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Serum osteocalcin in relation to calcaneal bone mineral density in elderly men and women: a 5-year follow-up

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Abstract A 5-year follow-up study investigated serum concentrations of total (tOC) and intact (iOC) osteocalcin in relation to calcaneal bone mineral density (BMD). The study comprised two cohorts, 75- and 80-year-olds, both resident in the city of Jyväskylä, Finland. Baseline OC and BMD were obtained for 161 men and 233 women, of whom 83 men and 189 women participated in follow-up bone measurements. The mean concentration of tOC increased from 9.6 ± 4.3 to 13.2 ± 8.5 μg/l ($P = 0.001$) in men and from 11.2 ± 4.9 to 14.0 ± 6.1 μg/l ($P < 0.001$) in women, whereas mean iOC decreased from 6.4 ± 3.0 to 5.9 ± 3.0 μg/l ($P = 0.273$) and from 7.7 ± 3.7 to 6.9 ± 3.4 μg/l ($P = 0.021$) in men and women, respectively. TOC and iOC levels correlated inversely with BMD and change in BMD in both sexes (r ranged from −0.223 to −0.422 and $P = 0.048 \leq 0.001$). When we divided the baseline tOC and iOC values into four quartiles, the decrease in BMD was significantly greater in the third tOC quartiles in women and in the fourth tOC and iOC quartiles in men when compared with the lower quartiles. During the 5-year period, 19 men and 59 women sustained at least one fracture. These individuals with fractures had significantly higher iOC values and tended to have higher tOC values compared with the nonfracture group at baseline ($P = 0.038$ and 0.087, respectively). Our results indicate that baseline serum tOC and iOC were associated with bone loss and predicted fracture in the two cohorts of independently living elderly men and women.

Key words bone mineral density · bone turnover · elderly · fracture · osteocalcin · prospective study

Introduction

Osteocalcin (OC), also known as bone Gla protein, is a small protein that is released into the circulation and is usually perceived as a specific marker of bone formation when formation and resorption are uncoupled [1]. Changes in OC in circulation have been thought to reflect changes in the rate of bone turnover.

There is clear evidence that bone loss persists in elderly women and men [2–4]. This age-related bone loss is partly caused by a decline in osteoblast function [5,6]. However, a decrease in bone formation at the cellular level may not reflect the overall rate of bone turnover [1]. Although the formation marker OC is considered a general marker of bone turnover when its level is elevated, elevated bone resorption markers are more closely associated with bone loss, which becomes evident when OC is adjusted for increased bone resorption [7,8]. Some studies have shown that OC can be used to estimate both short- and long-term bone loss [9,10] and to identify fast and slow losers [11]. Undercarboxylated (ucOC) but not total OC (tOC) was used as an independent marker of hip bone mineral density (BMD) in elderly institutionalized women [12]. However, other studies found both tOC and ucOC to be negatively correlated with femoral neck BMD in elderly women [13] and tOC with different skeletal sites in both men [14] and women [14,15].

Despite incompatibility regarding the different OC assays used in different studies, the relationship between the level of OC and BMD seems to be clearer than that between OC and fracture. According to some studies, ucOC is a marker of hip fracture risk in elderly institutionalized women [16,17] and in elderly healthy and independently living women [18]. A high concentration of intact OC (iOC) was found to be associated with spine fractures in postmenopausal women [19], whereas in another study no significant difference in tOC and ucOC between subjects with and without hip fracture was found [13]. tOC was not found to predict hip fracture in the French EPIDOS study [20]. OC was associated with fractures in women who had sustained a fracture within the 6 years preceding assessment after

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adjusting for age and bone mineral content, but no significant differences in OC were found between fracture and nonfracture women during a 5-year follow-up period [21]. After adjusting for lower BMD and increased bone resorption, reduced bone formation as assessed by OC was associated with prior osteoporotic fractures [7].

