Efficacy of chemical processes in collagen fibrils of articular cartilage is osmolality dependent

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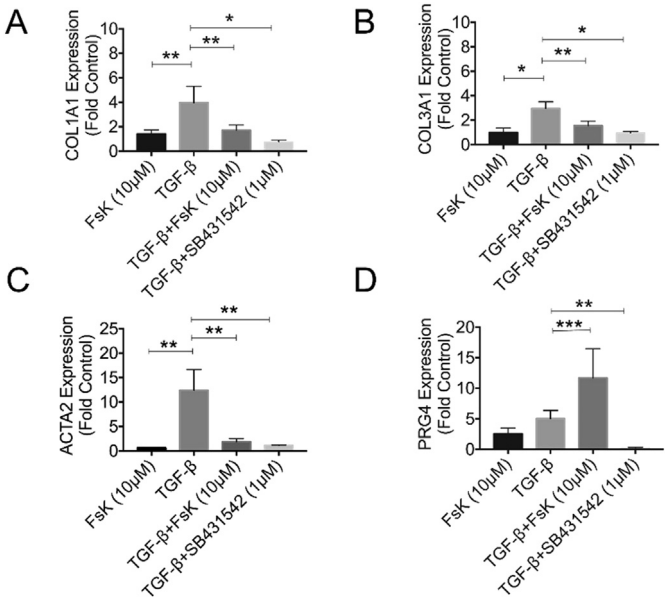

Fig. 1. Impact of Forskolin (FsK) treatment (10μM) on transforming growth factor beta (TGF-β)-induced expression of collagen type I (COL1A1), collagen type III (COL3A1), α_2 smooth muscle actin (ACTA2) and proteoglycan-4 (PRG4) in osteoarthritic fibroblast-like synoviocytes. SB431542 is a specific inhibitor of TGF-β signaling. *p<0.001; **p<0.01; ***p<0.05. FsK treatment reduced TGF-β induced COL1A1, COL3A1 and ACTA2 expression and increased TGF-β induced PRG4 expression.

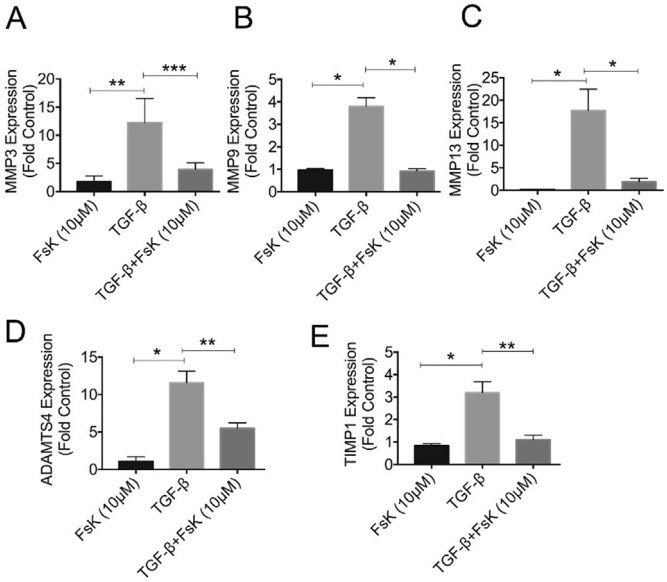

Fig. 2. Impact of Forskolin (FsK) treatment (10μM) on transforming growth factor beta (TGF-β)-induced expression of matrix metalloproteinases 3, 9, and 13 (MMP3, 9 and 13), aggrecanase-1 (ADAMTS4) and tissue inhibitor of metalloproteinase-1 (TIMP1). *p<0.001; **p<0.01; ***p<0.05. FsK treatment reduced TGF-β induced MMP3, MMP9, MMP13, ADAMTS4 and TIMP1 expression.

