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.

P91
AUROTITHOMALATE INHIBITS COX-2 AND mPGES-1 EXPRESSION IN ACTIVATED CHONDROCYTES AND IN HUMAN CARTILAGE

R. Nieminen1, K. Vuolteenah01, T. Moilanen2, E. Moilanen1
1The Immunopharmacology Research Group, Medical School, University of Tampere, and Tampere University Hospital, Finland, 2The Immunopharmacology Research Group, Medical School, University of Tampere and Tampere University Hospital, and Coxa Hospital for Joint Replacement, Tampere, Finland

Purpose: Aurothiomalate is used in the treatment of arthritis. Prostaglandin E2 (PGE2) production is increased in various forms of arthritis. The key enzymes in inflammatory PGE2 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 PGE2 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. PGE2 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 PGE2 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 expression in chondrocytes and in human OA cartilage. The results suggest a novel anti-inflammatory mechanism for aurothiomalate.

P92
C2C AND COLL2-1 BIOMARKERS REVEAL INCREASED TYPE II COLLAGEN CATABOLISM IN BIGLYCAN/FIBROMODULIN DOUBLE DEFICIENT MICE

L.G. Armeve1, M. Deberg2, M. Oliveira1, A. Labasse2, J.-M. Aeberli1, Y. Henrotin1
1Nestlé Research Center, Lausanne, Switzerland, 2University of Liège, Liège, Belgium

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 Coll2-1NO2 biomarkers to evaluate collagen catabolism in an animal model of osteoarthritis.

Methods: Serum levels of the type II collagen biomarkers C2C, Coll2-1 and Coll2-1NO2 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 Coll2-1NO2. Mice were sacrificed at day 141. C2C, Coll2-1 and Coll2-1NO2 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, Coll2-1NO2 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, Coll2-1NO2 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 Coll2-1NO2 suggest that the 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 Coll2-1NO2 is less suitable as it requires the additional presence of oxidative stress.

P93
THE PREDICTIVE VALUE OF MOLECULAR MARKERS OF BONE METABOLISM IN EARLY KNEE OSTEOARTHRITIS

J. Kumm, A. Tamm, M. Lintrop, A. Tamm
University of Tartu, Tartu, Estonia

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

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>C2C (mg/ml)</th>
<th>Coll2-1 (nM)</th>
<th>Coll2-1NO2 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
<td>WT</td>
<td>DKO</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>200±30</td>
<td>222±31</td>
<td>0.06</td>
</tr>
<tr>
<td>66</td>
<td>32±16</td>
<td>52±14</td>
<td>0.001</td>
</tr>
<tr>
<td>81</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>95</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>141</td>
<td>30±16</td>
<td>50±26</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 1. Serum levels (mean±SD) of C2C, Coll2-1 and Coll2-1NO2 biomarkers in wild-type (WT) and biglycan/fibromodulin double-deficient mice (DKO).
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 diag-
nosed with knee OA grade I. Twenty-one subjects (13%) had OA
grade II or III, predominantly in the PF region. Radiographic find-
ings 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 osteocal-
cin (S-OC) and propeptide of type I procollagen (S-P1NP) were
measured. At baseline and 3 years later validated bone resorp-
tion 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 differ-
ces. 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
tended 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 base-
line S-OC and presence of osteophytes localizing in any knee
joint compartment (rho=0.226, p=0.034). More detailed analy-
sis 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 base-
line CTx-I) that correlated with TF osteophytes in lateral com-
partments (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 be-
 tween 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 osteoarthrosis some years later.

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

**P94**

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

N. Charny1, P. Richardot1, A.B. Jensen2, J.-M. Delaissé2,
P. Garnero3

1Molecular Markers, SYNARC, Lyon, France, 2Clinical Cell
biologi, Vejle Hospital, Vejle, Denmark, 3SYNARC and INSERM
U664, Lyon, France

**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,
sperm 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, nitro-
sylated BSA and free nitrotyrosine, indicating high specificity
for both nitrosylation and type III collagen sequence. Immuno-
histochemistry of synovial tissue from patients with knee OA,
showed strong IIINys staining in the extracellular matrix, particu-
larly 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 pa-
tients 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 de-
tects specifically nytrosylated type III collagen N-telopeptide (II-
INys). 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 alter-
ations of synovial tissue in patients with RA or OA.