Methods: Fat conditioned medium (FCM) was made by culturing small pieces of infrapatellar fat in medium for 24 hours. The fat was obtained from osteoarthritic knees during total knee replacement. P2 synoviocytes, isolated from synovium obtained during total knee replacement, were seeded in high density and cultured in 9 different batches of FCM, representing different infrapatellar fat pad donors. Synoviocytes were also cultured in 10 ng/ml TGFβ1 as positive control. After 1 day, gene expression of myofibroblast markers PLOD2 (a cross-linking enzyme), α-smooth muscle actin (αSMA) and collagen type I was determined, after 4 days collagen content was measured. Furthermore, effects of FCM on proliferation (d1, 4, 5) and migration of the synoviocytes (scratch-wound assay, after 16 hours) were analyzed. Fat was also processed for immunodetection of CD68 (macrophages), CD86 (classically activated macrophages) and CD206 (alternatively activated macrophages).
Results: Fat Conditioned Medium induced synoviocytes to produce significantly more collagen (Figure 1) and express more PLOD2 (Figure 2) than when cultured in control medium. No clear effects were seen on collagen type I and αSMA gene expression. Synoviocytes cultured in FCM had a higher proliferation rate and migrated faster. Large numbers of CD68+ and CD206+ cells were present in the fat tissue. CD86+ cells were present in lower numbers.

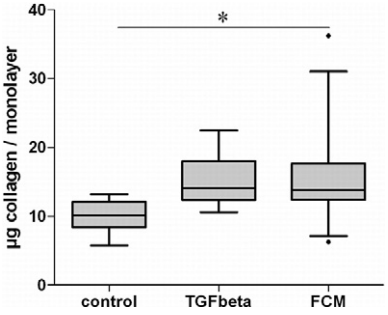

Figure 1. Collagen production by synoviocytes in monolayer. Average effect of 9 different batches of FCM is shown. *Indicates $p<0.05$.

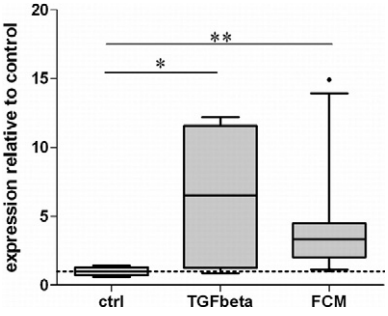

Figure 2. PLOD2 gene expression by synoviocytes in monolayer. Average effect of 9 different batches of FCM is shown. *Indicates $p<0.05$, **indicates $p<0.01$.

Conclusions: In conclusion, infrapatellar fat produces factors that can contribute to the development of synovial fibrosis by increasing collagen production, PLOD2 gene expression, cell proliferation and cell migration, all characteristics of a fibrotic process. A possible source for these factors could be the many alternatively activated (or wound healing) macrophages that were present in the fat tissue, since these cells are thought to be involved in tissue repair.

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MODULATION OF HYALURONIC ACID SYNTHESIS BY COLLAGEN HYDROLYSATES IN RABBIT SYNOVIOCYTES (HIG-82)

