glucocorticoids have also serious long-term adverse events, as mellitus diabetes and osteoporosis.
GILZ is involved in osteogenic differentiation and reduces adipogenic differentiation of mesenchymal stem cells. Previously, we have shown that genistein promoted adipogenesis, but inhibited leptin production. In regards to theses observations, the aim of this study was to determine GILZ's implication in corticosteroid-induced leptin secretion.
Methods: Human synovial fibroblasts were isolated from OA patients during knee surgery. Lentiviral vectors were generated by co-transfecting Lenti-X 293T cells (Clonetech, Belgium) with a pSPAX2 plasmid (Addgene, Plasmid #12260), a VSV-G encoding vector and a GILZ (TSC22D3) shRNA plasmid (#TRCN0000013793 (shRNA1), #TRCN0000 364625 (shRNA2) or #TRCN0000369187 (shRNA3), Sigma-Aldrich, USA) or with a non target sequence encoding plasmid (Sigma, Belgium, SHC002). Cells were treated with prednisolone, aldosterone, Compound A, mifepristone, eplerenone and spironolactone. ELISA measured leptin production in culture supernatant. GR, GILZ and GAPDH were analyzed in total cell extracts by Western Blot.
Results: 1. Glucocorticoid (GR) and mineralocorticoid (MR) receptors were both expressed by OA synovial fibroblasts. Glucocorticoid prednisolone induced GILZ expression in OA synovial fibroblasts. Mineralocorticoid aldosterone also induced GILZ expression, but in a lower extend. Both GILZ expressions were dependent on GR. Indeed, GR antagonist (mifepristone), but not MR antagonists (eplerenone and spironolactone), inhibited prednisolone- and aldosterone-induced GILZ expressions. Previously, we observed similar modulation for leptin secretion, with a stimulation by prednisolone and aldosterone, through GR but not MR.
2. GILZ expression was already observed by western-blot after 24 h-stimulation with prednisolone. Under prednisolone stimulation, leptin secretion gradually increased day after day, with a similar profile of expression than GILZ, hypothesizing a link between GILZ and leptin expression. Opposite to prednisolone, Compound A, a dissociative glucocorticoid receptor agonist, did not induce neither leptin nor GILZ expression in human OA SF. Theses observations suggested a link between GILZ and leptin expression.
3. Upon prednisolone stimulation, GILZ-shRNA reduced GILZ expression with 3 different shRNA. When GILZ was down regulated, prednisolone-induced leptin was significantly decreased compared to the controls. Decrease of leptin expression was correlated to the degree of GILZ extinction. GR down-regulation was not affected by GILZ-silencing after seven days of stimulation.
Conclusions: Leptin and GILZ's modulations shared similar properties. GILZ silencing led to a significantly decrease expression of leptin under prednisolone stimulation. These results suggest that GILZ could be involved in prednisolone-induced leptin in OA synovial fibroblasts.

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PIGMENTED VILLONODULAR SYNOVITIS (PVNS)- LIKE CHANGES IN PERIPROTHETIC INTERFACE MEMBRANES

