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Tibolone inhibits bone resorption without secondary positive effects on cartilage degradation

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Abstract

Background: Osteoarthritis is associated with increased bone resorption and increased cartilage degradation in the subchondral bone and joint. The objective of the present study was to determine whether Tibolone, a synthetic steroid with estrogenic, androgenic, and progestogenic properties, would have similar dual actions on both bone and cartilage turnover, as reported previously with some SERMS and HRT.

Methods: This study was a secondary analysis of ninety-one healthy postmenopausal women aged 52–75 yrs entered a 2-yr double blind, randomized, placebo-controlled study of treatment with either 1.25 mg/day (n = 36), or 2.5 mg/day Tibolone (n = 35), or placebo (n = 20), (*J Clin Endocrinol Metab.* 1996 Jul;81(7):2419–22) Second void morning urine samples were collected at baseline, and at 3, 6, 12, and 24 months. Urine CrossLaps® ELISA (CTX-I) and Urine CartiLaps® ELISA (CTX-II) was investigated as markers of bone resorption and cartilage degradation, respectively.

Results: Tibolone significantly ($P < 0.001$) suppressed bone resorption by approximately 60%. In contrast, no effect was observed on cartilage degradation.

Conclusion: These data suggest uncoupling of the bone and cartilage effects of the synthetic steroid, Tibolone. Bone resorption was significantly decreased, whereas cartilage degradation was unchanged. These effects are in contrast to those observed some SERMs with effects on both bone and cartilage degradation. These effects may in part be described by the complicated pharmacology of Tibolone on testosterone, estrogen and progesterone receptors.

Background

Osteoarthritis (OA) is the most common form of arthritis [1]. One hallmark of the disease is progressive degeneration of articular cartilage, generation of osteophytes and subsequent joint space narrowing. This progression of disease may involve both bone and cartilage parameters, which in some instances may be tightly coupled.

The relationship between bone and cartilage degradation in OA is a complex. A apparent co-existence between the two processes exists [2,3], although the cellular and molecular mechanism remains to be further investigated and identified [4]. Several groups have demonstrated an accelerated incidence of OA in women following menopause [5,6] which in part may be caused by increased

bone resorption [7,8]. Studies investigating gender and age as risk factors of developing OA indicate that OA increases with age and women are at a higher risk than men [9], which supports the notion that sex hormones are related to the incidence of OA. In addition, pre-clinical studies in monkeys [10] and rats [11] have shown that estrogen depletion results in increased bone turnover, leading to altered subchondral bone structure and decreased articular cartilage integrity.

In clinical settings a number of studies have provided evidence for the coupling between bone and cartilage degradation. A selective estrogen-receptor modulator (SERM) was shown to protect against both bone and cartilage degradation, by restoring turnover of both compartments to pre-menopausal level during a 12 month period [12]. In similar context, cartilage degradation was assessed in 384 postmenopausal women, and was found to be significantly lower in women using hormone replacement therapy (HRT) compared to control [6,12], and the cartilage degradation was significantly higher in postmenopausal women when compared to an age-matched group of premenopausal women [6].

Tibolone is a synthetic steroid [(7- α ,17- α & 17-hydroxy-7-methyl-19-norpregn-5(10)-en-20-yn-3-one, Org OD 14, Livial, Organon, The Netherlands] with a combination of estrogenic, androgenic, and progestogenic properties, is capable of relieving climacteric symptoms [13-15] with almost no stimulatory effect on the endometrium [16]. Tibolone is metabolized in the intestine and liver into 3 active metabolites: 3 α - and 3 β -hydroxy metabolites and Δ^4 -7 α -methylnorethisterone. The first two metabolites bind to estrogen receptors, primarily ER α receptors, and have estrogenic effects on bone, thermoregulatory centers in the central nervous system, and the vagina [17-19]. The Δ^4 -7 α -methylnorethisterone metabolite binds to the progesterone and androgen receptors. Tibolone is primarily used to treat women with climacteric complaints for whom bleeding is unacceptable, or who have experienced side-effects during conventional HRT. The estrogenic characteristics of tibolone imply an estrogen-like effect on bone as well, which have been documented and characterized previously [20,21].