Given the considerable controversy in this area and given the lack of data in elderly men, this study aimed to evaluate the serum concentration of tOC and iOC in relation to calcaneal BMD and changes in BMD and fracture occurrence in two cohorts of elderly men and women during a 5-year follow-up study.

Study cohorts and methods

Study populations

The study was part of the Evergreen project, a large gerontological research program. The study populations have been reported elsewhere [4,22,23]. Briefly, the baseline study population was drawn from the national population register. It consisted of all Finnish men and women who were 75 (born in 1914) or 80 (born in 1910) years old and residents in the city of Jyväskylä. In the younger cohort, 103 men and 191 women participated in the baseline bone measurements; of these, 59 men and 125 women participated in the follow-up study. In the older cohort, 58 men and 142 women participated in the baseline bone measurements, and 24 men and 64 women participated in the follow-up study. Only a few of the subjects who participated in the BMD measurements were institutionalized or not ambulatory. Informed consent was obtained in advance from all the participants.

Assessment of fractures

The occurrence of fractures was followed for 5 years after the baseline measurements. Information about fractures was first obtained either by a postal questionnaire or a telephone call as well as at home interview. The information was then checked against radiographic data from medical records at the local health center in the city of Jyväskylä and at the Central Hospital of Central Finland as well as at private clinics. All the documentation relating to fractures was evaluated by a physician. To ensure validity, the medical records of those persons who reported no fractures, or who had died, or who could not be contacted, or failed to respond were also checked. Detailed information about how the fracture assessments were carried out has been described in our previous publication [4].

Medication affecting bone metabolism

At follow-up, the subjects were asked about medication affecting bone metabolism. Nine women had taken estrogen for at least 1 year since the onset of menopause (1–20 years; mean, 6 years); all had discontinued the therapy at least 5 years before the baseline study. Nineteen persons were using insulin due to diabetes (2 men and 17 women), 6 women were continuing to take cortisone (1–12 years; mean, 6 years) and 10 women (2–44 years; mean, 20 years) and 1 man (14 years) to take thyroxin as medication for hypothyreosis. Of the 6 women who were taking cortisone, 2 had fallen without sustaining a fracture. Of those taking thyroxin, 2 women had fallen and both had sustained a fracture. All those using insulin, cortisone, and thyroxin were excluded from final analysis owing to the potential effects on bone metabolism.

Reagents and procedure for human osteocalcin immunoassays

Serum samples were obtained in the morning after the subjects had been fasting for 12 h and were frozen at −70°C until the assessments were conducted. The OC assessments were performed blind and assessed randomly with respect to the time the sample was taken. The amount of iOC and tOC was measured using fully automated two-site time-resolved fluorescence assays based on well-characterized monoclonal antibodies (MAbs). The iOC assay recognizes full-length OC. Conversely, the tOC assay has also been shown to detect the N-terminal midfragment of OC. The characteristics of the assays have been reported in detail elsewhere [24,25].

Measurement of the serum samples was performed using the AutoDELFIA 1235 automatic immunoassay system (Wallac Oy, Turku, Finland). Serum samples or standards in 10-μl volume were added to streptavidin microtiter wells (Wallac Oy). The biotinylated and Eu-labeled antibody was then added simultaneously to 50 μl of DELFIA buffer (Wallac Oy); 5 mmol/l of EDTA was added to the DELFIA assay buffer (Wallac Oy) for the tOC assay. After shaking for 2 h at 25°C, the plates were washed six times with DELFIA wash solution and 200 μl of DELFIA enhancement solution was added. After shaking for 30 min at 25°C, fluorescence was measured. The intra- and interassay coefficients of variation (CVs) were less than 5% and less than 8%, respectively.

Bone mineral measurements

BMD was measured in the right calcaneus by applying the single-energy photon absorption method as described earlier [22]. Briefly, the foot was placed in a

water-filled Plexiglas box between a radioactive source (^{125}I) and a NaI scintillation detector. Four scans were taken from two orthogonal directions across the mid-calcaneus to measure both the width and depth of the bone. This method enables the results to be expressed per volume unit of bone (g/cm^3). The CV between repeated measurements for different days was 1.5%.

