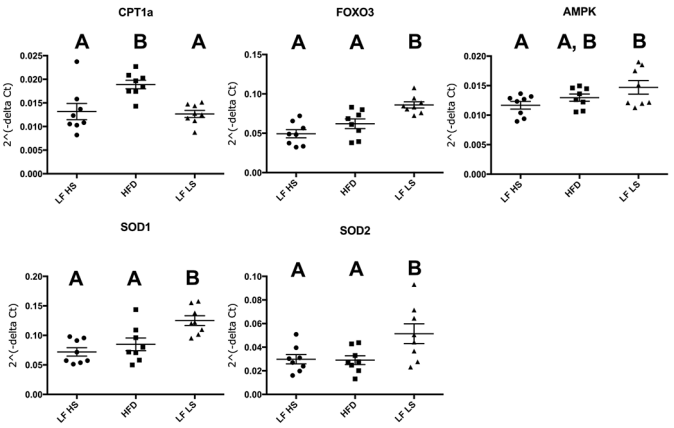

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GENE THERAPY FOR FOLLISTATIN DECREASES SYSTEMIC INFLAMMATION AND PAIN SENSITIVITY FOLLOWING KNEE INJURY IN HIGH-FAT DIET INDUCED OBESE MICE

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Purpose: Both obesity and joint trauma are risk factors for osteoarthritis (OA) and OA-related pain. In addition, muscle may have a role in knee OA development as changes in muscle strength could affect kinematics of joint loading. Furthermore, because of its key function in glucose storage and utilization, muscle strength and physiology are particularly vulnerable to obesity-associated inflammation. However, the mechanical, inflammatory, and metabolic links among obesity, muscle, and OA remain unclear. Follistatin (FST), an activin-binding protein, has been used as an effective treatment for muscle degenerative diseases by increasing muscle growth due to its ability to inhibit myostatin, a negative regulator of muscle mass. Moreover, FST has been reported to reduce the infiltration of inflammatory cells in the synovial membrane of the knee joint. Therefore, we hypothesized that overexpression of FST using a gene therapy approach will mitigate obesity-associated inflammation and pain sensitization in a high-fat diet (HFD) induced obesity model of mouse OA.

Methods: Six-week old male C57BL/6 mice were fed either a chow control-diet or HFD (60% fat by kcal, n = 16/dietary group). At 7-weeks of age, either an AAV9-mediated FST 314 gene vector or GFP-labeled control vector (n = 10 for each dietary groups) was delivered via tail vein at a final dose of 6×10^{11} vg/ mouse. At 16-weeks of age, mice in each group (n = 10) underwent surgery to destabilize the medial meniscus (DMM) on the left knee. The remaining mice served as non-surgical controls. At time of surgery, Dual Energy X-ray Absorptiometry was conducted to measure body composition and bone mineral density (BMD). Six-weeks post-surgery (22-weeks of age), blood was collected and analyzed using a 31-plex Luminex® assay. Peripheral thermal sensitivity was determined using a hot/cold plate, while mechanical pain algesia at the knee was evaluated by a pressure-pain test. A two-way ANOVA between groups or a paired t-test within each group was performed to determine statistical significance, as appropriate ($\alpha = 0.05$).

Results: Prior to surgery, HFD resulted in increased body weight for both GFP- and FST-treated animals compared to chow ($p < 0.05$). However, overexpression of FST significantly decreased percentage of body fat in both chow and HFD treated mice as compared to their own corresponding GFP-controls. There was a main effect of both diet ($p = 0.028$) and FST-treatment ($p = 0.007$) on bone mineral density, such that HFD decreased BMD (HFD-GFP: $0.46 \pm 0.23 g/cm^2$ vs. chow-GFP: $0.51 \pm 0.15 g/cm^2$) while FST increased BMD (HFD-FST: $0.52 g/cm^2 \pm 0.17$, chow-FST: $0.56 g/cm^2 \pm 0.17$). Six weeks post-surgery, serum inflammatory profiles for HFD-GFP animals demonstrated increased systemic inflammation compared to chow diet, which was attenuated in the HFD-FST animals across fourteen markers (Table 1). HFD increased hot-plate paw-withdrawal latency time in GFP-treated animals compared to all other groups ($p < 0.05$), and the phenotype was rescued by FST-treatment in the HFD animals. Similarly, reduced cold plate responsiveness was also observed in HFD animals, which was rescued by FST-treatment. No differences in thermal peripheral pain were found with surgery. Additionally, FST treatment protected HFD animals from mechanical algesia at the knee post-DMM surgery (Fig. 1).

