week 16 post the first Lupron treatment in the older monkeys but no increase was observed in the younger monkeys. No differences in serum CTxII due to OVX were noted in either group. However in the younger monkeys, Lupron treatment resulted in increases in COMP and CPII. COMP was significantly increased compared to pre Lupron treatment two weeks after the first Lupron injection and remained significantly elevated through week 12. CPII in the younger monkeys did not increase until 12 weeks after the first Lupron injection. In the older monkeys, no differences in COMP and CPII due to OVX were noted.

Conclusions: In summary, this data demonstrated that animal age affects cartilage biomarker levels. Secondly, medical ovariectomy was able to cause increases in cartilage turnover and the response varied depending upon the age of the animal. These data suggest some of these biomarkers could be useful in monkeys to monitor the response to experimental OA drugs.

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AUROTHIOMALATE INHIBITS COX-2 AND mPGES-1 EXPRESSION IN ACTIVATED CHONDROCYTES AND IN HUMAN CARTILAGE

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Purpose: Aurothiomalate is used in the treatment of arthritis. Prostaglandin E_2 (PGE_2) production is increased in various forms of arthritis. The key enzymes in inflammatory PGE_2 synthesis are inducible cyclooxygenase-2 (COX-2) and inducible microsomal prostaglandin E synthase-1 (mPGES-1). Two other forms of prostaglandin E synthase (PGES), microsomal PGES-2 (mPGES-2) and cytosolic PGES (cPGES) are ubiquitously expressed. In the present study, we investigated the effects of aurothiomalate on interleukin-1β (IL-1β) -induced COX-2 and PGES expression and subsequent PGE_2 production in immortalized murine H4 chondrocytes and in human osteoarthritic (OA) cartilage.

Methods: Cartilage tissue was obtained from the leftover pieces of total knee replacement surgery from patients with OA. PGE_2 production was measured by RIA, protein expression was measured by Western blot and mRNA expression was measured by quantitative PCR.

Results: Aurothiomalate inhibited IL-1β -induced PGE_2 production in chondrocytes and in human cartilage. Aurothiomalate inhibited also COX-2 and mPGES-1 expression but did not have an effect on mPGES-2 or cPGES. Aurothiomalate enhanced COX-2 mRNA degradation in IL-1β -treated chondrocytes. This mechanism may well explain the inhibitory effect of aurothiomalate on COX-2 expression.

Conclusions: Aurothiomalate suppressed IL-1β -induced COX-2 and mPGES-1 expression in chondrocytes and in human OA cartilage. The results suggest a novel anti-inflammatory mechanism for aurothiomalate.

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C2C AND COLL2-1 BIOMARKERS REVEAL INCREASED TYPE II COLLAGEN CATABOLISM IN BIGLYCAN/FIBROMODULIN DOUBLE DEFICIENT MICE

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Purpose: Compared to wild-type mice (WT), biglycan/fibromodulin double deficient mice (DKO) develop premature and severe knee OA. In this study, we aimed at comparing 1) type II collagen catabolism between the 2 genotypes and 2) the usefulness of C2C, Coll2-1 and Coll2-1NO$_2$ biomarkers to evaluate collagen catabolism in an animal model of osteoarthritis.

Methods: Serum levels of the type II collagen biomarkers C2C, Coll2-1 and Coll2-1NO$_2$ were determined in 15 WT and 15 DKO at the age of 66 and 141 days for C2C and at the age of 49, 81, 95 and 141 days for Coll2-1 and Coll2-1NO$_2$. Mice were sacrificed at day 141. C2C, Coll2-1 and Coll2-1NO$_2$ immunohistochemistry was performed on coronal histological sections of knee joints.