Bone resorption marker assessment

At the follow-up assessment, we collected 24-h urine samples from a subgroup of the women in the younger cohort ($n = 63$). Pryidinoline (Pyr) and deoxypyridinoline (Dpyr) were assessed using reverse-phase high-performance liquid chromatography (HPLC) with minor modifications [26] according to the method of Eyre et al. [27]. In addition, creatinine (Cr) was quantified in the unhydrolyzed urine samples by the Jaffe procedure using a commercial reagent kit (Boehringer Mannheim GmbH, Mannheim, Germany). The results of Pyr and Dpyr were given in comparison with the standards injected at three different concentrations and expressed as the total amount excreted during 24 h and per creatinine. The CV was 3.1% for Pyr and 5.3% for Dpyr.

Statistical analyses

Student's *t* test (two-tailed) for paired and for independent samples was applied to examine the significance of the differences between the means of the two measurements. One-way analysis of variance followed by the least-squares difference (LSD) test was employed to test the differences between the means in the tOC and iOC quartiles. Two-way analysis of variance was used to test the differences between cohort and sex. The Pearson correlation was used to compare tOC and iOC with Pyr and Dpyr. Partial correlation coefficients were used to control for the effect of body weight when examining the relationship between tOC, iOC, and BMD. The statistical analyses were done using an SPSS-WIN software package.

Results

Comparison of baseline and follow-up

The mean concentration of tOC increased from 9.6 ± 4.3 to 13.2 ± 8.5 μg/l ($P = 0.001$) in men and from 11.2 ± 4.9 to 14.0 ± 6.1 μg/l ($P < 0.001$) in women, whereas iOC decreased from 6.4 ± 3.0 to 5.9 ± 3.0 μg/l ($P = 0.273$) and from 7.7 ± 3.7 to 6.9 ± 3.4 μg/l ($P = 0.021$) in the men and women, respectively, during the 5-year period (Fig. 1).

Comparison of cohorts and sex

When we looked separately at the changes in tOC and iOC in the two cohorts and sexes, we found that, in the younger cohort, 71% of the men showed an increase in tOC during the 5-year period; the corresponding figure for the women was 66%. On the other hand, 63% of men and 62% of women showed a decrease in iOC during the 5-year period. In the older cohort, 75% of men and 61% of women showed an increase in tOC during the 5-year period. The decrease in iOC was 71% for men and 50% for women.

Significant differences were found between the two cohorts for both tOC and iOC whereas no sex difference was found in either the baseline or follow-up measurements when two-way analysis of variance was used to test for the differences between cohort and sex. The men in the younger cohort had significantly higher tOC values than those in the older cohort at both baseline (10.3 ± 4.7 vs 7.7 ± 2.6 μg/l, $P = 0.014$) and follow-up

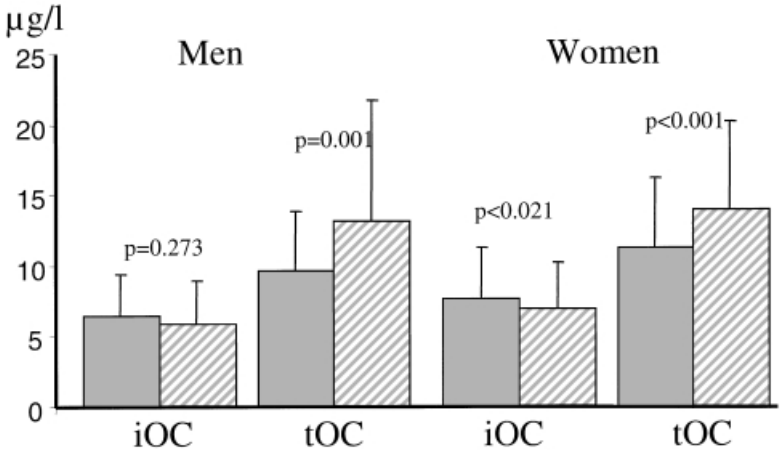

Fig. 1. Comparison of total (tOC) osteocalcin between baseline (*solid bars*) and follow-up (*hatched bars*) assessments in men and women. The *P* values indicate the significance of the difference between two consecutive bars