Conclusions: Overexpression of FST decreased body fat, pain sensitivity, and obesity-associated systemic inflammation, while increasing whole body BMD in mice with HFD-induced obesity. These findings further support the important role of muscle in obesity and inflammation, which are well-known risk factors associated with OA onset and progression. We are currently evaluating the relationship among these factors in these mice, and whether FST treatment will mitigate OA development due to obesity and joint injury.

Serum Markers Decreased with FST Treatment in HFD Animals compared to HFD animals with GFP.

Marker	HFD-GFP Mean ± SEM (ng/mL)	HFD-FST Mean ± SEM (ng/mL)	p-value
CXCL1/KC	0.25 ± 0.03	0.16 ± 0.02	0.050
CXCL10/IP-10	0.09 ± 0.01	0.06 ± 0.04	0.005, #
IL-1α	1.03 ± 0.17	0.53 ± 0.08	0.022*
IL-1β	0.06 ± 0.02	0.03 ± 0.01	0.028*
Leptin	137.93 ± 17.43	17.69 ± 4.64	0.001*
Insulin	25.22 ± 4.88	4.30 ± 0.65	0.001*

* indicates $p < 0.05$; # indicates $p < 0.05$ between DMM animals and control surgery animals.

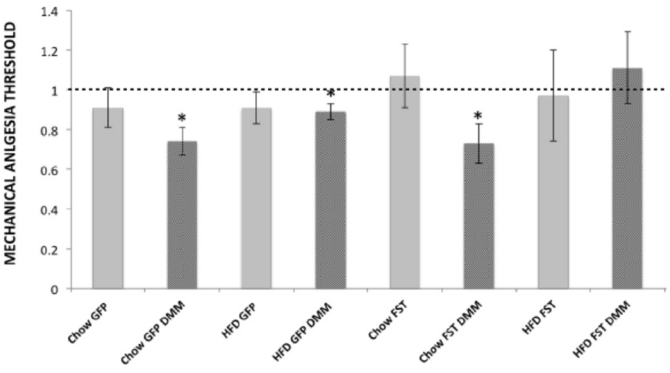

Fig. 1. HFD-FST protected against mechanical algesia at the knee with DMM surgery. *indicates $p < 0.05$ DMM limb vs. non-operated limb. Data are shown as the ratio of DMM to non-operated limbs for each group; line indicates symmetry between limbs (ratio = 1).

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EFFECT OF MODERATE INCREASING EXERCISE ON THE MECHANICAL BALANCE OF THE KNEE JOINT IN YOUNG RATS

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Purpose: It is hypothesized that the amount, duration and magnitude of mechanical loading are important factors that maintain the cartilage tissue in physiological condition. The underlying subchondral bone attached to the cartilage tissue and the cartilage-bone interface are influenced by the mechanical loading as well. In this study, we aimed to investigate adaptation of the rat knee joint to the mechanical demands. For this purpose, we applied load in the form of exercise using a moderate-intensity increasing running protocol. A series of analyses was performed to elucidate the response of cartilage and bone to this physical activity.

Methods: Male Wistar rats (Charles River, Germany) with an age of 8 weeks were placed in 2 groups: a moderate running group that runs for

8 weeks with a slowly increasing running velocity - from 10 m/min for 10 min, up to 20 m/min for 1 hour (n = 10), and a control group without running (n = 10). Running takes place on a 5 lane motorized rodent treadmill (LE-8700; Panlab Harvard Apparatus). At starting point and after 8 weeks cartilage qPCR, micro-CT, histology and plasma FIB 3-2 (Artialis) was performed.