Results: The mean serum concentrations of Coll2-1 and C2C were significantly higher in DKO than in WT at all time points (see Table 1). The DKO/WT ratios for these 2 biomarkers remained approximately constant with time and were ~1.63 for C2C and ~1.15 for coll2.1. In contrast, Coll2-1NO$_2$ displayed cyclic variations with a DKO/WT ratio ~1.60 at day 49 and 95 but =0.86 at intermediate time points (day 81 and 141) (see Table). In both genotypes, immunostainings with Coll2-1, Coll2-1NO$_2$ and C2C labelled some fibroblasts in tendons and menisci as well as the chondrocytes above the tidemark in articular cartilage whereas chondrocytes in the growth plate remained unstained. For the 3 biomarkers, extracellular stainings was limited to fibrocartilage areas in tendons and menisci from both genotypes and to some of the focal lesions of the biglycan/fibromodulin deficient cartilage. No extracellular staining was observed in WT cartilage.

Conclusions: Our study demonstrates a higher type II collagen catabolism in the biglycan/fibromodulin double-deficient mouse. The different DKO/WT ratios observed with C2C, Coll2-1 and Coll2-1NO$_2$ suggest that these 3 serum biomarkers give complementary rather than redundant information on type II collagen catabolism. C2C and Coll2-1 are useful to directly monitor collagen degradation in animal models of osteoarthritis whereas Coll2-1NO$_2$ is less suitable as it requires the additional presence of oxidative stress.

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THE PREDICTIVE VALUE OF MOLECULAR MARKERS OF BONE METABOLISM IN EARLY KNEE OSTEOARTHRITIS

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Purpose: Osteoarthritis is considered to have heterogeneous metabolism, involving all joint tissues. However, it is not known in whom the disease will progress. The latter is characterized

P92 – Table 1. Serum levels (mean±SD) of C2C, Coll2-1 and Coll2-1NO$_2$ biomarkers in wild-type (WT) and biglycan/fibromodulin double-deficient mice (DKO).

Age (days)	C2C (ng/ml)			Coll2-1 (nM)			Coll2-1NO2 (nM)		
	WT	DKO	p	WT	DKO	p	WT	DKO	p
49	/	/	/	200±30	222±31	0.06	1.38±0.22	2.22±0.42	0.000
66	32±16	52±14	0.001	/	/	/	/	/	/
81	/	/	/	245±40	281±61	0.06	1.90±0.38	1.63±0.32	0.053
95	/	/	/	305±32	362±60	0.005	1.81±0.54	2.89±0.67	0.000
141	30±16	50±26	0.03	264±24	303±35	0.008	2.37±0.24	2.04±0.41	0.027

mainly by the presence of osteophytes and thinning of joint cartilage.

To examine by serum molecular markers the response of bone tissue in early stage knee OA.

Methods: The study group consisted of 147 subjects aged 32-55 (mean 45) years (51 male, 96 female) with chronic knee pain. Half of the female subjects studied were postmenopausal. Altogether 20 and 32 female subjects matched by age and place of residence served as controls.

Bilateral radiographs were graded for tibiofemoral(TF) and patellofemoral(PF) knee OA at baseline and in the 3 year follow-up. At baseline, 55(34%) individuals in the study group had knee joint symptoms but no radiographic findings, 85(53%) were diagnosed with knee OA grade I. Twenty-one subjects (13%) had OA grade II or III, predominantly in the PF region. Radiographic findings of osteophytes and JSN were distributed in the OA group as follows: one-third had only osteophytes, another third had only JSN and the rest had both simultaneously.

At baseline a general marker of bone turnover, serum osteocalcin (S-OC) and propeptide of type I procollagen (S-P1NP) were measured. At baseline and 3 years later validated bone resorption marker serum C-terminal cross-linked telopeptides of type I collagen (S-CTx-I) was assessed by ECLIA (Roche Diagnostics). Statistics: non-parametric methods.

Results: All three markers revealed significant gender differences. S-CTx-I levels were significantly higher in men. Medians for CTx-I for men and women were 0.416 and 0.371 ng/ml (p=0.033). S-OC levels were higher in women (medians for men and women 18.6 and 22.6 ng/ml, p=0.012). S-P1NP levels trended to be higher in women (p=0.08). CTx-I and P1NP levels were significantly higher in female postmenopausal symptomatic subjects compared to female controls.