Bone and cartilage degradation can be assessed by biochemical markers. Bone resorption by osteoclasts is for the major part mediated by the protease cathepsin K, which results in the specific degradation fragment of collagen type I, CTX-I [22,23]. CTX-I fragments have been used as a surrogate measure of bone resorption for *in vitro*, pre-clinical and clinical studies [22,24]. With respect to cartilage degradation, CTX-II, is a matrix metalloprotease (MMP) generated fragment of collagen type II which is predominately found in articular cartilage [25,26]. CTX-II

levels has been shown to predict structural progression of osteoarthritis under several clinical settings [27].

As several lines of evidence suggest a tight coupling between bone and cartilage turnover [28], and protective effects of estrogens and some SERMs [28], the objective of the present study was to determine whether Tibolone, a synthetic steroid with estrogenic, androgenic, and progestogenic properties, would have similar dual actions on both bone and cartilage turnover.

Methods

This study is a secondary analysis of the effects published previously [21]. Ninety-one healthy postmenopausal women aged 52–75 yrs entered a 2-yr double blind, randomized, placebo-controlled study of treatment with either 1.25 mg/day (n = 36), or 2.5 mg/day tibolone (n = 35), or placebo (n = 20) as previously described [21]. Second void morning urine samples were collected at baseline, and at 3, 6, 12, and 24 months. The biochemical marker CTX-II was measured in urine by Urine CartiLaps[®] ELISA (Nordic Bioscience A/S) as marker of cartilage degradation.

As described in the original publication, [21], after being introduced thoroughly about the trial, participants gave their informed consent to participate (Helsinki Declaration II). The study was approved by the ethical committee of Copenhagen County, Denmark.

Statistical analysis

To assess longitudinal changes, the values were calculated for each person and expressed as the percentage of the initial baseline value. The data of the creatinine-corrected values of CTXI and CTXII, and the relative changes were logarithmically transformed to obtain normality and symmetry of variances. The trapezoidal method was applied for calculation of the time-averaged mean change from baseline. Analysis of variance (ANOVA) was used for comparison of baseline data between treatment groups. The two-tailed Student's *t*-test was applied for pairwise comparison of data from the active treatment groups with placebo.

For all tests $p < 0.05$ was considered significant. All statistical calculations were performed using the SAS software package (release 9.1, SAS Institute Inc., Cary, NC, USA).

Results

Table 1 shows the baseline demographic data of the participants within each treatment group who completed the 2-year study protocol as published previously [21]. The group receiving 2.5 mg of tibolone was of slightly higher height ($p = 0.04$). In all other aspects of age, weight, BMI, years since menopause, and the biochemical markers of

Table 1: Values shown are mean (SD) or ^ageometric mean (\pm 1 SD range)

	Placebo (n = 13)	1.25 mg (n = 29)	2.5 mg (n = 28)
Age (yrs)	68.3 (6.0)	66.5 (7.0)	64.0 (6.7)
YSM (yrs)	20 (6.2)	19.6 (6.1)	17.5 (6.5)
Height (cm)	158.5 (5.3)	159.7 (5.9)	162.9 (6.1)
Weight (kg)	61.8 (6.8)	62.5 (9.5)	61.1 (6.8)
BMI (kg/m ²)	24.7 (3.2)	24.5 (3.4)	23.1 (2.8)
^a CTXI (μ g/mmol)	0.26 (0.18–0.37)	0.32 (0.21–0.49)	0.31 (0.22–0.45)
^a CTXII (μ g/mmol)	0.13 (0.08–0.26)	0.17 (0.09–0.32)	0.13 (0.07–0.24)

urinary CTX-I and CTX-II there were no significant differences among the groups.

The effect of tibolone on bone resorption

Bone resorption was investigated by measurement of urinary CTX-I during the 2 years of therapy every 3rd month in the groups receiving 2.5 mg or 1.25 mg of tibolone, or placebo. Tibolone inhibited bone resorption highly significant with a plateau after 6, as seen in figure 1, upper panel. The relative change from baseline in urinary CTX-I during 2 years of therapy with 1.25 and 2.5 mg of tibolone is presented in figure 1, lower panel. Values shown are placebo-corrected time-averaged mean change during the treatment period and given as mean \pm 1 SEM. The level of significance denotes difference from the placebo group: *** $p < 0.001$. Data are modified with permission from [21].

