

Vitamin D Status, Estradiol and Bone Metabolism in Elderly Female Patients With Hip Fracture

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ABSTRACT

AIM: In postmenopausal women increased bone turnover is as-

sociated with hip fracture apart from bone mineral density. The aim of this study was to evaluate bone metabolism in consideration of vitamin D and hormonal status in elderly female hip fracture patients compared to controls.

MATERIAL AND METHODS: Circulating 25-hydroxyvitamin D₃ (25(OH)D), sex hormones, BTM [osteocalcin (OC); propeptide of type-I collagen (PINP); C-terminal telopeptide of type I collagen (CTX-1); tartrate resistant acid phosphatase 5b, (TRAP5b)] and osteoclastogenic cytokines [soluble receptor activator of nuclear factor κB ligand (sRANKL); osteoprotegerin (OPG)] were measured in patients with a low-energy hip fracture and controls without an incident hip fracture (each group *n* = 47 females).

RESULTS: 57.4 % of the patients, but only 10.6% controls had a severe vitamin D deficiency. T-Score at the lumbar spine was comparable in both groups (-1.8 ± 1.5 vs -1.6 ± 1.4 , *p* = 0.535). In hip fracture patients 25(OH)D (*p* < 0.001), albumin (*p* < 0.001), osteocalcin (OC) (*p* < 0.001) and sRANKL (*p* < 0.001) levels were lower compared to controls, whereas estradiol (*p* < 0.001), cortisol (*p* < 0.001), CTX-1 (*p* < 0.001) and OPG (*p* < 0.001) were higher. Linear regression analysis revealed an independent association between 25(OH)D and CTX-1 in female fracture patients (*p* = 0.023). Higher estradiol levels after hip fracture correlated inversely with OC (*p* = 0.018) and TRAP5b (*p* = 0.004). Multivariable regression analysis demonstrated that 25(OH)D, OC, estradiol (each *p* < 0.001) and albumin (*p* = 0.002) were independently associated with fracture. Higher estradiol levels correlated inversely with OC in fracture patients (*p* = 0.018).

CONCLUSION: 25(OH)D was an independent risk factor of hip fracture and demonstrated an independent association with CTX-1. Unchanged or lower levels of bone formation markers were not associated with 25(OH)D and were partly related to higher levels of estradiol presumably induced by trauma.

Key words: Hip fracture; Vitamin D; Bone metabolism; Bone turnover; Bone turnover markers; OPG; sRANKL; Estradiol

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Abbreviations:

DXA: Dual X-ray absorptiometry

BTM: Bone turnover marker

25(OH)D: 25-hydroxyvitamin D

PTH: parathormon

OC: osteocalcin

PINP: propeptide of type-I collagen

CTX-1: C-terminal telopeptide of type I collagen

TRAP5b: tartrate resistant acid phosphatase 5b

sRANKL: soluble receptor activator of nuclear factor kB ligand

OPG: osteoprotegerin

INTRODUCTION

Hip fractures are considered as the most severe osteoporotic fractures associated with high morbidity and mortality^[1-3]. Fracture toughness in elderly patients depends not only on bone mineral density, but also on different aspects of bone quality. According to Viguet-Carrin *et al.*^[4] bone quality is characterized by bone trabecular micro-architecture, geometry and shape, the extracellular bone matrix and remodeling by bone turnover. Observational cohort studies revealed that increased bone formation and resorption markers were associated with increased fracture risk^[5-7]. In postmenopausal women hypogonadism and consecutively lower estrogen levels are associated with increased bone turnover markers (BTM)^[8]. Estrogen is a key mediator in bone health. It inhibits bone resorption by direct and indirect actions on osteoclasts and maintains bone formation by reducing osteoblast apoptosis^[9]. Moreover, osteoclastogenesis is regulated by receptor activator of nuclear factor kB ligand (RANKL) and osteoprotegerin (OPG). RANKL is a potent activator of osteoclasts and also exists in a soluble form (sRANKL)^[10,11]. The decoy receptor OPG is the natural opponent of RANKL displaying anti-resorptive properties^[12]. However, the implication of sRANKL and OPG levels on fracture vulnerability is still discussed controversially^[13-17].

Vitamin D is an important factor for bone health. The influence of vitamin D status on trabecular micro-architecture and the etiology of femoral neck fractures are well described^[18]. Although severe vitamin D deficiency is highly prevalent in elderly patients of many countries^[19] its influence on BTM in elderly hip fracture patients is scarcely investigated. Likewise, studies analyzing the hormonal status in postmenopausal women early after hip fracture and potential interactions with bone metabolism are limited.

Therefore, the main aim of this study was to evaluate BTM in consideration of the vitamin D status in elderly female low-energy hip fracture patients compared to patients without an incident hip fracture. A secondary endpoint was to analyze the influence of potential postraumatic differences in the hormonal status on BTM.