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Purpose: PVNS is a tumour-like mesenchymal lesion of uncertain histogenesis that may occur at both intra-articular and extra-articular sites. There have been infrequent reports about PVNS or similar changes after total endoprothesis (TEP). In this study, we describe and quantify the occurrence of PVNS-like lesions in the neosynovia following total endoprothesis.
Methods: Periprothetic membrane specimens from 477 patients undergoing replacement of their knee TEP were paraffin embedded, sectioned and hematoxylin-eosin stained. For evaluation of iron deposits the classic Prussian blue reaction was used. Wear particles were detected by conventional and polarisation microscopy. In order to compare PVNS-like lesions with genuine PVNS, 10 cases of the collective of PVNS-like periprothetic membranes as well as 10 representative PVNS cases were stained with antibodies against CD3, CD4, CD8, CD20, CD34, CD68, CD138, mast cell tryptase (MCT), collagen IV and Mib-1/Ki67.
Results: The neosynovia displayed a broad spectrum of histological changes. In 5,0 % (24 of 477) of all periprothetic membranes we found histological lesions showing some features of PVNS. In 3,8 % (18 of 477) of the cases the histology closely resembled PVNS with marked villous hyperplasia, fibrosis, iron deposits and a mixed cellular infiltrate composed of mononuclear cells, fibroblasts, foam histiocytes and multinucleated giant cells with varying hemosiderin deposits. Wear particles were detected in 63,3 % (302 of 477) of all perprothetic membranes and in 100% of the cases with features of PVNS (p-value <0,002). Regarding the age of the patients, no statistically significant difference was found between neosynovia with and without features of PVNS (69,4 +/-11,3 and 67,5 +/-11,3 respectively). The elapsed time between primary arthroplasty and revision surgery was 9,9 +/-6,4 years, a typical value for periprothetic membranes of the wear type. Immunohistochemistryshowed a similar pattern for PVNS and PVNS-like lesions, with a lower positivity for B- and T-Cells and especially for cytotoxic T-cells in PVNS-like lesions.
Conclusions: PVNS- like lesions found in the neosynovia of periprothetic membranes represent an exuberant fibrohistiocytic reaction and are likely induced by wear particles. Their development might be governed by the same pathological principle responsible for the classical PVNS in synovial membranes, probably involving chronic inflammatory/ irritation-induced synovial hyperplasia. These findings might provide an additional clue for the understanding of the pathological mechanism of classical PVNS.

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SYNOVIAL TISSUE EXHIBITS MOLECULAR HETEROGENEITY IN RESPONSE TO INTRAARTICULAR KNEE JOINT SURGERY

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Purpose: Severe injury to the knee joint, such as anterior cruciate ligament (ACL) tears and/or menisci damage often results in accelerated osteoarthritis (OA). It is thought that there may be an injury-induced, mechanical abnormality of the injured joint and subsequent interplay between altered mechanics and biological changes, such as inflammation, which may lead to OA. In recent years, the role of synovium in the pathophysiology of OA has gained importance. Clinical evidence suggests that inflamed synovium can add to adjacent articular cartilage damage, potentially contributing to OA. Histological analysis of synovial tissue biopsies from OA patients has demonstrated abnormalities: thickening, increased vascularity, and inflammatory infiltration. We have previously demonstrated that normal synovial tissue is a homogenous tissue for biomarkers of inflammation. However, following ACL reconstruction (ACL-R), the synovium exhibits increased mRNA levels for select inflammatory markers. Currently, it is unknown whether inflammation in the synovium tissue is uniformly distributed. Microscopic observation of synovium for pathological changes may provide relevant prognostic information, which can help in the early identification of the disease and possibly influence the therapeutic decisions. The hypothesis was that synovial tissue from different locations of the knee joint would demonstrate a uniform pattern in inflammatory marker expression.
Methods: Six skeletally mature, female Suffolk Cross sheep were allocated into three groups: ACL-R ($n = 2$), surgical sham ($n = 2$), and non-operated controls ($n = 2$). Surgeries involved an arthrotomy to the right stifle joint. For the ACL-R, the stifle joint was opened on the lateral side and the patella was dislocated medially to expose the ACL. A dry pneumatic drill was fitted over the guide pin and used to core out the uninjured ACL on its femoral insertion. The bone core was then released and immediately fixed in place using two crossed Kirschner wires. For the surgical sham, a similar arthrotomy and surgical approach to the ACL-R was used. The stifle joint was opened on the lateral side and the patella was dislocated medially to expose the ACL, followed by a similar standard closure. There was no injury to the ligaments in sham animals. The control group did not have the intervention. Animals were sacrificed 2 weeks after surgery and synovium tissue was collected from four different locations (Fig. 1), and snap frozen for mRNA analysis using real time qPCR. Part of the synovium was fixed for histological analysis and evaluated for microscopic changes with Hematoxylin and Eosin staining. ANOVA with Bonferroni post-hoc analysis was used to determine differences in mRNA expression between groups, using SPSS 19.0.
Results: Analysis of the synovium tissue exhibited that pro-inflammatory interleukins (IL) were generally elevated in the operated animals but not in the shams or non-operated controls. IL-8 and IL-1β were found to be distinctly higher in the lateral region of the synovium (Fig. 2A-B). Furthermore, matrix metalloproteinase (MMP)-13 was found to be higher in the lateral region (Fig. 2C). In contrast, IL-6 mRNA expression levels in the four locations of the synovium was similar (Fig. 2D). The surgical sham animals did not exhibit the same response as the ACL-R animals. Histological observations of the four locations of

the synovium showed varying responses to the sham and ACL-R surgery. Although no statistically significant differences were detected, the medial and lateral regions exhibited trends for increased synovitis when compared to patellar and posterior side (Fig. 3).