The effect of tibolone on cartilage degradation

Cartilage degradation was measured by measurement of urinary CTX-II during the 2 years of therapy every 3rd month in the groups receiving 2.5 mg or 1.25 mg of tibolone, or placebo. Tibolone did not affect cartilage degradation, as presented in figure 2, upper panel. The relative change from baseline in urinary CTXII during 2 years of therapy with 1.25 and 2.5 mg of tibolone is presented in figure 2, lower panel. Values shown are placebo-corrected time-averaged mean change during the treatment period and given as mean \pm 1 SEM.

Discussion

Experimental and clinical observations suggest that the structural integrity of articular cartilage is dependent on normal subchondral bone turnover, intact chondrocyte function and ordinary biomechanical stresses [3,29-32]. Because there is an apparent inter-relationship between the subchondral bone and the articular cartilage, interventions with effect on bone turnover may possibly have secondary or direct effects on cartilage turnover.

The presented data suggest that bone resorption can be strongly attenuated without the secondary positive effects on cartilage degradation. In fact, a trend toward increased

cartilage degradation that may have become statistical significant if more samples had been available was observed. These data indicate that the tight coupling between bone and cartilage metabolism can be disassociated under some circumstances. This uncoupling is an important difference compared to that other estrogen like molecules and SERMS that have displayed protective effects on both bone and cartilage degradation. In the present study the observed effect size of 2.5 mg of Tibolone was an increase of 19% [95% confidence interval -17%;+70%] in CTXII. This is in contrast to other interventions that all have shown a decrease in CTXII, e.g. risedronate a decrease of 30% [33,34], estrogen replacement therapy a decrease of 25% [35], strontium ranelate a decrease of 15–20% [36] and levormeloxifene with a decrease of 50% [12].

Bone and OA

An increasing amount of attention has been devoted to the role of the bone in the pathogenesis of osteoarthritis. Subchondral bone sclerosis, alterations in the trabecular structure, lesions (previously called development of bone marrow edema) and osteophytes are important features of the pathology of OA [2,10,29,37-39]. The present understanding remains on an observational level, in which an increasing range of experimental evidence is emerging. In support of the important role of bone turnover in the pathogenesis of OA, several independent lines of experimental evidence are found [3,29-32]. In brief, examinations of peri-articular bone in knees and hips with OA have confirmed that the subchondral bone is abnormal in OA joints, which altered trabecular structure, sclerosis of the subchondral plate [40], as well increased bone turnover [41,42]. Cross-sectional studies have also established that women with advanced knee or hip OA have a higher bone mineral density (BMD) near or at the site of joint OA [43]. Plausible proof of a link between bone and cartilage recently came from an animal model of OA, where extensive inhibition of bone resorption resulted in a 50% decrease in cartilage pathology score assessed by Mankin score [29,38]. In addition, accelerated bone turnover has in both traumatic and estrogen deficiency models (ovariectomy (OVX)) been shown to augment articular cartilage erosion [7,11,12,44,45], in which increased bone