METHODS

Ethical statement and subjects

The study was approved by the local Ethics Committee at the University of Leipzig (243-11-11072011). Written informed consent was obtained from all patients or their legal representative. 47 female patients with femoral neck or trochanteric fracture and 47 female controls without an acute hip fracture were enrolled from July 2011 to March 2014 at the University of Leipzig and at an out-patient doctor's office, respectively. For both groups the inclusion

criteria was an age between 61 - 85 years. Only hip fracture patients with a low-energy trauma were included; patients with multiple injuries were excluded. The control group consisted of patients presenting at the orthopedic out-patient clinic for lower back pain, but were otherwise healthy. Structural disease, i.e. vertebral fracture, spondylodiscitis, etc was excluded in these patients. Subjects with a pathologic fracture or a history of a pathologic fracture, a previous fracture within the last 12 months or oral glucocorticoid medication were also excluded in both groups. Patients were included throughout the year. In the fracture group 73% of patients were included in fall and winter months (October – March) and 27% in spring and summer months (April – September). In the control group this distribution was more balanced with 41% and 59%, respectively.

Vitamin D deficiency was classified as severe (< 10 ng/mL), moderate (10-19.9 ng/mL), insufficient (20-29.9 ng/mL) and normal (\geq 30 ng/mL)^[20]. Hypoalbuminemia was considered at serum levels below 35 g/L. Bone mineral density of the spine and hip was measured with DXA and expressed as T-scores (NHANES III). Patient characteristics were summarized in (Table 1).

Blood samples and assays

Fasting blood samples were collected between 7.00 and 10.00 am. Samples were taken preoperatively and within 20 hours after admission to the hospital from the hip fracture patients. Blood was centrifuged at 4°C within 20 minutes after sampling and stored at -80°. Samples were thawed only once. Serum creatinine, urea, calcium, phosphorus, intact parathormone (PTH), albumin, alkaline phosphatase, estradiol, testosterone, cortisol were determined with standard assays of the fully automated analytical system Cobas (Roche, Mannheim, Germany). 25(OH)D was determined with the LIAISON® 25-OH Vitamin D assay (DiaSorin, Stillwater, MN, USA).

Concentrations of C-terminal telopeptide of type I-collagen (CTX-1, cross-laps), osteocalcin and propeptide of type-I collagen (PINP) in serum were measured by the cobas 601 system (Roche). MicroVue osteoprotegerin and MicroVue tartrate resistant acid phosphatase 5b (TRAP5b) enzyme immuno assays were purchased from Quidel Corporation (San Diego, US) and sRANKL enzyme immuno assays from BIOMEDICA Medizinprodukte GmbH (Vienna, Austria). All were measured at the SpectraMax M5 multi-mode microplate reader (Molecular Devices, Sunnyvale, US). The osteoprotegerin (OPG) assay has a limit of detection (+ 3 standard deviation, SD) of 0.13 pmol/l, and a precision coefficient of variation within-runs of < 3.6 % und between-runs < 6.2%. The TRAP5b assay has a limit of detection of 0.2 U/l and a precision coefficient of variation within-runs of < 2.3 % und between-runs < 3.1 %. The sRANKL assay has a limit of detection 0.01pmol/l and a precision coefficient of variation within-runs of < 5.0 % und between-runs < 3.0 %.

Statistical analysis

Normally distributed values were given as mean \pm SD, non-normally distributed parameters, as median and the interquartile range (25th–75th percentile). The Shapiro-Wilk-test was used to test for normal distribution. In addition, the following statistical tests were applied: chi-square test for binominal, student's t-test, exact Fisher test for normally distributed, Mann-Whitney-U-Test and Spearman correlation for non-normally distributed parameters, binary multivariable regression analysis and linear multivariable regression analysis to assess the association of 25(OH)D and bone turnover markers. All statistical computations were performed using SPSS version 20.0 (Chicago, IL, USA). P values less than 5% were considered as significant.

RESULTS

Severe Vitamin D deficiency and hip fracture

Age and body mass index (BMI) were slightly different between hip fracture patients and controls. Osteoporosis according to a T-score (spine) < -2.5 was found in 35.6% of hip fracture patients and 29.8% of controls with no statistical difference (Table 1).

Severe vitamin D deficiency was more prevalent in the fracture group compared to controls (Table 2).

Consistently, controls supplemented 25(OH)D more often than fracture patients (Table 1). In the fracture group 25(OH)D levels were moderately lower during fall and winter months (7.8 ng/ml) compared to summer time (13.0 ng/ml). No seasonal variations of 25(OH) D levels were observed in the control group (17.7 ng/ml vs. 18.7 ng/mL).

Serum levels of 25(OH)D were significantly lower in the fracture group (Table 3). Apart from age and BMI, low 25(OH)D levels were an independent predictor of hip fracture (Table 4).

Severe vitamin D deficiency and bone turnover markers

25(OH)D3 levels showed an inverse correlation with PINP ($r = -0.330$; $p = 0.023$) and OPG ($r = -0.364$; $p = 0.012$) in controls. In fracture patients 25(OH)D3 inversely correlated with CTX-1 ($r = -0.329$; $p = 0.024$).

Linear regression analysis with backward selection including age, BMI, all BTMs, sRANKL and OPG confirmed an independent association of 25(OH)D3 and OPG ($B = -1.147$; 95% CI = $-2.005 - -0.290$; $p = 0.010$; $R^2 = 0.242$) in controls and between 25(OH)D3 and CTX-1 ($B = -0.009$; 95% CI = $-0.017 - -0.001$; $p = 0.023$; $R^2 = 0.111$) in hip fracture patients. No correlations between BTMs, sRANKL/OPG and BMI were found in both groups.

