EXTENDED REPORT

Cartilage turnover assessed with a newly developed assay measuring collagen type II degradation products: influence of age, sex, menopause, hormone replacement therapy, and body mass index

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Background: Cartilage normally has a slow turnover but in arthritis increased metabolism results in degradation of the tissue.

Objective: To assess cartilage turnover in a sample of the general population by an assay measuring cartilage derived urinary collagen type II (CTX-II) C-telopeptide degradation products.

Methods: CTX-II concentrations were measured in urine samples from 615 healthy men and women aged 20–87 years, and the influence of age, sex, menopause, hormone replacement therapy (HRT), and body mass index (BMI) was assessed.

Results: CTX-II concentrations showed age dependent variations, with notable differences between men and women. Mean (SD) CTX-II concentration in postmenopausal women (220 (118) ng/mmol, n=25) was significantly higher than in an age matched group of premenopausal women (112 (79) ng/mmol, n=26, p<0.001). CTX-II concentration in women not receiving HRT (118 (57) ng/mmol, n=50) was significantly lower than in an age and BMI matched group of women not receiving HRT (215 (99) ng/mmol, p<0.001). In subjects with a BMI ≥25 kg/m², CTX-II concentrations were significantly higher than in those with a BMI <25 kg/m² (185 (114) v 148 (91) ng/mmol, p<0.001).

Conclusions: Cartilage turnover, as assessed by measuring urinary degradation products of CTX-II varies considerably with age, and significant differences between CTX-II levels in men and women as well as in pre- and postmenopausal women are found. Further studies are required to validate the marker for assessing cartilage degradation in arthritis.

Osteoarthritis (OA) and other arthritic diseases involving a degradation of the hyaline articular cartilage represent a major healthcare problem in the general population. Despite the high prevalence of OA, early diagnosis and monitoring of disease progression is hampered by the lack of specific and sensitive diagnostic tools. Radiographic assessments are used to quantify joint space narrowing, osteophytes, and sclerosis of subchondral bone. These changes in the joint structure, however, appear in fairly advanced stages of the disease and extended examination periods are needed to demonstrate significant changes on radiographs.

The potential use of biochemical markers in the diagnosis and monitoring of OA has recently been reviewed. Although the clinical applicability of biochemical markers awaits verification, they potentially represent useful diagnostic and monitoring tools. We have recently reported a laboratory assay called CartiLaps, which we propose as a specific marker of cartilage degradation. CartiLaps is highly specific for the epitope EKGPDP derived from the C-telopeptide of collagen type II (CTX-II). Type II collagen is almost exclusively found in cartilage, where it constitutes a major structural component of the tissue. Thus, CTX-II potentially represents a specific marker of cartilage degradation that may be suitable for the clinical monitoring of OA.

In vitro, cultures of human cartilage explants have been shown to produce CTX-II when catabolism is induced. Furthermore, urinary CTX-II levels are significantly raised in diseases with increased cartilage turnover, such as OA and rheumatoid arthritis (RA), whereas they remain unaffected in Paget’s disease, known to affect bone and not cartilage. Measurements of CTX-II have been shown to correlate with radiological measures of structural damage, pain, and physical function in patients with knee OA. In one longitudinal study involving patients with early RA, measurements of CTX-II were correlated with joint space narrowing, and high levels of baseline CTX-II were associated with an increased risk of disease progression over a one year study.

Our study aimed at assessing CTX-II levels in a large cross section of a population of healthy men and women. We intended to determine how age, sex, menopause status, hormone replacement therapy (HRT) and body mass index (BMI) influence urinary CTX-II concentrations in a healthy population. We included the simultaneous investigation of urinary collagen type I (CTX-I), a marker of bone degradation, and serum osteocalcin, a biochemical marker of bone formation.

MATERIALS AND METHODS

Participants

A total of 763 healthy men and women responded to an invitation to participate in an epidemiological study in the county of Northern Jutland, Denmark. The purpose of the original study was to evaluate risk factors for the development of osteoporosis. The inclusion criterion was: healthy male and female volunteers aged 20–100. The exclusion criteria were: systemic diseases or treatments (presently or previously).