Results: A total 24 km running within 8 weeks of this running protocol illustrates chondrocyte sensitivity and cartilage response to the mechanical loading (Fig. 1). At the end of the experiment, aggrecan was 1.55-fold up-regulated while MMP-2 was 2.38-fold down-regulated ($P < 0.05$) (Fig. 1A). The histological appearance of the chondrocytes also showed load-dependency, with more hypercellularity and hypertrophy in the running group (Fig. 1B). FIB3-2 as a plasma biomarker, interacts with the tissue inhibitor of metalloproteinase 3 (TIMP-3) and the elevated amount of FIB3-2 is expected in osteoarthritis samples. FIB3-2 is also known to be cleaved by several MMPs family including MMP-2. FIB3-2 levels dropped in the running group (from 52.7 ± 13.2 nM to 30.2 ± 8.4 nM) as compared to the control group (45.3 ± 15.0 nM to 33.3 ± 9.7 nM) (Fig. 1C). MicroCT analysis revealed an enhancement in bone response as a result of early moderate physical training where epiphysis bone parameters in the running group including thickness and bone volume fraction of subchondral bone tibia plateau as well as trabecular bone mass significantly increased compared with control animals (Fig. 2).

Conclusions: Gradual increase of running up to a moderate level to 1120 m/h for one hour enhances aggrecan expression, reduces catabolic enzymatic activity of MMP-2 as well as increases subchondral bone thickness in the epiphysis area and leads to hypercellularity and hypertrophy of the chondrocytes. Conclusively, a moderate exercise program can significantly influence both bone remodeling and cartilage tissue adaptation.

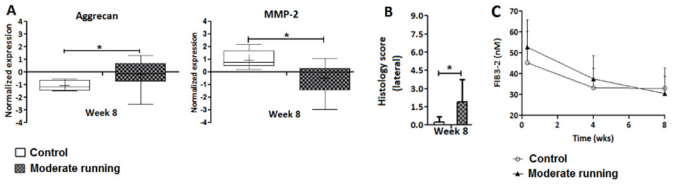

Fig. 1. (A) Aggrecan and MMP-2 gene expression. The values are expressed as a Log_2. *GAPDH* and ACTB1 were used as reference genes (B) Changes in the chondrocyte population (hypercellularity and cell clustering) determined by Safranin-O histological staining. (C) Plasma levels of Fib3-2, before start of the running regime (t = 0), at weeks 4 and 8.

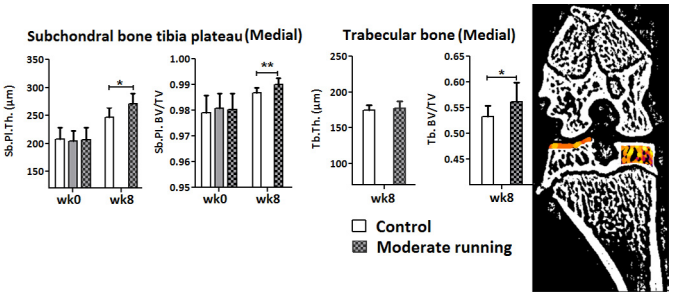

Fig. 2. Micro-CT result on tibial epiphysis. Shubchondral bone thickness (Sb. Pl. Th.), shubchondral bone volume fraction (Sb. Pl. BV/TV), trabecular bone thickness (Tb. Th.), and trabecular bone volume fraction (Tb. BV/TV) * p-value ≤ 0.05, ** p-value ≤ 0.01.

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EXERCISE INDUCES TRANSIENT INFLAMMATORY AND PRO-FIBROTIC REMODELING OF THE INFRAPATELLAR FAT PAD

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Purpose: The pro-inflammatory phenotype of the infrapatellar fat pad (IFP) in OA knee joints has raised considerable interest in understanding how this tissue contributes to the risk of OA development and progression. Some aspects of IFP pathology, such as trauma-related fibrosis, calcification, and impingement, are associated with knee pain and often referred to as Hoffa's disease. However, the biological response of the IFP to increased mechanical loading in the healthy knee is poorly understood. The IFP is predicted to contribute to knee OA risk by modulating the production of pro- and anti-inflammatory paracrine signaling molecules. Exercise reduces pro-inflammatory adipokine production and the cross-sectional area of subcutaneous adipocytes. Therefore, our goal was to understand the effect of exercise on IFP remodeling and inflammation. We hypothesized that increased physiologic joint loading would reduce IFP adipocyte size and the expression of pro-inflammatory cytokines and adipokines.