We were not able to demonstrate significant correlations between knee OA scores and bone markers neither in men nor women. However, in women we observed an association between baseline S-OC and presence of osteophytes localizing in any knee joint compartment (rho=0.228, p=0.034). More detailed analysis revealed a correlation between S-OC and TF osteophytes in lateral compartments in premenopausal subjects (rho=0.327, p=0.032).

The same was observed with baseline P1NP (but not with baseline CTx-I) that correlated with TF osteophytes in lateral compartments (rho=0.269, p=0.045) in women.

In women, S-CTX-I levels were significantly correlated with height and S-OC with age. In men, we did not find an association between knee joint osteophytes and bone markers, but all markers correlated with BMI.

Conclusions: 1. Gender differences have an important effect on the levels of bone markers. Postmenopausal women have significantly increased bone turnover.

2. In case of early knee OA bone turnover is accelerated. Increased synthesis of type I collagen seems to refer to the proliferative bone response expressed by osteophytes.

3. Baseline S-OC and S-P1NP levels seem to have predictive value for definite type of osteophytosis some years later.

4. For interpretation of the results of bone markers one has to consider the important contribution of age, gender and BMI.

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NITROSYLATED N-TELOPEPTIDE OF TYPE III COLLAGEN (IIINYS): A NEW SPECIFIC BIOCHEMICAL MARKER OF OXIDATIVE-INDUCED SYNOVIAL TISSUE METABOLISM IN ARTHRITIS

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Purpose: Nitric oxide (NO) is an important mediator of joint inflammation and destruction in rheumatoid arthritis (RA) and osteoarthritis (OA). Peroxynitrite induced by NO can react with amino acids including tyrosine (Y) to form nitrotyrosine. Increased NO-related species have been found in the joint of patients with OA or RA, especially in synovial tissue. The aim of this study was to develop an immunoassay recognizing nitrosylated N-telopeptide of type III collagen - one of the main constituent of synovial membrane - to monitor oxidative-related joint damage in arthritis.

Methods: We produced a polyclonal antibody raised against a nitrosylated sequence specific of the N-telopeptide of human type III collagen (IIINys). Using IIINys antibody, we performed immunohistochemistry of synovial tissue from 11 patients with knee OA undergoing total joint replacement. We also developed a competitive ELISA to measure IIINys levels in the synovial fluid, serum and urine of healthy controls and patients with OA or RA.

Results: The IIINys antibody did not recognize the non-nitrosylated sequence of type III collagen N-telopeptide, nitrosylated BSA and free nitrotyrosine, indicating high specificity for both nitrosylation and type III collagen sequence. Immunohistochemistry of synovial tissue from patients with knee OA, showed strong IIINys staining in the extracellular matrix, particularly around the synoviocytes and within macrophage-like cells. The ELISA for serum IIINys demonstrated intra and inter-assay CV below 15% and recovery of diluted serum samples ranged from 96.6 to 118.3% (mean: 99%). Detectable levels of IIINys were measured in the synovial fluid, serum and urine of 11 patients with knee OA. Compared to 30 healthy postmenopausal women, serum IIINys levels were increased by an average of 195% (p<0.0001) in 30 postmenopausal women with early RA.

Conclusions: We have developed an immunoassay which detects specifically nytrosylated type III collagen N-telopeptide (IIINys). The strong immunoreactivity of IIINys in the synovial tissue of patients with OA and the marked increased serum IIINys levels in patients with RA, suggest that this new biochemical marker should be useful for the investigation of oxidative-induced alterations of synovial tissue in patients with RA or OA.

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WHOLE BLOOD LEAD LEVELS AND PUTATIVE OSTEOARTHRITIS BIOMARKERS IN AFRICAN AMERICAN AND CAUCASIAN WOMEN

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Purpose: The importance of bone turnover is increasingly recognized in osteoarthritis (OA) pathogenesis.Lead (Pb) is a heavy metal that affects many aspects of bone including osteoclasts, osteoblasts, and calcium and vitamin D metabolism. As such, Pb could potentially be involved in OA given the importance of bone in this process. The effect of Pb on cartilage and other joint tissues is not known. No previous studies have evaluated effects