Bone turnover markers and acute hip fracture

Circulating levels of BTMs revealed higher bone resorption and lower bone formation in fracture patients compared to controls, but only OC and CTX-1 reached a level of significance. Levels of the osteoclastic activator sRANKL were lower ($p < 0.001$) and levels of the osteoclastic inhibitor OPG higher ($p < 0.001$) in fracture patients compared to controls (Table 3).

Circulating CRP ($p = 0.039$), cortisol ($p < 0.001$) and estradiol ($p < 0.001$) levels were higher in patients compared to controls. No correlation was found between albumin and BMI in patients and controls. Multivariable regression analysis corrected for age, BMI and 25(OH)D levels revealed an independent association of fracture with estradiol and albumin, but not CRP and cortisol (Table 4). Estradiol also correlated inversely with OC ($r = -0.348$; $p = 0.018$) and TRAP5b ($r = -0.412$; $p = 0.004$) in fracture patients. In addition, TRAP5b correlated inversely with CRP ($r = -0.344$; $p = 0.019$) (Figure 1).

In the control group an inverse correlation between estradiol and OC ($r = -0.313$; $p = 0.034$) was observed. OC was also independently associated with hip fracture (Table 5). No significant correlations were found for cortisol and markers of bone metabolism in both groups.

DISCUSSION

In the present study severe vitamin D deficiency was an independent risk factor of hip fracture. Low levels of 25(OH)D were independently associated with higher levels of the bone resorption marker CTX-1 in patients with hip fracture. Bone formation markers were lower or remained unchanged in the fracture group.

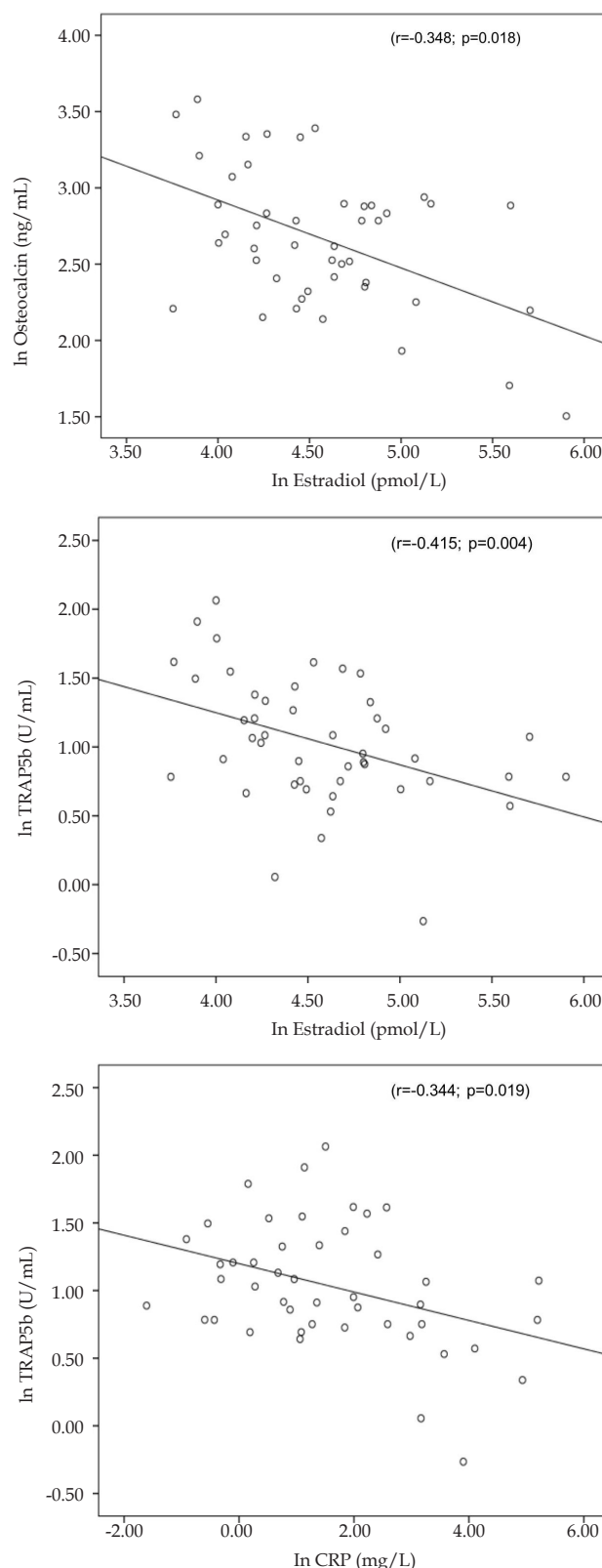


Figure 1 Spearman correlation analysis between In [Osteocalcin] and In [Estradiol], In [TRAP5b] and In [Estradiol], In [TRAP5b] and [CRP] in hip fracture patients.

Observational studies revealed that bone resorption as well as bone formation markers remained high late after menopause and were a major determinant of osteoporosis^[21]. Consequently increased bone turnover was also shown to be associated with increased hip fracture risk^[5-7]. Interestingly, we found lower levels of the bone

formation marker OC in our hip fracture patients compared to controls. A longitudinal observational study demonstrated that pre-injury OC levels were higher in patients suffering a hip fracture compared to controls that did not sustain a fracture^[22]. These results are in accordance with other studies analyzing patients after hip fracture^[23-27]. Ivasca *et al*^[26] determined OC and PINP before and 4 h after hip fracture. OC and to a lesser extent PINP decreased after trauma. The authors speculated that this trauma-induced decrease of OC is related to stress-induced increased glucocorticoid levels since glucocorticoid administration suppresses synthesis of OC^[28]. Indeed, cortisol levels were higher in our patients compared to controls, but they were not associated to OC. Our results are supported by Napal *et al*^[29] demonstrating a decrease of OC levels in response to stress observed in acute disease and tissue injury as myocardial infarction, fractures or surgery. In addition, Olmos *et al*^[30] exhibited that the decrease of serum OC is independent of the increase in cortisol secretion which is also in line with our findings.