435
THE EFFECT OF TREADMILL RUNNING ON KNEE ARTICULAR CARTILAGE IN RATS

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Purpose: Appropriate mechanical stress is needed to maintain normal articular cartilage metabolism. Therefore exercise therapy is recommended as an effective treatment for osteoarthritis (OA). On the contrary, excessive exercise is detrimental for articular cartilage causing OA progression. In our previous study, hydrostatic pressure stimulation acts as catabolic or anabolic effect dependent on its intensity for chondrocytes *in vitro*. However, how exercise intensity affecting articular cartilage remains unclear *in vivo*. The purpose of this study was to investigate the metabolism of articular cartilage in knees of rats running on treadmills.
Methods: Twelve-week-old male Wistar rats ran on a treadmill at 12 m/min or 20 m/min for 45-min at a single time (n=16 in each group). Rats in control group were kept sedentarily in cages (n=16). All were sacrificed after running. Articular cartilage was removed from patellar, distal femur, and proximal tibia immediately after sacrifice. The cartilage was homogenized and total RNA was extracted. We analyzed gene expression of Sox9 and NF-κB as transcription factors, aggrecan and col2a1 as anabolic factors, and ADAM-TS5, MMP-13, and col10a1 as catabolic factors was analyzed with quantitative reverse transcriptase polymerase chain reaction (RT-PCR).
Results: Gene expression of sox9 was significantly higher in the 20 m/min group than the others. NF-κB expression was significantly higher in the 12 m/min group than control group. Gene expression of ADAM-TS5 in the 20 m/min group was significantly higher than the others. Agrrecan gene expression was increased in the 20 m/min group. There was no significant difference among all groups in gene expression of Col2a1, MMP13, or Col10a1.
Conclusions: Homeostasis in articular cartilage is constantly maintained by the balance of anabolic and catabolic factors. Collapse of this balance due to excessive mechanical stress, aging or something causes OA. According to previous report, running on treadmill at around 12 m/min prevented OA in the destabilization of the medial meniscus (DMM) model.In the 12 m/min group of our study, almost all gene expression didn't change compared with control group. 12 m/min running on treadmill at a single time does not adversely affect the homeostasis of articular cartilage. On the other hand, normal rats became OA by 20 m/min constant running in previous study. In present study, the 20 m/min running increased the expression of ADAM-TS5 and sox9. This results show that cartilage metabolism in the joint may be activated from the early stage with excessive exercise. In addition, our results indicate that gene expression of transcription factor was increased to maintain homeostasis in the cartilage, following up-regulation of ADAM-TS5 as catabolic factor by excessive mechanical stress. Anabolic factors may also subsequently increase to maintain homeostasis. In conclusion, even only a single treadmill running affects the metabolism of articular cartilage.

Matrix Biochemistry

436
EFFICACY OF CHEMICAL PROCESSES IN COLLAGEN FIBRILS OF ARTICULAR CARTILAGE IS OSMOLALITY DEPENDENT

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Purpose: Ageing of articular cartilage is considered a major risk factor in osteoarthritis (OA), of which a part might be explained by advanced glycation that provides accumulation of cross-links such as Pentosidine within the collagen molecules. This process leads to brittleness of the tissue and potential breakdown. Furthermore, irreversible proteolytic activity of mainly metalloproteinase matrix proteins (MMPs) leads to collagen fibril cleavage, which as well leads to breakdown of cartilage. Previous research showed that mechanical stretching in molecular and fibrillar forms as well as in cornea can protect collagens against enzymatic cleavage. In this research, we investigate the effects of stretching of collagen fibrils of articular cartilage on enzymatic activity of collagenase as well as non-enzymatic cross-linking activity.
Methods: Two separate equine femoral condyles were used to extract adjacent osteochondral plugs (diameter=8.5 mm) for cross-linking (n=5) and enzymatic (n=6) experiments. Upon thawing the cartilage layer was split into 4 quarter disks and they were osmotically equilibrated (400 mOsm/kg water and 4 °C) for about one hour. Micro-indentation (tip radius~50 μm and tip stiffness~ 50 N/m, Piuma, Optics, The Netherlands) was applied to obtain the average effective Young's modulus in a 1.5*1.5 mm^2 area containing 81 equally spaced indentation points. The disks for enzymatic experiment were then mechanically pre-conditioned in iso- (400 mOsm/kg water) and hyper-tonic (4000 mOsm/kg water) conditions. Additionally, the disks for cross-linking experiments underwent hypo-