Abbreviations: BMI, body mass index; CTX-II, collagen type II; ELISA, enzyme linked immunosorbent assay; HRT, hormone replacement therapy; OA, osteoarthritis; RA, rheumatoid arthritis
known to influence bone metabolism, malignancy, and clinically relevant abnormal baseline laboratory values. Participants with mild or manifest OA were thus not excluded. Six hundred and fifteen participants aged 20–87 met the selection criteria and had CTX-II and bone marker measurements performed. Table 1 shows the number of male and female participants, split according to age and menopausal status. The local ethics committee in the county of Northern Jutland approved the study. All participants gave their written informed consent before inclusion.

**Demographic characteristics**

Body weight and height were measured to the closest 0.1 cm and 0.1 kg, respectively, on participants wearing light indoor clothes and no shoes. BMI was calculated as BMI = m/h², where m is the weight of the patient in kilograms and h is the height of the patient in metres. The influence of BMI on urinary CTX-II concentrations was studied in participants aged ≥35 years.

Menopausal status and the use of hormones were evaluated by questionnaires. Women were considered postmenopausal if they had not experienced menstrual bleeding within the past 12 months. Blood samples were collected after an overnight fast. Urine samples were collected as second morning void after an overnight fast.

Of the 763 responders, a group of 50 women, not included in the original study owing to their use of HRT, underwent all screening investigations and enabled us to evaluate the influence of HRT on urinary CTX-II concentration.

**Urinary CartiLaps ELISA/CTX-II**

Monoclonal antibody mAbF46, specific for CTX-II C-telopeptide fragments, was used in a competitive enzyme linked immunosorbent assay (ELISA) format developed for measurement of urine samples. Briefly, the assay was performed as follows: biotinylated CTX-II C-telopeptide derived peptide (EKGPDP) was coated on a streptavidine microtitre plate, and sample and primary antibody (mAbF46) were added. After overnight incubation the amount of bound antibody was quantified using a peroxidase labelled secondary antibody and a chromogenic peroxidase substrate. The concentration of the CartiLaps ELISA (ng/l) was standardised to the total urine creatinine (mmol/l). Measurement precision of the assay was 7.1% and 8.4% for the intra- and interassay variations, respectively.

**Urinary CrossLaps ELISA/CTX-I**

The CrossLaps ELISA (Nordic Bioscience A/S, Herlev, Denmark) is a competitive ELISA using a polyclonal rabbit antiserum. The antibody targets a sequence in the C-terminal telopeptide α1 chain in the human CTX-I. The inter- and intra-assay variations were <5% and <7%, respectively. The assay was performed as recommended by the manufacturer.

**Serum osteocalcin**

Serum osteocalcin was measured by N-MID ELISA (Nordic Bioscience A/S, Herlev, Denmark). The inter- and intra-assay variations were both <7%. The assay was performed as recommended by the manufacturer.

**Statistical analysis**

Data on physical characteristics given in text are shown as means (SD). Results in graphs are expressed as means with SEM. Differences in study variables between groups were tested by independent t tests and analysis of variance after log
transformation of data. Spearman’s correlations were calculated to compare the association between CTX-II concentrations and bone markers. Comparison of CTX-II concentrations in subjects stratified according to BMI was made after adjustment for age. Statistical analysis was performed using SPSS 10.0 data analysis software (SPSS Inc, Chicago, IL, USA). Differences were considered as significant if \( p < 0.05 \).

**RESULTS**

**Age dependent variations in urinary CTX-II concentrations**

Figure 1 depicts the association between age and levels of urinary CTX-II as well as urinary CTX-I and serum osteocalcin in women and men. The essential observations were as follows: the mean concentrations of all three parameters were high in both men and women aged 20–25. Mean concentrations of the three biomarkers measured in the young adults tended to decrease with age, with minimum values seen in participants aged 40–45. Biomarkers of bone turnover (CTX-I and osteocalcin) remained constant in men older than 45 (figs 1B and C). In women, mean CTX-I and osteocalcin concentrations from women aged 50–55 were significantly higher than mean concentrations obtained from women aged 40–45 (\( p < 0.001 \)) (figs 1B and C). In women, mean CTX-I and osteocalcin concentrations from women aged 50–55 were significantly higher than mean concentrations obtained from women aged 40–45 (\( p < 0.001 \)) (figs 1B and C).