In accordance with our results, Resmini *et al*^[22] found increased CTX-1 levels in postmenopausal women with a hip fracture. High levels of CTX-1 were shown to persist in postmenopausal women^[21] and increased bone turnover paralleled with low bone mineral density (BMD)^[21,31]. Others found no difference of CTX-1 levels in hip fracture and control patient^[24,25]. However, these studies performed no DXA and, in addition, 25(OH)D levels were equal in both groups impeding a reliable comparison to our results.

The role of TRAP5b in fracture patients is not yet clear. It is a marker of bone resorption that also indicates the number of osteoclasts^[32]. As demonstrated by others^[24], TRAP5b differed not between our hip fracture patients and controls. In contrary, higher TRAP5b levels were demonstrated in 18 hip fracture patients compared to 35 patients with hip osteoarthritis and 19 subjects with normal bone mineral density^[23]. The different results might be explained by a potentially variable inflammatory status of our hip fracture patients compared to controls which was not addressed in the previous study^[23]. We found

Table 1 Baseline characteristics of hip fracture patients and controls.

| | fracture (n = 47) | controls (n = 47) | p value |
|---------------------------------------|-------------------|-------------------|----------|
| Age (years) | 76.1 ± 5.5 | 71.1 ± 6.3 | < 0.001* |
| BMI (kg/m ²) | 25.4 ± 4.1 | 28.7 ± 5.2 | 0.001* |
| T-score lumbar spine | -1.8 ± 1.5 | -1.6 ± 1.4 | 0.535 |
| History of fracture, n (%) | 17 (36.2) | 7 (14.9) | 0.049* |
| Vitamin D supplementation, n (%) | 1 (2) | 14 (30) | <0.001* |
| Calcium supplementation, n (%) | 3 (6) | 8 (17) | 0.198 |
| Bisphosphonate supplementation, n (%) | 0 (0) | 3 (6) | 0.242 |
| Smoking, n (%) | 8 (17) | 2 (4) | 0.091 |
| Alcohol (≥ drinks/week), n (%) | 4 (9) | 2 (4) | 0.68 |
| Living in a nursing home, n (%) | 5 (10) | 0 (0) | 0.061 |

Values are given as mean ± SD; t- or exact Fisher test, *p < 0.05.

Table 2 Vitamin D deficiency and Hypalbuminemia in hip fracture patients and controls.

| | fracture (n = 47) | controls (n = 47) |
|--|-------------------|-------------------|
| Vitamin D deficiency (levels of 25(OH)D) | | |
| severe (< 10 ng/mL), n (%) | 27 (57.4) | 5 (10.6) |
| moderate (10-19.9 ng/mL), n (%) | 18 (38.3) | 22 (46.8) |
| insufficient (20-29.9 ng/mL), n (%) | 1 (2.1) | 15 (31.9) |
| normal (≥ 30 ng/mL), n (%) | 1 (2.1) | 5 (10.6) |
| Hypoalbuminemia, n (%) | 14 (29.8) | 0 (0) |

higher CRP levels in our fracture group which were inversely related to TRAP5b. Moreover, timespans of blood sample collection varied considerably. We and Sypniewska *et al*^[24] collected the samples within the first 20 hours after admission to the hospital, whereas the span was 48 hours in the study of Resmini *et al*^[23].

Table 3 Comparison of circulating biochemical markers between hip fracture patients and controls.

| | fracture (n = 47) | controls (n = 47) | p value |
|-------------------------------|----------------------|---------------------|----------|
| 25(OH)D (ng/ml) | 8.5 (5.4-13.7) | 18.5 (13.4-23.7) | <0.001* |
| PTH (pmol/l) | 5.6 (4.1-6.2) | 4.6 (3.6-6.4) | 0.266 |
| Calcium (mmol/l) | 2.3 (2.2-2.4) | 2.4 (2.3-2.5) | 0.005* |
| Phosphorus (mmol/l) | 1.0 (0.9-1.1) | 1.1 (1.0-1.2) | 0.039 |
| Creatinine (µmol/l) | 72.0 (59.0-101.0) | 72.0 (64.0-82.0) | 0.989 |
| C-reactive protein (mg/l) | 3.9 (1.3-13.3) | 2.8 (1.2-4.6) | 0.039* |
| Albumin (g/l) | 37.8 (33.5-40.9) | 45.6 (43.6-47.5) | <0.001* |
| Cortisol (nmol/l) | 777.8 (565.9-1037.0) | 532.3 (401.2-629.2) | < 0.001* |
| Estradiol (pmol/l) | 91.0 (67.1-123.4) | 40.4 (25.9-59.6) | < 0.001* |
| Osteocalcin (ng/ml) | 14.8 (10.5-18.1) | 22.0 (16.9-28.7) | < 0.001* |
| PINP (µg/l) | 37.0 (27.0-53.0) | 44.0 (31.6-60.0) | 0.191 |
| Alkaline phosphatase (µkat/l) | 1.2 (1.0-1.6) | 1.2 (1.0-1.4) | 0.209 |
| CTX-1 (pg/ml) | 429.0 (302.0-617.0) | 306.0 (248.0-407.0) | <0.001* |
| TRAP5b (U/l) | 2.7 (2.1-3.8) | 2.6 (2.1-3.2) | 0.532 |
| sRANKL (pmol/l) | 0.005 (0.005-0.012) | 0.035 (0.005-0.089) | <0.001* |
| OPG (pmol/l) | 8.8 (6.3-11.0) | 6.1 (4.8-7.7) | < 0.001* |
| sRANKL/OPG ratio | 0.001 (0.001-0.002) | 0.005 (0.001-0.017) | <0.001* |