Conclusions: The observations suggest that synovium tissue exhibits heterogeneity with respect to the molecular expression of select inflammatory markers like IL-1β and IL-8. Furthermore, expression of the MMP-13 also followed a trend similar to these IL. However, IL-6 does not mimic this pattern of expression. The synovium tissue does show varied inflammatory response to the ACL-R. As the surgical shams did not exhibit alterations to levels of expression, we conclude that the synovium at the lateral location is sensitive to the inflammatory changes associated with ACL-R.

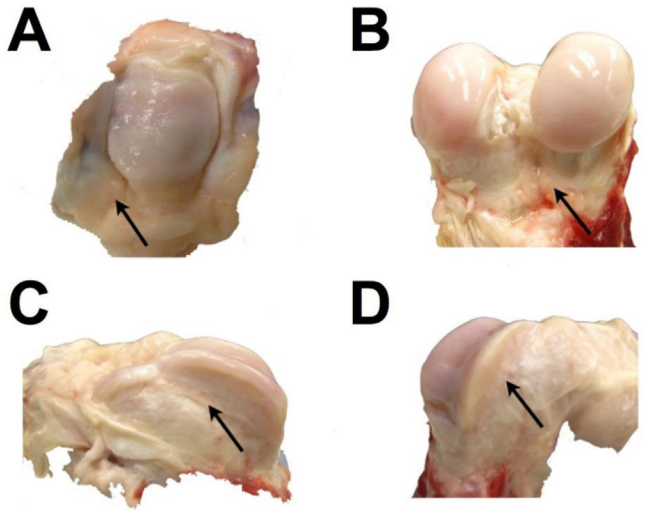

Figure 1. Images of the four sampling locations: patellar (A), posterior (B), medial (C), and lateral regions (D). Arrows indicate the site of collection of synovium biopsy.

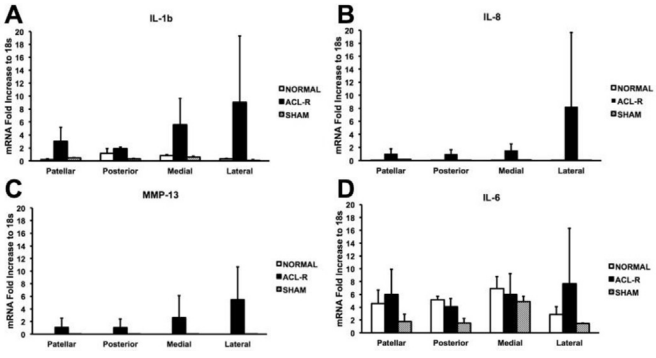

Figure 2. Analysis of mRNA levels at 2 weeks after ACL-R ($n = 2$) or sham ($n = 2$) surgery and the control group ($n = 2$) for IL-1β (A), IL-8 (B), MMP-13 (C), and IL-6 (D).

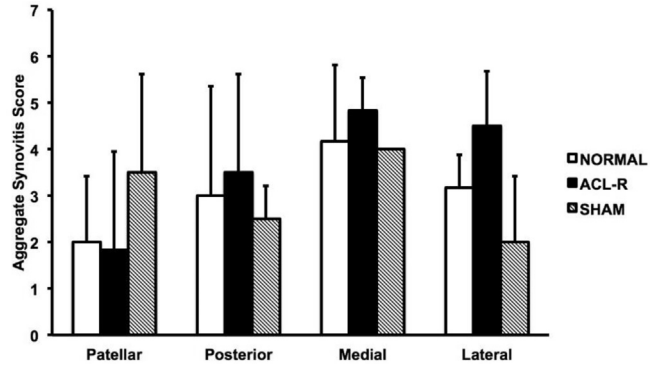

Figure 3. Aggregate synovitis score at 2 weeks after ACL-R ($n = 2$) or sham ($n = 2$) surgery and the control group ($n = 2$).

