.3 ± 1.4 and 4.2 ± 2.0), though not all changes reached statistical significance. Similar, expression of *MMP3* was upregulated directly after KJD (4.4 ± 0.8) and downregulated 10 weeks post-KJD (-3.3 ± 0.8). Biochemical analysis of the tibia cartilage directly after KJD revealed a lower PG content compared to the OA joint (20.1±10.3 mg/g vs 23.7±11.7 mg/g). At 10 weeks post-KJD this difference in PG content was gone (24.8±6.8 mg/g vs 25.4±7.8 mg/g). The PG synthesis rate directly after KJD appeared significantly lower vs. OA (1.4±0.6 nmol/h.g vs 5.9±4.4 nmol/h.g; $p < 0.001$)). Conversely, 10 weeks post-KJD this difference disappeared (3.7±1.2 nmol/h.g vs 2.9±0.8 nmol/h.g), and the synthesis rate in the distracted knee was increased compared to directly after distraction ($p < 0.01$) indicating a shift upon follow-up.

Conclusions: Further in-depth investigation of the material is ongoing and also includes the other joint tissues such as the bone and the synovial tissue. Irrespective, these first results on cartilage changes suggest that the shift from a catabolic to an anabolic state occurs within the weeks **after** joint distraction. As such, the post-distraction period seems to be essential in identifying key-players that support intrinsic cartilage repair.

Acknowledgements: Financial support was received from: TTW Technology Foundation: Perspectief P15-23, Dutch Arthritis Society (ReumaNederland) Long term Research Program LLP9 and LLP12



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MECHANOBIOLOGICAL RESPONSES IN SYNOVIUM: INSIGHTS INTO THE BENEFITS OF EXERCISE AND THE ROLE OF INFLAMMATION IN KNEE OSTEOARTHRITIS

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Purpose: Exercise improves pain and joint function in patients with knee osteoarthritis (OA), including after total knee arthroplasty. However, many patients limit exercise due to pain, reporting increased pain with activity and relieved with rest. The mechanisms leading to the clinical benefits of exercise over time despite pain during activity in knee OA are poorly understood. Joint pain signals emanate from multiple innervated joint tissues, especially synovium. The synovium is a critical tissue for maintenance of joint health and undergoes marked changes during OA. Knee synovium is stretched during walking (flexion-extension) at predictable frequencies, but the mechanobiology of synovium during the gait cycle is unknown. Since synovial inflammation (synovitis) is strongly associated with pain symptoms and pain sensitization in OA, responses to exercise may depend on the degree of inflammation in OA joints. Here, we assessed synovial tissue mechanobiological responses to high and low frequency mechanical stretch loading and whether these responses are altered by the burden of inflammation.
Methods: Synovial tissue samples were collected during total knee arthroplasty from 6 patients with end-stage knee OA. Each sample was split into 3 equally matched pieces and subjected to cyclic mechanical 10% stretch for 30 minutes at low (20 Hz, to model very slow walking 40 steps/min) or high (60 Hz, to model fast, more painful walking 120 steps/min) frequency or static (non-stretch control). Inflammation burden was determined as high (n=3) or low (n=3) using the semi-quantitative OMERACT grading system for musculoskeletal ultrasound and synovial tissue histology (sub-intimal leukocyte infiltration). Following mechanical stimulation, tissue samples were incubated in fresh culture media for 6 hours prior to whole tissue RNA isolation for RNA sequencing. RNA sequences were aligned and sorted by coordinates, to the NCBI human genome. Quantification of genes was performed in featureCounts, DESeq2 was used to normalize feature counts and find differentially expressed genes, and pathway enrichment was determined using KEGG analysis.
Results: The mean age of patients included in the study was 66.5 (+/- 9.65) and mean BMI was 36.1 (+/- 5.61), with 4 males and 2 females. All patients had end-stage knee OA with Kellgren-Lawrence grade 3-4. Gene lists were compared between stretch conditions (control vs low frequency; control vs high frequency), and then within stretch conditions and between tissues with high versus low levels of inflammation. 1885 mechanoresponsive genes were significantly dysregulated by low frequency stretch versus 168 mechanoresponsive genes by high frequency stretch. Common pathways that were highly enriched by both low and high frequency stretch were genes involved in phagocytosis, Toll-like receptor signaling, NF-kappa-B signaling, and TGF-beta signaling. Pathways uniquely enriched by low frequency stretch include genes involved in osteoclast differentiation, necroptosis, NOD-like receptor signaling, and those suggestive of adaptive immunity (Th1 and Th2 differentiation, B cell receptor signaling). Pathways uniquely enriched by high frequency stretch include genes involved in innate immunity including cytokine receptor interaction, chemokine signaling, and natural killer cell-mediated cytotoxicity. In secondary analyses, we explored differences in mechanically-induced gene expression between patients with high versus low burden of synovial tissue inflammation. Venn analyses showed that only 1.8% (17) of genes are mechanoresponsive in both low and high inflammation groups and are therefore purely mechanoresponsive, while 98.2% (945) of significantly mechanoresponsive genes are influenced by the inflammation status of the tissue. Thus, suggesting that inflammation strongly influences gene expression in response to stretch.
Conclusions: This work explores a mechanobiological basis for further understanding the clinically-relevant effects of exercise on knee joints with OA, including mechanisms controlling joint homeostasis, immune cell function and interaction, and pain. We provide evidence that synovium is mechanoresponsive, with differential transcriptional network activation in response to mechanical loading frequencies that model less-painful (slow) vs painful (fast) walking. Further, we provide contextual understanding of these effects in the presence of synovial inflammation, which affects a large proportion of knee OA patients and appears to modulate synovial responses to mechanical loading.