Values were given as median and interquartile range (IQR), Mann-Whitney-U-Test, * p < 0.05.

Table 4 Associations of albumin, CRP and hormones with hip fracture.

| Model | Adjusted Odds Ratio (OR) | 95% confidence interval (CI) | p-value | R ² | |
|-------|--------------------------|------------------------------|---------------|----------------|-------|
| 1 | Age | 1.257 | 1.099 – 1.437 | 0.001* | 0.693 |
| | BMI | 0.689 | 0.569 – 0.836 | <0.001* | |
| | 25(OH)D | 0.743 | 0.649 – 0.851 | <0.001* | |
| 2 | Albumin | 0.56 | 0.387 – 0.809 | 0.002* | 0.844 |
| 3 | CRP | 1.083 | 0.952 – 1.231 | 0.225 | 0.746 |
| 4 | Cortisol | 1.002 | 0.999 – 1.005 | 0.11 | 0.713 |
| 5 | Estradiol | 1.057 | 1.025 – 1.090 | <0.001* | 0.831 |

Multivariable regression analysis correcting for age, BMI and 25(OH)D levels (* p < 0.05). Each variable of models 2 to 5 was included separately. In all models age, BMI and 25(OH)D levels remained independently associated with hip fracture.

Table 5 Associations of markers of bone metabolism with hip fracture.

| Model | Adjusted Odds Ratio (OR) | 95% confidence interval (CI) | p-value | Nagelkerke R ² | |
|-------|--------------------------|------------------------------|-----------------|---------------------------|-------|
| 1 | Age | 1.257 | 1.099 – 1.437 | 0.001* | 0.693 |
| | BMI | 0.689 | 0.569 – 0.836 | <0.001* | |
| | 25(OH)D | 0.743 | 0.649 – 0.851 | <0.001* | |
| 2 | OC | 0.786 | 0.681 – 0.908 | 0.001* | 0.846 |
| 3 | PINP | 0.969 | 0.944 – 0.995 | 0.019* | 0.735 |
| 4 | CTX-1 | 1.001 | 0.997 – 1.006 | 0.49 | 0.697 |
| 5 | TRAP5b | 0.9 | 0.546 – 1.481 | 0.677 | 0.693 |
| 6 | sRANKL | 0.001 | 0.000 – 283.553 | 0.291 | 0.701 |
| 7 | OPG | 1.062 | 0.851 – 1.326 | 0.593 | 0.694 |

Multivariable regression analysis correcting for age, BMI and 25(OH)D levels (* p < 0.05). Each variable of models 2 to 7 was included separately. In all models age, BMI and 25(OH)D levels remained independently associated with hip fracture.

We found lower sRANKL and higher OPG levels and thus a lower sRANKL/OPG ratio in our patients compared to controls. In accordance to our study, Köttsdorfer *et al.*^[33] also measured lower sRANKL and higher OPG levels after long bone fracture in 33 male and 31 female patients with a mean age of 59 years. Wang *et al.*^[34] found significantly higher sRANKL and OPG levels in 36 elderly patients with an intertrochanteric fracture compared to controls. They hypothesized that the number of osteoblasts increased immediately after fracture. Resmini *et al.*^[23] found higher OPG levels, but no difference in sRANKL levels in postmenopausal women with hip fracture compared to patients with hip osteoarthritis and subjects with normal bone mineral density. Large longitudinal observational studies revealed that higher levels of pre-fracture OPG were associated with increased hip fracture risk^[14,17]. It was concluded that higher pre-fracture levels of OPG reflect a compensatory mechanism that is related to hip fracture risk^[14]. Thus, higher levels of OPG in patients before and after hip fracture suggest that this cytokine is not altered substantially early after the trauma event. Only one study found an association of pre-fracture low sRANKL levels and nontraumatic fractures^[13], whereas others found no association^[14,15].

Our hip fracture patients were further characterized by a more frequent severe vitamin D-deficiency and dramatically lower 25(OH)D levels compared to controls. Consistently, controls supplemented more often vitamin D (30% vs 2%). In addition, fracture patients were included more frequently over winter months. However, 25(OH)D levels were lower in the fracture group throughout the year. 25(OH)D levels were inversely and independently associated with CTX-1 in the fracture group as in the study of Napoli *et al.*^[35]. In a randomized double blind study of 297 postmenopausal women, CTX-1 levels were reduced after 1 year vitamin D supplementation^[36]. The data were confirmed by others^[37]. An independent relationship was also found for 25(OH)D and OPG in our control group. In opposition to our results, no correlations between 25(OH)D and OPG were demonstrated by Wanby *et al.* in control and fracture patients^[27]. Different to our study, fracture patients had higher 25(OH)D levels compared to our patients.