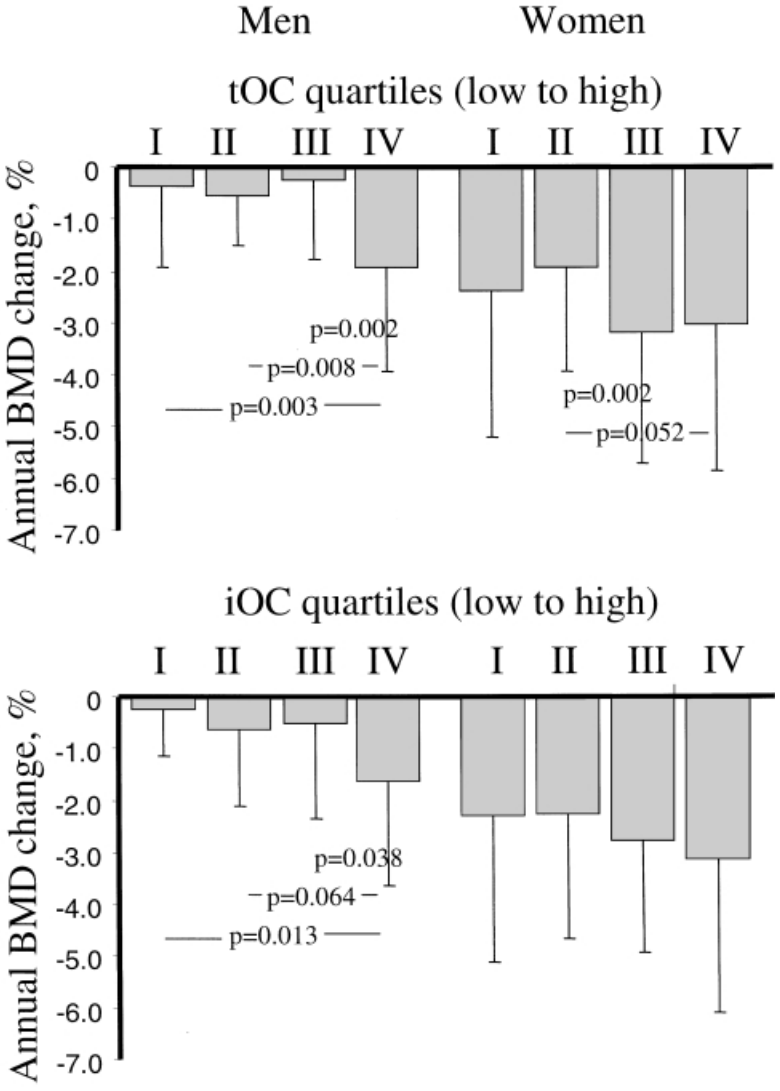

Fig. 2. Annual change in bone mineral density (BMD) during 5-year follow-up in combined 75- and 80-year-old men and women with baseline tOC and intact (iOC) osteocalcin in quartiles (mean, *n*, 95% confidence intervals). The *P* values indicate the significance of the difference between two bars

(14.9 ± 9.2 vs 9.0 ± 4.1 μg/l, $P = 0.003$) and higher iOC values at baseline (7.0 ± 3.2 vs 5.1 ± 1.9 μg/l, $P = 0.005$). Among women no significant differences in tOC and iOC were found between the younger and older cohort at baseline. However, women in the younger cohort had significantly higher tOC values than those in the older cohort at the follow-up measurement (15.2 ± 6.4 vs 11.6 ± 4.7 μg/l, $P < 0.001$).