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INSULIN SELECTIVELY SUPPRESSES TNFα/IL-1β-INDUCED CATABOLIC ENZYMES IN OSTEZOARTHRITIC FIBROBLAST-LIKE SYNOVZIOCYTES

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Introduction: Obesity is well recognized as a major risk factor for osteoarthritis (OA). Classically, it has been proposed that mechanical overload due to obesity causes cartilage damage leading to OA. Our recently published report, however, established that mice fed a high fat diet, a model of type 2 diabetes mellitus (T2DM), have synovial hyperplasia and accelerated progression of posttraumatic OA that is primarily associated with altered metabolism, and not weight gain. These results indicate that systemic factors, such as the low-grade inflammation and insulin resistance induced by obesity/diabetes, may be involved via effects on the synovium that are independent of mechanical overload.

The pathogenesis of OA is not fully understood, though some aspects of the molecular mechanism of cartilage degradation are generally accepted. MMP1 and MMP13 are major collagenases that cleave type 2 collagen while aggrecanases, such as ADAMTS4 and ADAMTS5, degrade aggrecan in OA. The expression of these proteinases is induced by pro-inflammatory cytokines such as TNFα and IL-1β. Importantly, obesity and associated T2DM are a chronic inflammatory state involving TNFα and IL-1β. Thus, OA and the metabolic dysfunction of obesity and T2DM share pro-inflammatory features that may explain the accelerated OA progression in the obese population. With these links between OA and T2DM, we questioned whether insulin has an effect on the catabolic response in FLSs. Here we demonstrate that insulin selectively suppresses TNFα/IL-1β-dependent expression of catabolic enzymes in osteoarthritic FLSs.

Methods: Synovial samples: Human FLSs were obtained from 4 OA patients undergoing total knee arthroplasty after obtaining informed consent under approval from the University of Rochester Institutional Review Board.

Pro-inflammatory cytokines and insulin: For gene and protein expression, cells were treated with cytokines and insulin for 24 hr before harvesting.

Quantitative real-time PCR: Total RNA was extracted from FLSs using an RNeasy Mini kit and was converted to cDNA using the iScript kit. Quantitative real-time PCR was performed using iTaq SYBR Green Master Mix.

Western blot analysis: Cell media supernatants were subjected to SDS-PAGE, electroblotted onto PVDF membranes, and probed with antibodies against MMP1 and MMP13, followed by a HRP-conjugated secondary antibody. Immune complexes were detected with a chemiluminescent reagent using a Bio-Rad image scanner.

Statistical analysis: Comparisons between more than two groups were made by the ANOVA test. Statistical analyses were carried out using Prism statistical software, with $p < 0.05$ was considered significant.

Results: TNFα and IL-1β markedly induced expression of MMP1, MMP13 and ADAMTS4 in FLSs. Interestingly, the effect of TNFα and IL-1β was selective since expression of ADAMTS5 was very modest.

Insulin at 100nM inhibited TNFα-dependent induction of MMP1, MMP13 and ADAMTS4, and IL-1β-dependent induction of MMP1, and ADAMTS4 by 50%. In contrast, insulin had no effect on TNFα-dependent expression of ADAMTS5 and IL-1β-dependent expression of MMP13 and ADAMTS5.

Next, we evaluated MMP1 and MMP13 at the protein level in cell media supernatants after TNFα/IL-1β and insulin treatment. TNFα/IL-1β-induced MMP1 levels in supernatants while insulin at 100nM partially inhibited the effect of these cytokines. The effect of insulin on TNFα/IL-1β-induced MMP13 in supernatants was even more pronounced with the essential elimination of this protein by insulin. These results demonstrate that insulin suppresses TNFα/IL-1β-dependent release of MMP1 and MMP13 by FLSs into the culture media.

Discussion: TNFα and IL-1β markedly up-regulate expression of the catabolic genes MMP1, MMP13 and ADAMTS4. Additionally, we have made the novel observation that insulin suppresses TNFα-dependent induction of MMP1, MMP13 and ADAMTS4, and IL-1β-dependent