the current study were pair-fed a matched 10% kcal fat (lard) diet, suggesting the importance of diet in OA pathology. Future work is needed to compare the independent effects of leptin and obesity with or without inflammation on the underlying mediators of cartilage degeneration.

Figure 1. Knee OA scores in leptin receptor mutant Zucker fatty rats (A) and F344BN rats undergoing chronic systemic leptin treatment (B). Obesity in the absence of leptin signaling and increased systemic leptin in the absence of obesity were both sufficient to induce site-specific increases in spontaneous knee OA (*p<0.05).

86 SYNOVIAL WNT AND WISP1 EXPRESSION INDUCES CARTILAGE DAMAGE BY SKEWING OF TGF-BETA SIGNALING VIA THE CANONICAL WNT SIGNALING PATHWAY

M. van den Bosch, A. Blom, P. van Lent, H. van Beuningen, E. van de Loet, E. Blaney Davidson, P. van der Kraan, W. van den Berg, Radboud Univ. Nijmegen Med. Ctr., NCMIS, Nijmegen, Netherlands

Purpose: Many osteoarthritis (OA) patients show significant synovial involvement. Recently, we found strong upregulation of canonical Wnts 2b and 16 and Wnt-1 induced signaling protein 1 (WISP1), a downstream protein, in the synovium in two murine OA models. Wnt signaling has been implicated in OA through activation of the hypertrophy-inducing ALK1 pathway. On a functional level, pre-incubation with Wnt3a and/or WISP1 led to decreased CAGA-Luc reporter construct activity, confirming decreased AK5 signaling. Moreover, the expression of the anti-hypertrophic factor Sox9 was decreased after pre-incubation with Wnt3a and WISP1. In order to investigate whether Wnt3a skews the TGF-β signaling via the canonical signaling pathway, we pre-incubated chondrocytes with Wnt3a and/or WISP1 in the presence of DKK-1, a selective inhibitor of the canonical Wnt signaling pathway. Compared to the groups without DKK-1, we found increased Smad 2/3 phosphorylation and decreased phosphorylation of Smad 1/5/8 after Wnt3a stimulation.

Conclusions: Wnts produced in the synovium may play an important role in OA pathology by changing the chondrocyte phenotype, probably through modulation of the important TGF-β signaling pathway via the canonical Wnt signaling pathway. This points towards Wnt/WISP1 expression in the synovium as an interesting target for OA therapy.

87 MECHANICAL LOAD-INDUCED BONE REMODELING REQUIRES FSTL3 EXPRESSION

A. Blazek, P. Perera, D. Knapik, L.-C. Wu, A. Litsky, Z. Sun, D.-C. Kim, B. Lebelecibicglou, S. Agarwal, The Ohio State Univ., Columbus, OH, USA

Purpose: Physical activity is shown to strengthen bone, however, its molecular mechanisms remain unclear. Here, we identified a molecule, FSTL3 (NM_005860), that may drive mechanical load-dependent bone remodeling.

Methods: IACUC at the Ohio State University approved all protocols. Female Sprague Dawley rats (12-14 wks, n=10), wild-type (WT, 10-12 wks, n=10/group) or homozygous FSTL3-/- (C57Bl/6 mice (10-12 wks, n=10) were subjected to exercise by treadmill running at 12 M/min (rats) or 8 M/min (mice) for 45 min/day. Following 0, 2, 5 or 15 days of exercise, animals were sacrificed and gene expression analyzed by quantitative real time polymerase chain reaction (qTqPCR), Western blots, or immunohistochemistry of the trabecular bone/bone marrow at the distal ends of femurs. Bone mineral apposition rates in wild type or FSTL3-null mice was assessed by administration of Calcein and Alizarin complexones on day 3 and 12, during the total exercise period of 15 days. Statistical analysis was performed by one-way ANOVA with Tukey’s post hoc or T-test.

Results: Trabecular bones/bone marrow from exercised healthy mice for 2 or 5 days were subjected to the gene expression analysis of Fstl3, Follistatin (Fst), Inhibin-Alpha (Inha), Inhibin-Beta-A (Inhba), and -Beta-B (Inhhb) that are known to regulate ligands in TGF-β superfamily. The qTqPCR revealed that exercise dramatically stimulated Fstl3 mRNA expression in the trabecular bone and bone marrow cells, reaching 6 fold increase on day 2 and then declining to basal levels by day 3. However, there was no significant change in the expression of Inha, Inhba, and Inhhb. The expression of Fstl3 in osteoblasts at the protein level assessed by immunofluorescence in the sagittal sections of rat femurs exhibited significant expression of Fstl3 in the control non-exercised trabecular bone or minimal cells lining trabecular bones. However, a robust increase in Fstl3 expression was observed in the femurs of rats subjected to exercise at day 2 and 5, predominantly within and adjacent to trabecular bones. Osteocalcin used as a positive marker for osteoblasts in adjacent sections of the same bones, confirmed the osteoblastic phenotype of FSTL3 positive cells lining the bone. Female Fstl3+/+, Fstl3+/−, and Fstl3−/− mice (12 wks old) subjected to exercise for 15 consecutive days, exhibited significantly narrow distance between the Calcine and Alizarin incorporation and limited bone deposition in all three groups of non-exercised control mice. Fstl3+/+ and Fstl3+/− mice when subjected demonstrated a significant increase in bone deposition on the endosteal surface of the posterior side of the femur together with increase in the total MAR (Fig 1). Strikingly, such exercise-induced bone formation and hence increase in MAR was not observed in Fstl3−/− mice. The representative stress-strain curves of the cortical bone of the WT and Fstl3−/− femurs under three point bending test revealed that strain at ultimate strength of femurs from WT mice was approximately 25% greater than that of Fstl3−/− mice. Furthermore, the yield strain and yield energy were significantly upregulated by exercise in WT, but not in Fstl3−/− mice. Another evidence of FSTL3 in bone formation was more rounded shape and uniform deposition of calcine and alizarin complexone at the periosteal as well as endosteal surfaces of the femur in the Fstl3+/− mice.

Conclusions: The identification of FSTL3 as a mechaenorepressive protein may provide a new paradigm for investigating exercise-driven bone formation and a marker to monitor the effects of exercise on bone in health and in a wide spectrum of diseases. We further anticipate that FSTL3 may serve as a target to develop therapeutic drugs for treating bone diseases.