When we divided the baseline tOC and iOC values into four quartiles by sex, in men the annual change in BMD was significantly greater in those who were in the highest tOC and iOC quartiles compared with those in the lower quartiles ($P = 0.038$–0.002; Fig. 2). In women, those in the third and fourth tOC quartiles showed greater change in BMD than those in the second tOC quartile ($P = 0.030$ and 0.052; Fig. 2). No significant difference between iOC quartiles was found in annual change in BMD.

Comparison of participants and drop-outs

Only 68% of the men and 66% of the women in the younger cohort and 53% of the men and 48% of the women in the older cohort were able to participate in the follow-up bone assessments. The reasons for dropping out in the different groups were death (22%–42%), relocation (1%–4%), and refusal (5%–12%). To evaluate whether there were differences in the baseline assessments between those who were able to participate in both measurements and those who subsequently dropped out, we compared the BMD, tOC, and iOC results between these groups (Table 1). The results showed that men in the older cohort who participated in both assessments had significantly higher BMD and lower tOC values than their drop-out counterparts ($P = 0.013$–0.005). No significant differences in BMD, tOC, and iOC were found between those who partici-

Table 1. Bone mineral density (BMD), total (tOC), and intact (iOC) osteocalcin at baseline in 75-year-old (younger cohort) and 80-year-old (older cohort) men and women who participated in the follow-up assessments compared to whose who dropped out

	Men			Women		
Variable	Participated	Dropped out	*P*	Participated	Dropped out	*P*
Younger cohort	$n = 56$	$n = 44$		$n = 110$	$n = 64–66$	
BMD ($g \cdot cm^{-3}$)	0.145 (0.030)	0.147 (0.029)	0.814	0.123 (0.039)	0.132 (0.039)	0.150
tOC ($\mu g \cdot l^{-1}$)	10.34 (4.7)	11.41 (5.4)	0.290	11.28 (4.0)	10.60 (3.3)	0.566
iOC ($\mu g \cdot l^{-1}$)	6.97 (3.2)	7.55 (2.8)	0.322	7.71 (3.1)	7.35 (2.7)	0.660
Older cohort	$n = 23$	$n = 34$		$n = 54$	$n = 75–78$	
BMD ($g \cdot cm^{-3}$)	0.160 (0.025)	0.139 (0.034)	0.020	0.110 (0.026)	0.120 (0.038)	0.161
tOC ($\mu g \cdot l^{-1}$)	7.72 (2.6)	10.01 (3.3)	0.015	11.10 (6.5)	10.05 (5.4)	0.770
iOC ($\mu g \cdot l^{-1}$)	5.08 (1.9)	6.60 (2.2)	0.017	7.59 (4.6)	7.07 (3.6)	0.496
Total participants						
BMD ($g \cdot cm^{-3}$)	0.149 (0.029)	0.143 (0.031)	0.229	0.117 (0.030)	0.123 (0.030)	0.159
tOC ($\mu g \cdot l^{-1}$)	9.58 (4.3)	10.75 (4.6)	0.111	11.22 (4.9)	10.85 (4.4)	0.514
iOC ($\mu g \cdot l^{-1}$)	6.42 (3.0)	7.15 (2.6)	0.112	7.67 (3.7)	7.27 (3.2)	0.335

Data are means (SD)

Table 2. Correlation of tOC and iOC with BMD in 75-year-old (younger cohort) and 80-year-old (older cohort) men and women at baseline and after 5-year follow-up