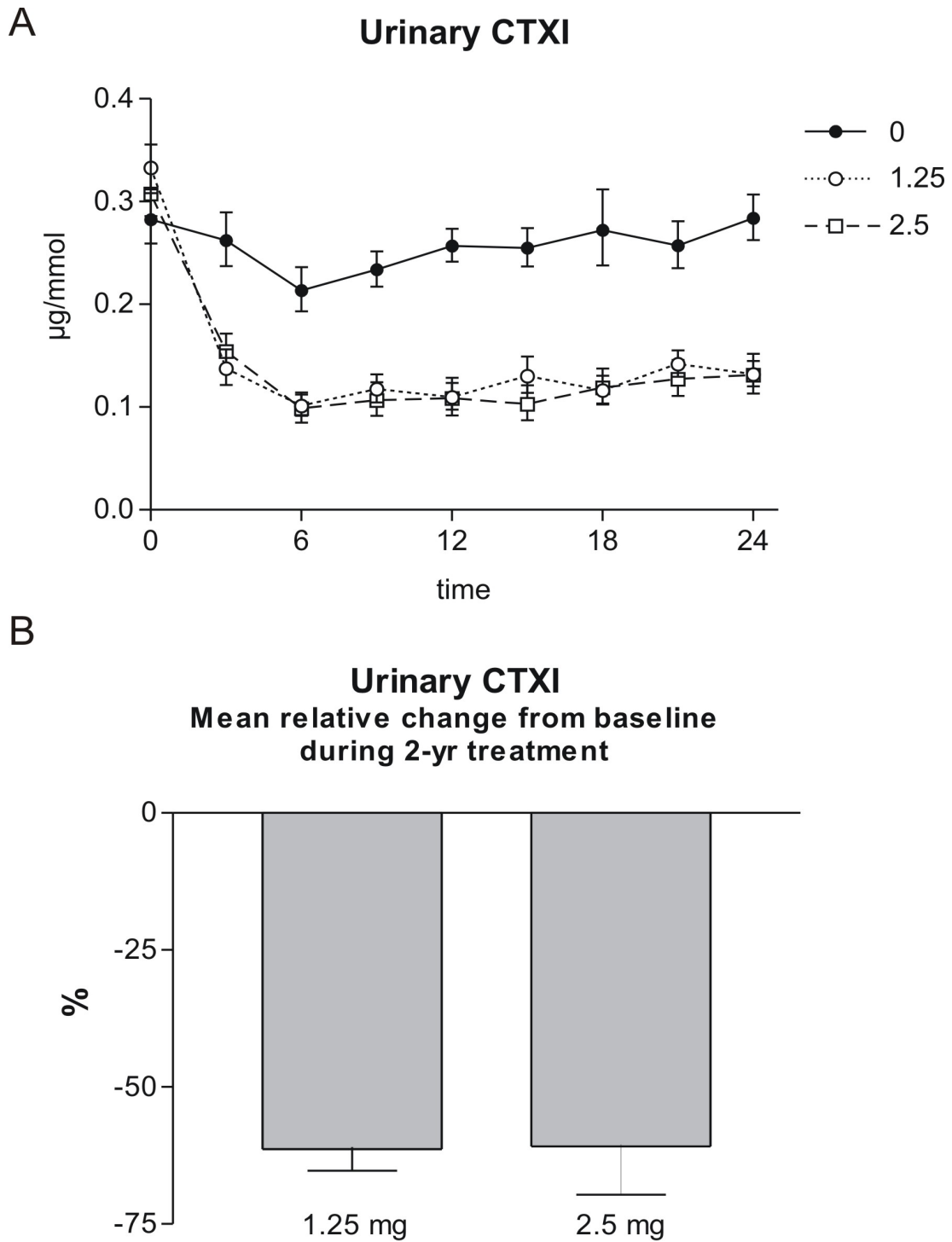


Figure 1
Bone resorption. Upper panel: Urinary CTXI during 2 years of therapy in the groups receiving 2.5 mg, 1.25 mg of tibolone, or placebo. Values shown are geometric mean \pm 1 SEM. **B. Lower panel: Mean relative change from baseline in urinary CTXI during 2 years of therapy with 1.25 and 2.5 mg of tibolone.** Values shown are placebo-corrected time-averaged mean change during the treatment period and given as mean \pm 1 SEM. The level of significance denotes difference from the placebo group: *** $p < 0.001$. Modified with permission from [21].