Methods: All procedures were conducted following an approved IACUC protocol. Studies were performed on 14 wk old male C57BL/6J mice. We increased physiologic loading of the IFP using voluntary wheel running and compared mice housed in standard cages (sedentary) to those after 3 and 14 days of running. IFP remodeling was evaluated by histomorphology and by Sirius red staining under epipolarized light to quantify fibrotic collagen deposition (n = 5). IFP inflammation was evaluated using a 42-gene customized RT-qPCR array including pro- and anti-inflammatory cytokines, adipokines, and inflammatory cell surface markers (n = 6). Cellular mediators of the IFP remodeling and inflammatory response to exercise were evaluated by flow cytometry. IFP stromal vascular fraction (SVF) cells were isolated by enzymatic digestion of IFPs pooled from both knees for 8 animals and repeated 3–4 times (n = 3–4). Isolated cells were stained for CD45.2, CD3, CD19, F4/80, CD11c, CD206, Ly6G/C and CD140a. Aqua zombi was used to determine cell viability. Results were reported as mean ± SEM, with significance evaluated by Student's t-test or 2-way ANOVA with multiple comparison correction ($p < 0.05$).

Results: Wheel running did not alter body weight or the overall IFP cross-sectional area evaluated mid-joint in the sagittal plane. 3 days of running increased IFP fibrosis, which was negatively associated with adipocyte cross-sectional area ($r^2 = 0.71$, $p = 0.07$). However, by day 14, IFP fibrosis and adipocyte size returned to sedentary levels (Fig. 1). Similar to the transient fibrosis response, the expression of numerous pro- and anti-inflammatory cytokines and chemokines were elevated at 3 days and returned to sedentary levels by 14 days (Fig. 2). In particular, wheel running increased the expression of IL-1β, Lta, IL-3, Itgb2, Ccr2 and Ccl2 ($p < 0.05$). Anti-inflammatory cytokines were also increased at 3 days, including IL1rn and Mrc1, suggesting a combination of inflammatory activation and resolution at this time point. We next, evaluated the effect of running on SVF cell populations. 3 days of running transiently reduced the viability of isolated SVF cells to 87.1% ($p < 0.0001$) from 96.2% and 96.4% in sedentary and 14-day samples, respectively. However, the immune cell population ($CD45.2^+$) was significantly increased after 3 days of running (Fig. 3). Consistent with the pro-inflammatory gene expression, the CD11c+ population of macrophages (F4/80+, Ly6C/G-) was increased at day 3. Although there were no significant differences in the immunoregulatory CD206+ population of macrophages with exercise, ~90% of IFP macrophages were CD206+. Running did not alter T-cells (CD3+) and slightly reduced B-cells (CD19+) at 3 and 14 days ($p = 0.04$ and 0.08, respectively). The overall effect of running on IFP adipokine expression was modest, with a transient increase in adiponectin expression at 3 days ($p < 0.01$), a trend for reduced leptin expression at 14 days (p=0.13), and no change in Nampt expression.

Conclusions: 14 days of running does not reduce IFP adipocyte size or adipokine expression in mice. Rather, running transiently upregulates IFP inflammation, activated macrophages, and fibrosis. These findings suggest a physiologic role for inflammation in load-induced IFP remodeling in young healthy knees.

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INCREASED CARTILAGE REMODELLING AND IMPAIRED CHONDROCYTE MECHANOTRANSDUCTION IN EARLY POST-TRAUMATIC OSTEOARTRITIS

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Purpose: A ligament rupture in the knee changes the joint kinetics and usually leads to the development of post-traumatic osteoarthritis (PT-