Although our patients were more than 5 years older compared to controls, they had a two-fold higher estradiol level which was independently associated with hip fracture. In contrast, a protective effect of higher estrogen levels on bone quality and a reduced fracture risk have been found^[38]. Thus, we suggest a trauma-induced increase of estradiol. Consistently, elevated estradiol levels were observed in patients after critical traumatic injury and associated with injury severity and poor outcome^[39,40].

Estradiol levels were inversely associated with levels of OC and TRAP5b in our fracture group. Higher levels of estradiol in our fracture group might have inhibited the rate of bone turnover explaining in part lower levels of OC and nearly unchanged TRAP5b in fracture patients.

Theoretically lower OC levels in our patients might also be explained by lower protein intake or poor nutritional status. Low protein intake affects building of bone matrix^[41] and decreases bone formation^[42]. Our fracture patients exhibited a lower BMI and lower albumin levels compared to controls. However, BMI was within normal range and we found no correlation of BMI with albumin in both groups. Alternatively, the low albumin levels might result from the traumatic injury. Indeed, albumin dropped immediately after tissue trauma, bottomed at 4 to 6 hours and recovered only slowly within days^[43]. Moreover, other factors involved in the acute phase or stress response, i.e. cytokines, might be responsible for lower OC levels which were not addressed in this study.

Our study has several limitations. First, it included only a low number of patients. Second, the cross-sectional design of our study and the lack of longitudinal data may impede interpretation of results found in our study. For instance pre-traumatic data were not available for our hip fracture patients. Thus, we could only suggest which altered parameters compared to controls were influenced by the traumatic event or already existed before hip fracture. Third, bone turnover markers measured maybe insufficiently specific for bone, since type I collagen is found in many different organs^[44].

In summary, 25(OH)D was identified as an independent risk factor of hip fracture and also demonstrated an independent association with CTX-1. Unchanged or lower levels of bone formation markers in the fracture group might be explained by higher levels of estradiol which presumably increased posttraumatically. No relation was seen between 25(OH)D and bone formation markers.

Authors' contributions

JKMF conceived the study, analyzed data and drafted the manuscript. JGD contributed to data acquisition, analyzed data and contributed to the manuscript draft. KHH contributed to data acquisition and revised the manuscript critically for important intellectual content. JK contributed to data acquisition, analyzed and revised the manuscript critically for important intellectual content. CK contributed to data acquisition, CJ revised the manuscript critically for important intellectual content, GA analyzed data and revised the manuscript critically for important intellectual content. All authors gave final approval for the published version.

REFERENCES

1. Johnell O, Kanis JA. An estimate of the worldwide prevalence, mortality and disability associated with hip fracture. *Osteoporos Int* 2014; **15**: 897-902. [PMID: 15490120]
2. Graham J, Bowen TR, Strohecker KA, Irgit K, Smith WR. Reducing mortality in hip fracture patients using a perioperative approach and "Patient-Centered Medical Home" model: a prospective cohort study. *Patient Saf Surg* 2014; **8**: 7. [PMID: 24490635]
3. Fakler JK, Grafe A, Dinger J, Josten C, Aust G. Perioperative risk factors in patients with a femoral neck fracture - influence of 25-hydroxyvitamin D and C-reactive protein on postoperative medical complications and 1-year mortality. *BMC Musculoskelet Disord* 2016; **17**: 51. [PMID: 26833068]; [DOI: 10.1186/s12891-016-0906-1]
4. Viguet-Carrin S, Garnero P, Delmas PD. The role of collagen in bone strength. *Osteoporos Int* 2006; **17**: 319-36. [PMID: 16341622]
5. Garnero P, Sornay-Rendu E, Claustrat B, Delmas PD. Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. *Bone Miner Res* 2000; **15**: 1526-36. [PMID: 10934651]
6. Finnes TE, Lofthus CM, Meyer HE, Eriksen EF, Apalset EM, Tell GS, Torjesen P, Samuelsen SO, Holvik K. Procollagen type I amino-terminal propeptide (PINP) and risk of hip fractures in elderly Norwegian men and women. A NOREPOS study. *Bone* 2014; **64**: 1-7. [PMID: 23496953]; [DOI: 10.1016/j.bone.2014.03.010]
7. Shigdel R, Osima M, Ahmed LA, Joakimsen RM, Eriksen EF, Zebaze R, Bjørnerem Å. Bone turnover markers are associated with higher cortical porosity, thinner cortices, and larger size of the proximal femur and non-vertebral fractures. *Bone* 2015; **81**: 1-6. [PMID: 26112819]; [DOI: 10.1016/j.bone.2015.06.016]
8. Sypniewska G, Chodakowska-Akolinska G. Bone turnover markers and estradiol level in postmenopausal women. *Clin Chem Lab Med* 2000; **38**: 1115-9. [PMID: 11156339]; [DOI: 10.1515/