tonic (100 mOsm/kg water) condition. Thereafter, enzymatic samples were incubated at 37 ºC and for 1 hour in collagenase type II (0.5 μM, Worthington Biochemical Corporation) in their corresponding osmolalities with a sufficient supply of Ca^{++}. Cross-linking samples were incubated in L-threose (50mM, Sigma Aldrich) instead for four consecutive days using the same incubation parameters as the enzymatic experiment. Post-treatment the samples were re-equilibrated (400 mOsm/kg water and 4 °C) for 1 hour and micro-indentation in the same area as in the initial step was performed. The relative difference in the average effective Young's modulus was used as an indication of the chemical activities that created either collagen cleavage and lowered the stiffness (collagenase type II) or created crosslinks and increased stiffness (L-threose). Moreover, surface color of the specimens after incubation in L-threose was quantified as an indication of non-enzymatic cross-linking efficiency.

Results: The loss in the average effective Young's modulus for the samples treated with collagenase type II was lower under hyper-osmolality condition (Figure 1). Within all groups, we observed an osmolality-dependent trend with respect to the cross-linking process (Figure 2). Increase of the osmolality is associated with a higher chance of collagen fibril cross-linking as suggested by the changes in the average effective Young's modulus. Furthermore, color analysis of cartilage surface confirmed the increase in cross-linking i.e. toward dark yellow, as the osmolality increases (Figure 3).

Conclusions: Chemical reactions are believed to be responsible for several types of collagen fibril alterations within articular cartilage, namely enzymatic cleavage occurring in OA and advanced glycation or non-enzymatic cross-linking occurring in ageing. One way to reduce these glycation reactions is to enhance the pre-stress in the collagen fibers, which can be accomplished by low osmolality in the external bath or by increased proteoglycan content. In the current study, we showed that collagen fibril pre-stress plays a role in controlling the degradation induced by bacterial collagenase as well, although in a reverse way compared to the glycation processes. Nevertheless, the effect of osmolality i.e. collagen fibril pre-stress on the enzymatic activity of MMPs is yet to be investigated as a complementary to this research. Therefore, mechanical forces in the macro scale could substantially affect the molecular processes within collagen fibrils in articular cartilage which could offer potential strategies to restrict them and consequently prolong the functional lifetime of articular cartilage.

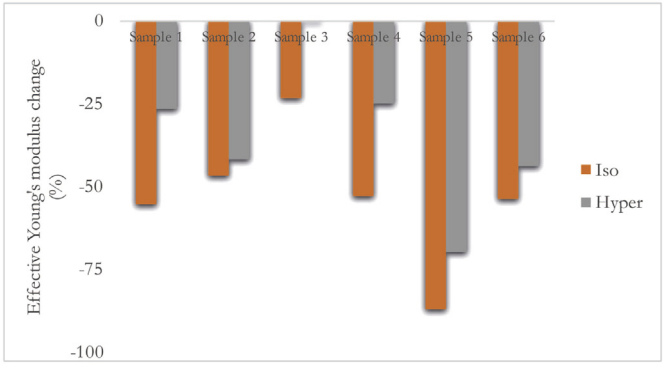

Figure 1: Changes in effective Young's modulus as a result of enzymatic treatment with collagenase II.

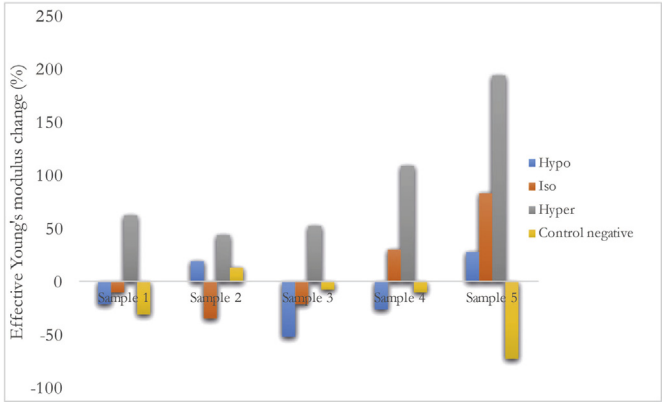

Figure 2: Changes in effective Young's modulus as a result of non-enzymatic cross-linking treatment.