Variable	BMD (baseline)	BMD (follow-up)	BMD (change)	BMD (baseline)	BMD (follow-up)	BMD (change)
	75-year-old men			75-year-old women		
tOC (baseline)	−0.300 (0.002)		−0.304 (0.020)	−0.270 (<0.001)		−0.035 (0.708)
tOC (follow-up)		−0.401 (0.002)			−0.402 (<0.001)	
iOC (baseline)	−0.295 (0.025)		−0.217 (0.029)	−0.284 (<0.001)		−0.030 (0.746)
iOC (follow-up)		−0.317 (0.015)			−0.377 (<0.001)	
	80-year-old men			80-year-old women		
tOC (baseline)	−0.345 (0.010)		−0.655 (0.002)	−0.373 (<0.001)		−0.377 (0.006)
tOC (follow-up)		−0.134 (0.542)			−0.290 (0.024)	
iOC (baseline)	−0.281 (0.038)		−0.585 (0.007)	−0.356 (<0.001)		−0.486 (<0.001)
iOC (follow-up)		−0.273 (0.208)			−0.256 (0.047)	
Total participants						
tOC (baseline)	−0.296 (<0.001)		−0.309 (0.006)	−0.298 (<0.001)		−0.232 (0.002)
tOC (follow-up)		−0.413 (<0.001)			−0.376 (<0.001)	
iOC (baseline)	−0.224 (0.005)		−0.286 (0.011)	−0.293 (<0.001)		−0.261 (<0.001)
iOC (follow-up)		−0.365 (0.001)			−0.369 (<0.001)	

Data are *r* values with *P* values in parentheses

pated in both assessments and the drop-outs in the other groups.

Correlations

tOC and iOC values correlated inversely with BMD in both cohorts and sexes at both baseline and follow-up, except for men in the older cohort at follow-up (Table 2). Baseline tOC and iOC also correlated inversely with the change in BMD among men in both cohorts and among women in the older cohort. These relationships remained largely unchanged after controlling for the effect of body mass. tOC and iOC correlated with Pyr and Dpyr in a subgroup of 80-year-old women ($r = 0.244–0.398$; $P = 0.058–0.001$). The correlation lost significance when Pyr and Dpyr were corrected for Cr.

Fracture

The fracture cases included in this study were fall related only; fractures caused by car accidents, for example, were not included. Among the study population who sustained a fracture during the 5-year follow-up period, 8 men and 37 women in the younger cohort and 12 men and 26 women in the older cohort had participated in baseline bone measurements. Of these, 2 men

Table 3. tOC and iOC at baseline in 75-year-old (younger cohort) and 80-year-old (older cohort) men and women with or without fractures during 5-year follow-up

	Men			Women		
Variable	Fracture	Nonfracture	*P*	Fracture	Nonfracture	*P*
	$n = 19$	$n = 131$		$n = 53$	$n = 230$	
tOC ($\mu g \cdot l^{-1}$)	11.2 (5.7)	10.0 (4.3)	0.280	11.8 (5.3)	10.9 (4.6)	0.235
iOC ($\mu g \cdot l^{-1}$)	7.2 (3.2)	6.7 (3.2)	0.517	8.3 (3.6)	7.3 (3.4)	0.070

Total participants	Fracture	Nonfracture	*P*
tOC ($\mu g \cdot l^{-1}$)	11.6 (5.4)	10.6 (4.5)	0.087
iOC ($\mu g \cdot l^{-1}$)	8.0 (3.5)	7.1 (3.2)	0.038

Data are mean (SD)

and 25 women in the younger cohort and 4 men and 9 women in the older cohort participated in follow-up bone measurements. Information about the site and number of fractures has been reported earlier [4]. Briefly, the most common fracture sites for men were the femoral neck (27%) and wrist/hand (23%), and for women the radius/ulna (25%) and femoral neck (20%). In the younger cohort, there were 11 hip fractures, 30 upper-limb fractures, 12 lower-limb fractures, and 10 fractures of other types; the corresponding figures for the older cohort were 15, 19, 6, and 16.