- CCLM.2000.167]
9. Khosla S, Oursler MJ, Monroe DG (2012). Estrogen and the skeleton. *Trends Endocrinol Metab* **23**: 576-81. [PMID: 22595550]; [DOI: 10.1016/j.tem.2012.03.008]
 10. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 1998; **93**: 165-76. [PMID: 9568710]
 11. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci U S A*. 1998; **95**: 3597-602. [PMID: 9520411]
 12. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lüthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997; **89**: 309-19. [PMID: 9108485]
 13. Schett G, Kiechl S, Redlich K, Oberhollenzer F, Weger S, Egger G, Mayr A, Jocher J, Xu Q, Pietschmann P, Teitelbaum S, Smolen J, Willeit J. Soluble RANKL and risk of nontraumatic fracture. *JAMA* 2004; **291**: 1108-13. [PMID: 14996780]
 14. LaCroix AZ, Jackson RD, Aragaki A, Kooperberg C, Cauley JA, Chen Z, Leboff MS, Duggan D, Wactawski-Wende J. OPG and sRANKL serum levels and incident hip fracture in postmenopausal Caucasian women in the Women's Health Initiative Observational Study. *Bone* 2013; **56**: 474-81. [PMID: 23735608]; [DOI: 10.1016/j.bone.2013.05.018]
 15. Chiba Y, Onouchi T, Ikeda T, Adachi J, Tamura Y, Horiuchi T. Implications of measuring soluble receptor activators of nuclear factor-kappaB ligand and osteoprotegerin in bone metabolism of elderly women. *Gerontology* 2009; **55**: 275-80. [PMID: 19158438]; [DOI: 10.1159/000196280]
 16. Fahrleitner-Pammer A, Dobnig H, Pischwanger-Soelkner C, Bonelli C, Dimai HP, Leb G, Obermayer-Pietsch B. Osteoprotegerin serum levels in women: correlation with age, bone mass, bone turnover and fracture status. *Wien Klin Wochenschr* 2003; **115**: 291-7. [PMID: 12793029]
 17. Jørgensen L, Hansen JB, Ahmed L, Bjørnerem Å, Emaus N, Joakimsen R, Mathiesen E, Størmer J, Vik A, Jacobsen BK. Osteoprotegerin is associated with hip fracture incidence: the Tromsø Study. *J Epidemiol* 2012; **41**: 1033-9. [PMID: 22538253]; [DOI: 10.1093/ije/dys063]
 18. Seitz S, Koehne T, Ries C, De Novo Oliveira A, Barvencik F, Busse B, Eulenburg C, Schinke T, Püschel K, Rueger JM, Amling M, Pogoda P. Impaired bone mineralization accompanied by low vitamin D and secondary hyperparathyroidism in patients with femoral neck fracture. *Osteoporos Int* 2013; **24**: 641-649. [PMID: 22581296]; [DOI: 10.1007/s00198-012-2011-0]
 19. Lips P, Hosking D, Lippuner K, Norquist JM, Wehren L, Maalouf G, Ragi-Eis S, Chandler J. The prevalence of vitamin D inadequacy amongst women with osteoporosis: an international epidemiological investigation. *J Intern Med* 2006; **260**: 245-54. [PMID: 16918822]; [DOI: 10.1111/j.1365-2796.2006.01685.x]
 20. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007; **357**: 266-81. [PMID: 17634462]; [DOI: 10.1056/NEJMra070553]
 21. Garnero P, Sornay-Rendu E, Chapuy MC, Delmas PD. Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis. *J Bone Miner Res* 1996; **11**: 337-49. [PMID: 8852944]; [DOI: 10.1002/jbmr.5650110307]
 22. Dai Z, Wang R, Ang LW, Yuan JM, Koh WP. Bone turnover biomarkers and risk of osteoporotic hip fracture in an Asian population. *Bone*. 2016; **83**: 171-7. [PMID: 26555636]; [DOI: 10.1016/j.bone.2015.11.005]
 23. Resmini G, Migliaccio S, Dalle Carbonare L, Sala U, Brama M, Fornari R, Maggi S, Siviero P, Malavolta N, Iolascon G. Differential characteristics of bone quality and bone turnover biochemical markers in patients with hip fragility fractures and hip osteoarthritis: results of a clinical pilot study. *Aging Clin Exp Res* 2011; **23**: 99-105. [PMID: 21743289]
 24. Sypniewska G, Sobanska I, Pater A, Kedziora-Kornatowska K, Nowacki W. Does serum osteoprotegerin level relate to fragility fracture in elderly women with low vitamin D status? *Med Sci Monit* 2010; **16**: CR96-101. [PMID: 20110921]
 25. Thaler HW, Oudshoorn C, Hartholt KA, van der Cammen TJ. Parameters of bone health and fracture risk in older female fall victims: what do they tell us? *Gerontol Geriatr* 2015; **48**: 539-42. [PMID: 26811110]; [DOI: 10.1007/s00391-014-0843-2]
 26. Ivaska KK, Gerdhem P, Akesson K, Garnero P, Obrant KJ. Effect of fracture on bone turnover markers: a longitudinal study comparing marker levels before and after injury in 113 elderly women. *J Bone Miner Res* 2007; **22**: 1155-64. [PMID: 17488197]; [DOI: 10.1359/jbmr.070505]
 27. Wanby P, Nobin R, Von SP, Brudin L, Carlsson M. Serum levels of the bone turnover markers dickkopf-1, sclerostin, osteoprotegerin, osteopontin, osteocalcin and 25-hydroxyvitamin D in Swedish geriatric patients aged 75 years or older with a fresh hip fracture and in healthy controls. *J Endocrinol Invest* 2016; **39**: 855-853. [PMID: 26850415]; [DOI: 10.1007/s40618-015-0421-5]
 28. Godschalk MF, Downs RW. Effect of short-term glucocorticoids on serum osteocalcin in healthy young men. *J Bone Miner Res* 1998; **3**: 113-5. [PMID: 3264991]; [DOI: 10.1002/jbmr.5650030117]
 29. Napal J, Amado JA, Riancho JA, Olmos JM, González-Macías J. Stress decreases the serum level of osteocalcin. *Bone Miner*. 1993 May; **21(2)**: 113-8. [PMID: 8358248]
 30. Olmos JM, Amado JA, Valero C, Hernández JL, González-Macías J. Factors other than glucocorticoids are involved in the osteoblast activity decrease caused by tissue injury. *Clin Endocrinol (Oxf)*. 2006 Mar; **64(3)**: 280-3. [PMID: 16487437]; [DOI: 10.1111/j.1365-2265.2006.02457.x]
 31. Sypniewska G, Chodakowska-Akolinska G. Bone turnover markers and estradiol level in postmenopausal women. *Clin Chem Lab Med* 2000; **38**: 1115-9. [PMID: 11156339]; [DOI: 10.1515/CCLM.2000.167]
 32. Halleen JM, Tiitinen SL, Ylipahkala H, Fagerlund KM, Väänänen HK. Tartrate-resistant acid phosphatase 5b (TRACP 5b) as a marker of bone resorption. *Clin Lab* 2006; **52**: 499-509. [PMID: 17078477]
 33. Köttstorfer J, Thomas A, Gregori M, Kecht M, Kaiser G, Eipeldauer S, Sarahrudi K. Are OPG and RANKL involved in human fracture healing? *J Orthop Res* **32**: 1557-61. [PMID: 25212894]; [DOI: 10.1002/jor.22723]
 34. Wang XF, Zhang YK, Yu ZS, Zhou JL. The role of the serum RANKL/OPG ratio in the healing of intertrochanteric fractures in elderly patients. *Mol Med Rep* 2013; **7**: 1169-72. [PMID: 23440406]; [DOI: 10.3892/mmr.2013.1335]
 35. Napoli N, Strollo R, Sprini D, Maddaloni E, Rini GB, Carmina E. Serum 25-OH Vitamin D in relation to Bone Mineral Density and Bone Turnover. *Int J Endocrinol* 2014; 487463. [PMID: 25110474]; [DOI: 10.1155/2014/487463]
 36. Grimnes G, Joakimsen R, Figenschau Y, Torjesen PA, Almås B, Jorde R. The effect of high-dose vitamin D on bone mineral density and bone turnover markers in postmenopausal women with low bone mass--a randomized controlled 1-year trial. *Osteoporos Int* 2012; **23**: 201-11. [PMID: 21909730]; [DOI: 10.1007/s00198-