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Purpose: Collagen hydrolysate (CH) has been used as one of the functional foods for joint health. Some clinical studies have shown that oral ingestion of CH exerted its beneficial effect on OA symptoms. However, the efficacy of collagen-derived peptides has not been tested in clinical studies. So the therapeutic mechanisms remain unclear. There are several types of hydroxyproline (Hyp) -containing peptides in human blood after orally administered CH. Therefore we assessed whether CH-derived peptides and amino acids would influence hyaluronan (HA) synthesis in synoviocytes.
Methods: Rabbit synovial fibroblasts (HIG-82) were cultured in F-12 medium with 10%FBS. After 2 days, culture medium was replaced with serum free F-12 medium containing Gly-Pro-Hyp, Pro-Hyp, Hyp (50μg/ml) and incubated for 24h (RNA extraction) or 48h (HA assay). RT-PCR was performed to determine mRNA levels of hyaluronan synthases (HAS-1 and HAS-2). Culture medium was harvested for HA assay. HA content was measured by cellulose acetate membrane electrophoresis. Molecular weight of HA was estimated by agarose gel electrophoresis.
Results: Gly-Pro-Hyp and Pro-Hyp significantly up-regulated HAS-1 mRNA level in HIG-82 cells. Hyp treatment depressed markedly HAS-2 mRNA expression. But the amount of HA in the culture medium was not changed after the treatment of Gly-Pro-Hyp and Hyp. Pro-Hyp significantly stimulated the secretion of HA. The concentration of HA with a molecular weight greater than 700 kDa in the medium was higher in the presence of Hyp
Conclusions: Collagen hydrolysate-derived peptides enhanced HA synthesis in synoviocytes. This phenomenon is associated with increase of HAS-1 mRNA expression. In contrast, amino acid Hyp depressed HAS-2 mRNA expression. But HA content in the medium was not changed, and the molecular weight of HA significantly increased after Hyp treatment. These findings suggest that oral ingestion of CH may exert its beneficial effect on OA symptoms through increased high molecular weight-HA production into the synovial fluid.

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THE REGULATION OF THE ADAMTS4 AND ADAMTS5 AGGRECANASES IN OSTEOARTHRITIS

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Purpose: To investigate the regulation of the ADAMTS4 and ADAMTS5 aggrecanases in human osteoarthritis (OA) synovium, with regard to cytokine stimulation with IL-1 and/or TNFalpha, and also the degree of NFkappaB dependence.
Methods: To investigate the role of TNFalpha and IL-1 in driving ADAMTS4 and ADAMTS5 expression in the human OA synovium, we used a model of cultures of synovial cells from digested human OA synovium. It was possible to effectively and specifically neutralize the endogenous production of IL-1beta and/or TNFalpha from the OA synovial macrophages. Cultures were either left untreated, incubated with the p75 TNF soluble receptor Ig fusion protein etanercept (Enbrel), incubated with a neutralizing anti-IL-1beta antibody, or incubated with a combination of Enbrel and anti-IL-1beta. After incubation for 48 h, cells were washed and RNA extracted using Tri-reagent, to be taken to RT-PCR analysis using oligonucleotide primers specific for ADAMTS4 and ADAMTS5, with GAPDH used for comparison of gene expression.
The experiments with regard to the degree of NFkappaB dependence of ADAMTS4 and ADAMTS5 made use of outgrown human OA synovial fibroblasts. Treatment with IL-1beta or TNFalpha, but not treatment with phorbol ester, resulted in upregulation of ADAMTS4, whereas ADAMTS5 was unaffected by these stimuli. In this model, it was possible to use adenoviral gene transfer of the endogenous inhibitor IkappaBalpha to specifically inhibit NFkappaB, without affecting other intracellular signalling pathways, or inducing significant apoptosis.
Results: In OA synovial cell cocultures, there was no effect of either Enbrel or the neutralizing anti-IL-1beta antibody on ADAMTS5 expression, nor was this aggrecanase at all affected by a combination of these treatments. Thus ADAMTS5 appears to be constitutive in OA synovial cells. In contrast, ADAMTS4 was significantly ($p<0.05$) inhibited by Enbrel, and more potently ($p<0.01$) inhibited by a combination of Enbrel and the neutralizing anti-IL-1beta antibody. This would indicate that in the human OA synovium, the upregulation of ADAMTS4 is dependent on TNFalpha and IL-1 produced by the synovial macrophages, whereas the level of ADAMTS5 is not changed by these cytokines.
In OA synovial fibroblasts, ADAMTS5 gene expression was not changed by adenoviral gene transfer of IkappaBalpha, irrespective of stimulus used. In contrast, ADAMTS4 induction by IL-1beta or TNFalpha was potently inhibited by NFkappaB downregulation.
Conclusions: Although both ADAMTS4 and ADAMTS5 cleave aggrecanase,