No significant differences in tOC and iOC were found between the fracture and nonfracture groups when the data were analyzed by sex (Table 3). However, when we pooled the data and compared the fracture to nonfracture groups, we found that the fracture group had a significantly higher iOC value and a tendency toward a higher tOC value compared with the nonfracture group at baseline ($P = 0.038$ and 0.087, respectively).

Discussion

In this population-based 5-year follow-up study, we found baseline serum tOC and iOC to be associated with both baseline and follow-up BMD levels. Annual loss of BMD was significantly greater in both men and women who were in the higher tOC quartiles at baseline when compared with those in the lower quartiles. Men in the highest baseline iOC quartile also showed greater annual loss of BMD compared with those in the lower quartiles. The results suggest that baseline tOC and iOC can be used as a predictor of bone loss in elderly men and women.

Previous studies have shown a slight decrease in serum OC with age before onset of menopause and a marked increase after menopause [19,28–30]. When we looked at the changes in tOC and iOC separately, we observed an increase in tOC in the younger but not in the older cohort of men and women after the 5-year period. There was an apparent inconsistency in the results for the men. When the 75-year-old men reached the same age as the older cohort (80 years old), they might be expected, theoretically, to show comparable levels of BMD and tOC. However, BMD was higher and tOC was lower in the older cohort of men compared to the younger cohort when they were the same age. These results indicate an existing selective bias among men in the older cohort. Those men who dropped out had significantly lower baseline BMD and higher tOC values compared to those who participated in both assessments. These results have important implications for understanding the relationship between BMD and bone biomarkers when studying an elderly population, especially in the case of elderly men. The fact that the men who participated in follow-up at the age of 85 were in a very good state of health points to the existence of a highly selective bias and thus does not reveal the true age-related change in bone density and metabolism. In the absence of such a bias, the results would have showed a more "logical" relationship between BMD and OC. The limited number of participants in the older cohort may also partly explain this inconsistency in the results. The clinical interpretation of OC measurements is often difficult because results obtained with different OC assays are highly variable due to differences in sample storage, assay design, antibody specificity, and the lack of a common standard [24,31]. OC results derived from different study designs regarding bone turnover merit more rigorous examination.

Our previous study conducted among the same population showed that if both sexes had similar BMD values they also had a similar likelihood of sustaining a fracture, despite the fact that the total number of fractures was higher and the annual change in BMD was greater in women [4]. This previous finding together with the results of the present study suggest that men and women may not be, as often assumed, different in terms of bone formation and that the commonly observed difference in annual change in BMD between men and women

may be due to a difference in bone resorption. Unfortunately, we only have follow-up results from bone resorption markers for the subgroup of 80-year-old women and are unable to verify this assumption.

It is not clear whether we should expect OC to predict fracture risk. Previous studies have shown that it is only after adjusting for increased bone resorption that OC becomes negatively associated with fractures [7,8]. Moreover, OC may mark the turning point between bone formation and resorption [30]. When resorption and formation are coupled, OC is a valid marker of bone turnover. If formation and resorption are uncoupled, OC is a specific marker of bone formation [1,9,33–35]. It is well documented that osteoporosis occurs when the rate of bone loss exceeds that of bone formation. This silent disease can continue for years before accumulated damage is sufficient to precipitate bone fracture. When bone mass falls below the level required to maintain skeletal integrity during performance of everyday activities, fractures may occur after even minimal trauma. Bone turnover is more reflective of the current status of the bone metabolism whereas fracture is associated more with bone history and status and with mechanical force. Thus, we should not expect an increased rate of bone turnover to be directly associated with the occurrence of fracture.

When the data were pooled, a significantly higher iOC value and a tendency toward a higher tOC value were found among the fracture compared with the nonfracture group, especially in the case of hip fractures. This result, which is in agreement with previous reports [7,26], suggests that a combination of BMD measurements and bone biomarkers may be useful in improving the assessment of fracture risk in the elderly. Our results indicate that baseline serum tOC and iOC levels were associated with bone loss and predict fracture in two cohorts of independently living elderly Finnish men and women.

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