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- 011-1752-5]
37. Rizzoli R. Dairy products, yogurts, and bone health. *Am J Clin Nutr* 2014; **99**: 1256S-62S. [PMID: 24695889]; [DOI: 10.3945/ajcn.113.073056]
 38. Tella SH, Gallagher JC. Prevention and treatment of postmenopausal osteoporosis. *J Steroid Biochem Mol Biol*. 2014; **142**: 155-70. [PMID: 24176761]; [DOI: 10.1016/j.jsbmb.2013.09.008]
 39. Dossett LA, Swenson BR, Heffernan D, Bonatti H, Metzger R, Sawyer RG, May AK. High levels of endogenous estrogens are associated with death in the critically injured adult. *J Trauma* 2008; **64**: 580-5. [PMID: 18332796]; [DOI: 10.1097/TA.0b013e31816543dd]
 40. Wagner AK, McCullough EH, Niyonkuru C, Ozawa H, Loucks TL, Dobos JA, Brett CA, Santarsieri M, Dixon CE, Berga SL, Fabio A Acute serum hormone levels: characterization and prognosis after severe traumatic brain injury. *J Neurotrauma* 2011; **28**: 871-88. [PMID: 21488721]; [DOI: 10.1089/neu.2010.1586]
 41. Bonjour JP. Dietary protein: an essential nutrient for bone health. *J Am Coll Nutr* 2005; **24 (6 Suppl)**: 526S-536S. [PMID: 16373952]
 42. Cabrerizo S, Cuadras D, Gomez-Busto F, Artaza-Artabe I, Marín-Ciancas F, Malafarina V. Serum albumin and health in older people: Review and meta analysis. *Maturitas* 2015; **81**: 17-27. [PMID: 25782627]; [DOI: 10.1016/j.maturitas.2015.02.009]
 43. Hübner M, Mantziari S, Demartines N, Pralong F, Coti-Bertrand P, Schäfer M. Postoperative Albumin Drop Is a Marker for Surgical Stress and a Predictor for Clinical Outcome: A Pilot Study. *Gastroenterol Res Pract* 2016: 8743187. [PMID: 26880899]; [DOI: 10.1155/2016/8743187]
 44. Garnero P (2014). New developments in biological markers of bone metabolism in osteoporosis. *Bone* **66**: 46-55. [PMID: 24909537]; [DOI: 10.1016/j.bone.2014.05.016]

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