When comparing the pattern of age dependent variations seen in the bone markers with that of urinary CTX-II concentrations, similar tendencies were found (figs 1A–C). The CTX-II concentration in women aged 50–55 was twice as high as the mean urinary CTX-II concentration found in women aged 40–45 (\( p < 0.001 \)). The corresponding differences in the bone markers were 1.6-fold (\( p < 0.01 \)). After the age of 55 years urinary CTX-II levels in both men and women showed slightly increasing mean values with increasing age (NS).

To evaluate tissue specificity of the CartiLaps assay, correlations between this marker and the bone markers were analysed by performing Spearman’s correlations. The correlations between urinary CTX-II and the two bone markers were weaker than the correlation between the two bone markers (table 2).

**Influence of menopause on CTX-II concentrations**

Women in the age group 40–60 showed a pronounced heterogeneity in CTX-II values (fig 2A). We analysed this further by stratifying women aged 49 to 53 according to their menopausal status. Urinary CTX-II concentration in postmenopausal women was significantly higher than that in premenopausal women (fig 2B). Thus, urinary CTX-II concentrations in women aged 40–55 were significantly higher for postmenopausal women (open circle) compared with premenopausal women (filled circle). (B) Influence of menopausal status on creatinine corrected urinary CTX-II concentrations in women. (A) A scatterplot presenting individual values of premenopausal (filled circle) and postmenopausal (open circle) women. (B) Influence of menopausal status itself in women aged 49–53. Mean urinary CTX-II concentrations in the absence or presence of HRT were 215 (99) ng/mmol and 118 (57) ng/mmol, respectively (\( p < 0.001 \)).

The women receiving HRT were stratified into two groups; one representing those who had been receiving HRT for \( < 4 \) years, the other those who had been receiving HRT for \( 4–10 \) years. Average age and BMI were similar in the two groups. Comparative analysis showed that women who had started treatment \( 4–10 \) years previously had significantly lower urinary CTX-II concentrations than women who had been receiving HRT for \( < 4 \) years (89 (48) ng/mmol vs 128 (50) ng/mmol, \( p < 0.05 \)) (fig 3B). To assess the influence of body weight on urinary CTX-II concentrations men and women aged \( \geq 35 \) years were stratified into two groups according to whether they had a BMI above or below 25 kg/m\(^2\). The average age was higher in the group of subjects with a BMI \( > 25 \) kg/m\(^2\) (56.0 (11.3) v 53.5 (12.9) years, \( p < 0.02 \)). After adjustment for age CTX-II concentrations in those with a BMI \( > 25 \) kg/m\(^2\) were significantly higher than those with a BMI \( < 25 \) kg/m\(^2\) (185 (114) v 148 (91) ng/mmol, \( p < 0.001 \)) (fig 4).

![Figure 2 Influence of age and menopausal status on creatinine corrected urinary CTX-II concentrations in women.](http://ard.bmj.com/annrheumdis/content/62/4/332/fig2)

| Table 2 Correlations (\( r \)) between urinary CTX-II, CTX-I, and serum osteocalcin. Results are Spearman’s correlation coefficients |
|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | CTX-II/CTX-I     | CTX-II/osteocalcin | CTX-I/osteocalcin | \( n \)       |
| Both sexes, all ages | 0.43**          | 0.31**          | 0.61**          | 615           |
| Women, aged 30+  | 0.28**          | 0.19**          | 0.62**          | 196           |
| Men, aged 50+  | 0.27**          | 0.21**          | 0.44**          | 117           |

\*\( p < 0.05 \); **\( p < 0.01 \).
The increased bone turnover in postmenopausal women is well established. As seen in this and other studies, this becomes apparent as a significant increase in both CTX-I and osteocalcin levels. A corresponding increase in the cartilage degradation marker CTX-II was seen, and this indicates an increase in cartilage degradation at menopause. These findings may be considered in accordance with the descriptive epidemiology of OA, in which the prevalence and incidence of the disease increase steeply with age in women around the menopause.