they appear to be very different enzymes with regard to their regulation. At least in human cells, ADAMTS4 responds to stimulation with IL-1 and/or TNFalpha, but ADAMTS5 does not. Another difference is that whereas the upregulation of ADAMTS4 depends on the transcription factor NFkappaB, ADAMTS5 is NFkappaB independent and lacks kappaB elements on its promoter. The differential regulation of ADAMTS4 and ADAMTS5 has implications for the potential development of disease-modifying osteoarthritis drugs. A therapeutic strategy that would inhibit the cytokine-driven inflammatory response would be likely to downregulate ADAMTS4, as would an inhibitor of NFkappaB. Neither strategy would be likely to influence ADAMTS5, however.
The design of small molecule aggrecanase inhibitors is an area of considerable interest for the pharmaceutical industry. For such approaches to meet with success, there is a need to appreciate that ADAMTS4 and ADAMTS5 are differentially regulated. The primary aggrecanase (ADAMTS4 or ADAMTS5) involved in human OA also needs to be conclusively identified.

Mechanobiology

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BIOMECHANICAL MODULATION OF COLLAGEN FRAGMENT INDUCED ANABOLIC AND CATABOLIC ACTIVITIES IN CHONDROCYTE/AGAROSE CONSTRUCTS

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Purpose: Examine the effect of collagen fragments on the anabolic and catabolic activities by chondrocyte/agarose constructs subjected to dynamic compression.
Methods: Constructs were cultured under free-swelling conditions or subjected to continuous and intermittent compression regimes, in the presence of the N-terminal (NT) and C-terminal (CT) telopeptides derived from collagen type II and/or 1400W (inhibits inducible nitric oxide synthase [iNOS]). The anabolic and catabolic activities were compared to the amino-terminal fibronectin fragment (NH2-FN-f) and assessed as follows: nitric oxide (NO) release and sGAG content were quantified using biochemical assays. TNFα and IL-1β release were measured by ELISA. Gene expression of MMP-3, MMP-13, collagen type II and fibronectin were assessed by real-time qPCR. 2-way ANOVA and the post hoc Bonferroni-corrected t-test was used to examine data.
Results: The presence of the NT or CT peptides caused a moderate to strong dose-dependent stimulation of NO, TNFα and IL-1β production and inhibition of sGAG content. In some instances, high concentrations of telopeptides were just as potent in stimulating catabolic activities when compared to NH2-FN-f. Depending on the concentration and type of fragment, the increased levels of NO and cytokines were inhibited with 1400W, resulting in the restoration of sGAG content. Depending on the duration and type of compression regime employed, stimulation with compression or incubation with 1400W or a combination of both, inhibited telopeptide or NH2-FN-f induced NO release and cytokine production and enhanced sGAG content. All fragments induced MMP-3 and MMP-13 expression in a time-dependent manner. This effect was reversed with compression and/or 1400W resulting in the restoration of sGAG content and induction of collagen type II and fibronectin expression.
Conclusions: Col-fs containing the N and C-terminal telopeptides have dose-dependent catabolic activities similar to FN-fs and increase the production of NO, cytokines and MMPs. However, the catabolic response was dependent on the concentration and type of fragment such that for conditions which represent cartilage degradation, collagen telopeptides were just as potent in increasing catabolic activities as the FN-fs. The application of dynamic compression could reverse the catabolic process induced by the fragments and enhance anabolic activities. However, the response was dependent on the length and type of compression regime applied. In addition, co-stimulation by dynamic compression in the presence of the iNOS inhibitor led to further time-dependent increases in the expression of matrix proteins and downregulation of cytokines and MMPs, linking reparative activities by both types of stimuli. The ability of chondrocytes to interact with matrix fragments and respond to biomechanical signals may be a key initiating event in the disease process. Further studies are needed to examine the complexity of the sequence of signalling events which interplay with biomechanical and matrix fragment signals for early OA therapeutic intervention.