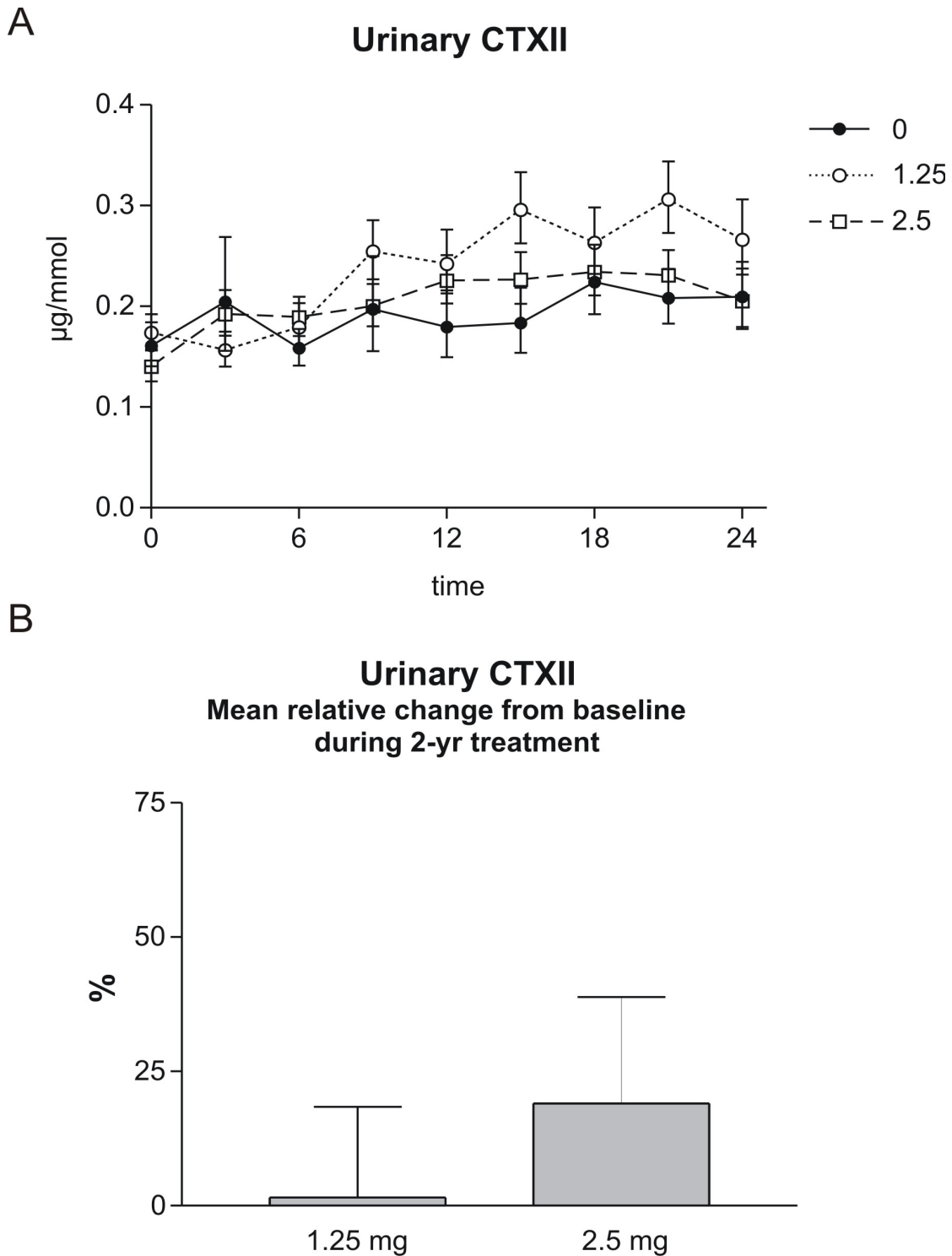


Figure 2
Cartilage degradation: Upper panel: Urinary CTXII (lower panel) during 2 years of therapy in the groups receiving 2.5 mg, 1.25 mg of tibolone, or placebo. Values shown are geometric mean \pm 1 SEM. Lower panel: Mean relative change from baseline in urinary CTXII during 2 years of therapy with 1.25 and 2.5 mg of tibolone. Values shown are placebo-corrected time-averaged mean change during the treatment period and given as mean \pm 1 SEM. Levels did not reach significance.

resorption alone results in increased articular cartilage damage [10,37,46].

Thereby, interventions that may positively affect bone turnover, may in theory have secondary positive effects on progression of OA. The present data strongly suggest that these processes can be uncoupled. This might in part be mediated through the androgenic properties of tibolone, as androgens in contrast to that of estrogens might have deleterious effects on cartilage health [45]. However the effects of androgens and the direct on articular cartilage compared to that of estrogens needs additional attention.