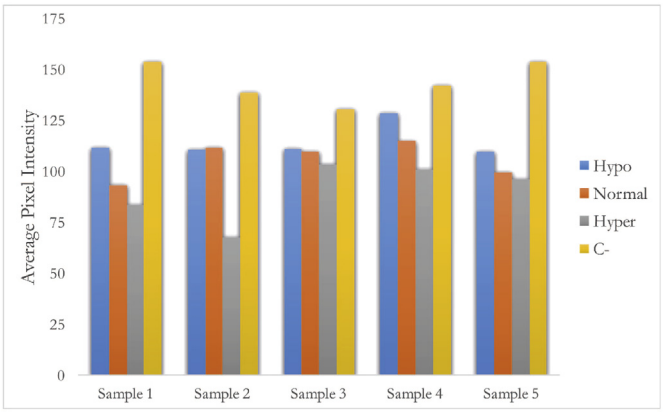

Figure 3: Analysis of cartilage surface color post-treatment with L-threose.

437
ESTABLISHMENT OF A HUMAN SYNOVIUM AND CARTILAGE CO-CULTURE

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Purpose: Osteoarthritis (OA) is a degenerative joint disease with a low-grade inflammatory component that leads to an altered turnover of extracellular matrix (ECM), not only in the cartilage, but also in the synovium and bone. The diseased tissues are believed to interact with each other and initiate and drive OA. However, the mechanism behind this interaction and the effect on the ECM turnover are unknown. The aim of this study was to establish an *ex vivo* co-culture model of the cartilage and synovium to study the interaction between the two tissues and its effect on the ECM turnover.

Methods: Human synovium and cartilage were obtained from end-stage OA patients undergoing total knee replacement. The synovium were cut into explants of 30±4 mg and the cartilage were punched with a 5 mm biopsy punches. After isolation of the two tissues, they were immediately added together in one well. Additionally both tissues were also cultured alone. The tissues were cultured for 14 days with OSM [10ng/mL] and TNFα [20ng/mL] (O+T), alone (w/o), or with O+T and GM6001 10 μM. GM6001 was only included in the co-culture system. The metabolic activity was measured with alamar blue weekly. Conditioned media were removed three times a week and fresh treatment added. The conditioned media were used for biomarker measurement. Four biomarkers, C3M, AGNx1, FFGV, and C2M, were measured by ELISA in the conditioned media.

Results: The human co-culture of the synovium and cartilage were cultured for 14 days. The explants were metabolic active throughout the study. However, the metabolic activity of the synovium dropped after 7 days on culture. Four biomarkers of the joint ECM turnover were measured in the conditioned medium and the accumulated biomarker over 14 days were calculated based on measurements of four time points. O+T increased the release of C3M 3.2-fold compared to w/o in the co-culture and 8.6-fold (P=0.016) compared to w/o in the synovium alone (Fig.1a). O+T increased the release of C2M 4.9-fold compared to w/o in the co-culture (P=0.012), 2-fold compared to non-treated synovium (P=0.041), and 2.4-fold compared to non-treated cartilage (P<0.001) (Fig. 1b). The MMP-mediated aggrecanse degradation, FFGV, was increased in 12.9-fold in response to O+T compared to w/o in the co-culture (P=0.003) and 7.1-fold in cartilage alone (P=0.002) (Fig. 1c). AGNx1, aggrecanase mediated degradation of aggrecan, was similarly released in response to O+T treatment compared to w/o, 2.2-fold increase in the co-culture (P=0.008) and 3.7-fold increase from cartilage alone (P<0.001) (Fig. 1d). Neither FFGV nor AGNx1 were released from synovium alone (Fig. 1c and d). No increased biomarker release was measured in the co-culture without external cytokine stimuli. The release of C3M, C2M and FFGV were MMP-depended as GM6001 inhibited the release (Fig. 1).