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LONG-TERM EFFICACY AND SAFETY OF INTRA-ARTICULAR SPRIFERMIN IN PATIENTS WITH KNEE OSTEOARTHRITIS: RESULTS FROM THE 5-YEAR FORWARD STUDY

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Purpose: The 5-year Phase II FORWARD study assessed the efficacy and safety of the potential disease-modifying osteoarthritis drug (DMOAD) sprifermin (recombinant human fibroblast growth factor 18) in patients with symptomatic, radiographic knee osteoarthritis (OA). The primary endpoint assessed at Year 2 showed dose-dependent modification of total femorotibial joint (TFTJ) cartilage thickness with sprifermin vs placebo (PBO) by quantitative magnetic resonance imaging (qMRI), and this was found to be sustained at Year 3 follow-up in a subsequent exploratory analysis. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) total scores improved by ~50% in all cohorts, including PBO, at Years 2 and 3. Post-hoc exploratory analyses identified a patient "subgroup at risk" of further structural and symptomatic progression, with thinner minimum joint space width (mJSW) and greater WOMAC pain at baseline, which displayed a clinically relevant differentiation in WOMAC pain score changes between sprifermin (100 μg every 6 months [q6mo]) and PBO at follow-up. Here, we report the long-term 5-year efficacy and safety results in the overall population and the "subgroup at risk".
Methods: Patients were randomised 1:1:1:1:1 to receive intra-articular sprifermin 100 or 30 μg q6mo, 100 or 30 μg q12mo, or PBO, for 18 mo. The treatment period-related analysis for the primary endpoint was 2 years, with follow ups over 3 years post treatment (at 3 and 5 years). The intent-to-treat (ITT) population included all randomized patients; the modified (m)ITT population all patients with a baseline and ≥1 qMRI reading up to Year 2. Post-hoc exploratory analysis was conducted in the "subgroup at risk" (minimum medial or lateral JSW of 1.5-3.5 mm and WOMAC pain 40-90 [0-100 scale; 100 = worst pain] at baseline). Treatment differences vs PBO were estimated using a repeated measures model controlling for baseline, treatment, time, pooled country and treatment by time interaction. Confidence intervals (CIs) were adjusted for multiplicity of treatments using Dunnett adjustment. Linear dose-effect trend tests were performed exploratively at each timepoint.
Results: 474 (86.3%) patients completed the primary 2-year observation period (including 18 mo of active treatment); 442 (80.5%) and 378 (69%) patients completed the 3 and 5-year extended follow-up periods, respectively. The significant dose-response effect of sprifermin on TFTJ cartilage thickness change seen at Year 2 was preserved to Year 5 (trend test, p<0.001), and the 0.05 mm greater mean increase in TFTJ cartilage thickness with sprifermin 100 μg q6mo vs PBO seen at Year 2 was sustained to Year 5 (95% CI 0.004, 0.095; p=0.015; Table 1). WOMAC pain scores were improved by ~50% from baseline to Year 5 in all cohorts, including PBO (Figure 1). Post-hoc analysis of the "subgroup at risk" (n=161) identified a potential differentiation in WOMAC pain scores between the sprifermin 100 μg q6mo (highest dose) and PBO groups at Year 2 (-5.82; 95% CI -18.87, 7.23), Year 3 (-8.75; CI -22.42, 4.92) and Year 5 (-10.08; 95% CI -25.68, 5.53; Figure 2). At Year 5, there was no notable difference in the incidence of adverse events (AEs), serious AEs or study discontinuation due to AEs in any sprifermin group vs PBO in the overall population or the "subgroup at risk". AEs were mostly moderate in severity. A total of 181 patients (33%) reported serious AEs, but none were deemed related to treatment by the investigators. Withdrawals from the study due to AEs were <10%,