Estrogen and OA

The putative positive of some estrogens of cartilage health may both be though indirect and direct effects on cartilage. Estrogens are anti-resorptives that directly and indirectly attenuates osteoclastogenesis and osteoclastic resorption [47]. At the same time, chondrocytes express estrogen receptors, and respond to estrogen [11,48-52].

The positive long-term beneficial effect of estrogen for prevention of OA was recently demonstrated in 180 female cynomolgus monkeys [10], receiving estrogen replacement therapy (ERT) treatment for 3 years. Significant less cartilage lesions of OA were seen in the ERT group compared to the control group. In addition, several preclinical models have provided evidence for protective effects of estrogen [11,12,46].

In clinical settings a number of studies have suggested positive effects on cartilage degradation. A SERM was shown to protect against both bone and cartilage degradation [53], and cartilage degradation was found to be significantly lower in women using hormone replacement therapy (HRT) compared to control [6,12,54], and the cartilage degradation was significantly higher in postmenopausal women when compared to an age-matched group of pre-menopausal women. These data are in alignment with the recent analysis from the WHI studies, which documented that women taking estrogen had 45% less total joint surgery compared to of placebo [55].

However in the present study Tibolone did not result in secondary positive effects on cartilage degradation. This may in part be described by the complicated pharmacology of Tibolone on testosterone, estrogen and progesterone receptors. This is in contrast to others SERM and estrogen like molecules that to a much larger extent is selective for the estrogen receptor.

Limitations of the current study

The present study has some limitations. Cartilage degradation was evaluated by the biochemical markers u-CTX-II. Levels of u-CTX-II have been shown to correlate with

cartilage damage in both animal models and clinical trials [7,11,27,56], however long term randomized clinical trials studies are needed to further investigate and document the putative positive effects of estrogens on the pathogenesis of osteoarthritis.

Biochemical markers of cartilage degradation measured in the systemic fluids, serum and urine, are the net results of the biological activity of all joints and tissues in which collagen type II present. CTX-II is generated by MMP activity [57] have been shown to be produced by catabolically stimulated articular cartilage, and present in damaged articular cartilage [25,56]. However, CTX-II may in addition be generated by the cartilage of non-synovial joints. The main contributors of cartilage degradation biomarkers have been shown to be; knees, hips, hands, vertebral facet joints in addition to spinal disc degeneration (DD) [58,59]. In addition, a smaller contribution of CTX-II may originate from the calcified cartilage in the subchondral bone area [28]. The contribution of each cartilage compartment to the total pool of cartilage degradation measured is important to further understand, for the interpretation of the effect on the total pool of any biochemical marker. Some insights into the relative contribution of the different joints to the total amount of CTX-II have been provided [58]. CTX-II was shown to be related to the number of joint affected, evaluated by radiological OA, in which generalised resulted in an approximately 100% increase in CTX-II [58]. In addition, CTX-II was very recently shown to predict medial knee articular cartilage loss evaluated by quantitative MRI [60]. With respect to the current study, each compartment may have contributed differently to the pool of CTX-II, also in response to therapy. Further research is needed to understand the effects of estrogen and estrogen related compounds on the individual joints.

The analysis was performed by re-analysis on stored samples, which to some extend may have influenced that measurements. However, in house data suggest that u-CTX-II is stable for more than 3 years under the appropriate conditions.

Conclusion

In summary, we describe for the first time that anti-resorptive treatments can result in an uncoupling of the bone and cartilage protective effects, measured by biochemical markers and the limitation associated with those. These findings may result in questioning of the coupling between bone and cartilage, and whether they indeed are separate or coupled processes.

Competing interests

All authors are full time employees of Nordic Bioscience, a company engaged in the development of biochemical

markers of bone and cartilage turnover. Morten a. Karsdal and Claus Christiansen hold stocks in Nordic Bioscience.

Authors' contributions

CC participated in designing the original study and reviewed the last version of the manuscript. IB performed statistical analysis and participated in drafting of the manuscript. DJL participated in data analysis and writing of the manuscript. MAK came up with the original idea for the study, drafted the first manuscript and finalized the last version of the manuscript.

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