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CHANGES IN THE CELLULARITY OF ARTICULAR CARTILAGE FOLLOWING CHRONIC ALTERED COMPRESSIVE LOADING OF THE RAT KNEE

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Purpose: Despite acceptance of the role of mechanical loads in the development and progression of osteoarthritis (OA), *in-vivo* quantitative assessments regarding the mechanism by which articular cartilage (AC) responds to different magnitudes and durations of sustained compressive loading are lacking. Using an *in vivo* model of chronic loading of the rat tibiofemoral joint, we investigated early load-induced alterations in the AC. We hypothesized that changes in chondrocyte distribution would result within 12 weeks of altered loading and that these changes would vary with the magnitude of the applied load.
Methods: Twenty-one male, Sprague-Dawley rats, 9 months of age, were randomized into 4 treatment groups: 0% (Sham), 50%, 80% body weight (BW), and Control. NIH guidelines for the care and use of animals were observed. Animals underwent surgery to implant transcutaneous bone plates and were fit with a varus loading device which applied compressive overloads of 0, 50 or 80% BW to the medial compartment of the tibiofemoral joint and an equivalent decrease in load to the lateral compartment. These altered loads were in addition to the normal forces experienced by the joint. Loading was applied 12 hr/day, 7 days/wk, for 12 wks. Following euthanasia, tibia plateaus were excised, formalin fixed, decalcified with 10% EDTA, and paraffin embedded. Serial, 5 μm-thick, coronal sections of the posterior half of the tibial plateau were deparaffinized and stained with Hematoxylin and Eosin prior to examination under light microscopy. Region specific (periphery, central and midline) measures were determined by dividing each compartment into 3 equally spaced regions (Fig. 1). Depth specific measures (superficial: 0-25%, mid: 25-50% and deep: 50-100%) were determined by dividing the AC thickness into three zones from the surface to the tidemark. Chondrocytes with visible nuclei were counted in each sub-area for each compartment. The cellularity of the AC (chondrocyte number/AC area) was determined for each region, depth, and sub-area. Three slides were evaluated for each leg. Analysis of variance was used to compare mean cellularity across experimental groups for sub-areas and the entire compartment (medial and lateral separately). Post-hoc pairwise comparisons were performed using Fisher's LSD procedure.

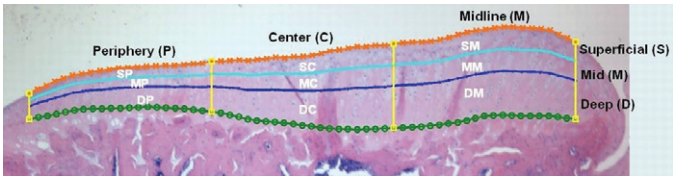

Figure 1. Articular cartilage of the tibial plateau illustrating the sub-areas evaluated in each compartment.

Results: Mean cellularity of the entire medial and lateral compartments were significantly different across groups (Medial: p=0.01; Lateral: p=0.03; Fig 2). Cellularity decreased with increased loading in the medial compart-

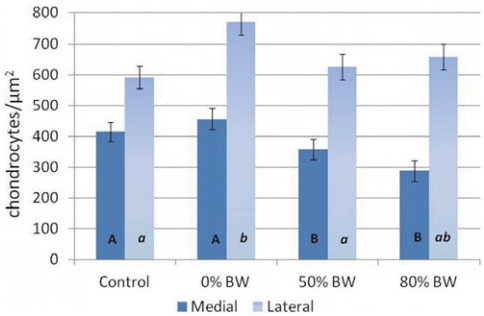

Figure 2. Cellularity of the medial and lateral compartments (mean±SE). Means not sharing a common letter are significantly different from one another ($p<0.05$).