After the age of 55 bone markers reached a new steady state in metabolism in both men and women. Measurements of CTX-II concentrations, however, demonstrated slightly increasing values with increasing age in both sexes from 55 years and upwards. These findings could reflect the well known increase in prevalence with age of OA.

As expected, high levels of the bone markers were found in the young adults, representing the high bone turnover that takes place at those ages. The high concentrations of CTX-II that were seen in the same age groups indicate a high metabolic activity in cartilage tissue and may reflect, to some extent, late closing growth plates. The growth plate is synthesised as cartilage, which subsequently becomes remodelled to bone. Thus in situations where the growth plate is still metabolically active, significant amounts of collagen type II fragments may originate from this process. Preliminary studies in rodents, which never reach a situation with closed growth plates, indicate a substantial contribution from growth plate metabolism, especially in younger animals, to systemic levels of CTX-II (Christgau S, unpublished information).

Comparisons of correlations between CTX-II and bone markers with the correlations between the bone markers indicate that although the different markers display similar age dependent variations, CTX-II is not a bone marker. Thus, the correlations between urinary CTX-II and the different bone markers were weaker than the correlation between urinary CTX-I and serum osteocalcin (table 2). The differences between CTX-II and the bone markers were even greater for subjects older than 50, where both OA and osteoporosis develop (table 2). These, together with previous findings therefore support the statement that CTX-II is not a bone marker.

To explain the sudden and marked increase in urinary CTX-II concentrations seen in perimenopausal women, the effect of the menopausal status itself on CTX-II concentrations was tested in a separate analysis. This showed significantly increased CTX-II concentrations in postmenopausal women than in age and BMI matched premenopausal women. The role of sex hormones in OA has long been in question and published reports give conflicting results. Epidemiological studies, however, have consistently reported that women who take HRT have a lower prevalence of OA than women not taking HRT. Also, long term users of HRT have been found to have a lower prevalence of hip OA than age matched short term users. To assess the influence of HRT on cartilage degradation, CTX-II concentrations measured in 50 women receiving HRT were compared with those of an age and BMI matched group of women not receiving therapy. Significantly lower concentrations of CTX-II were found in the group of women receiving HRT. Of the women receiving HRT, those receiving HRT for a longer period of time (4–10 years) had significantly lower CTX-II excretions than those receiving HRT for <4 years. These results appears to be in accordance with the abovementioned findings in the literature. Longitudinal studies designed specifically to assess the effects of menopause and HRT on OA prevalence and severity should be performed to assess this important issue.

Our study was also extended to investigate the influence of BMI on urinary CTX-II concentration. Cross sectional as well noteworthy differences, from the pattern of age dependent variations found in the bone markers (fig 1).
as longitudinal studies have shown that being overweight is a risk factor for the development of knee OA. Subjects with a BMI $\geq 25$ kg/m$^2$ (overweight) had significantly higher CTX-II concentrations than those with a BMI $<25$ kg/m$^2$ (fig 4). These findings do not provide conclusive evidence on the role of BMI in influencing CTX-II concentrations, but do suggest that BMI is a contributing pathogenic factor in cartilage degradation.

In summary, this study demonstrates measurements of collagen type II degradation products on a large population of generally healthy men and women aged 20–87. The age dependent variations in this marker of cartilage degradation have several similarities with the descriptive epidemiology of OA, the most common of the cartilage destructive diseases. Urinary concentrations of CTX-II are influenced by menopausal status, HRT and, to a lesser extent, BMI. The assay may prove applicable in the diagnosis and monitoring of OA and other cartilage destructive diseases, and may help the identification of new disease modifying treatments.

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REFERENCES