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THE IMPACT OF SMOKING ON BONE METABOLISM, BONE MINERAL DENSITY AND VERTEBRAL FRACTURES IN POST-MENOPAUSAL WOMEN

Running head: smoking and bone health

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ABSTRACT

Background. Smoking is recognized among the risk factors for osteoporosis, but only few studies have comprehensively explored its influence on bone metabolism and strength. We aimed to evaluate smoking effects on calcium-phosphate metabolism, bone mineral density (BMD) and fracture risk in post-menopausal women.

Methods. Our sample included 1067 post-menopausal women who arrived to our osteoporosis outpatient clinic. Anamnestic data, smoking habits (categorized as never, former, and current; and by smoking intensity and duration), biochemical parameters, lumbar/femoral BMD, and presence of vertebral fractures were recorded. In a subsample of 357 women, the changes in BMD after a 2-year follow-up period were also assessed.

Results. Current smokers had shorter reproductive age, lower body mass index, and higher prevalence of heavy alcohol consumption than former/never smokers. They also had lower PTH values and weaker linear association between serum vitamin D and PTH (current β=-0.11[SE=0.004]; former β=-0.14[SE=0.01]; never β=-0.20[SE=0.003]; $p<0.01$ for all). Baseline BMD did not reflect differences based on smoking habits, duration or intensity. However, after two years, only current smokers significantly worsened in femural BMD. After adjustment for confounders, the chance of having sustained vertebral fractures at the first evaluation increased by 74% (95%CI:1.07-2.83) in current compared with never smokers, especially among heavy smokers.

Conclusions. Smoking may negatively affect bone by inhibiting vitamin D-PTH axis, reducing estrogen exposure, promoting risky health behaviors, and accelerating bone loss, especially at the femur. No significant differences were observed in these outcomes among former smokers, suggesting that quitting smoking has beneficial effects on bone health.

Keywords: smoking habits; parathyroid hormone; vitamin D; vertebral fractures.

INTRODUCTION

Osteoporosis is a disease whose incidence in older women is particularly high. It tends to affect the quality of life and leads to disability and mortality[1], resulting mainly from the onset of fragility fractures.

Several studies considered unhealthy behaviours such as excess weight and physical inactivity[2, 3], heavy alcohol or caffeine intake[4] and qualitative or quantitative nutritional imbalance[5–7] as potential factors influencing the development of osteoporosis, although with some contrasting results[3, 8, 9]. In addition to those factors, smoking, in the past decades, has become increasingly important. In fact, since the sixties, the smoker population has progressively spread to include also women[10]. Smoking can have negative effects on bone health through a double mechanism[11]. Firstly, it can have a direct toxic effect on osteoblasts[12] and blood flow[13] which may affect bone health and lead to higher fracture risk, particularly femur fracture [14]. Secondly, smoking can have indirect effects on bone health as it often coexists with other health risk behaviours[15], and can affect sex hormones[10] and the physiological inverse association between vitamin D and parathyroid hormone (PTH). In fact, previous studies have found that serum concentrations of oestrogens, vitamin D and PTH[16], as well as calcium absorption levels, were lower in smokers than in non-smokers, despite contrasting results on calcium [17, 18] and PTH[17]. The inhibition of vitamin D-PTH axis, in particular, could have an overall negative effect on bone health since it may impair PTH-induced activation of vitamin D and the regulation of calcium and phosphate homeostasis[19]. As a consequence, many works have shown that smokers have a lower bone mineral density (BMD), steeper bone loss[15, 20–23] and a higher risk of femoral and vertebral fractures[14, 24] compared to non-smokers. One particular study on middle-aged to older adults found that current smokers, irrespective of smoking intensity, had a significantly higher risk of vertebral fractures[25]. More recently, a prospective study

confirmed that result in older women, and showed the beneficial effect of smoking cessation in decreasing vertebral fracture risk[26]. However, as the meta-analysis conducted by Kanis and colleagues suggests, only 23% of the smoking-related risk of hip fracture can be explained by low BMD[14]. Hence, a comprehensive evaluation on the implications of smoking on bone health is needed to explore its potential direct and indirect effects which ultimately lead to increase fracture risk.

In light of such considerations, the aim of our study was to investigate the effects of smoking, firstly, on phosphocalcic metabolism and secondly, on bone mass and on the risk of vertebral fractures in post-menopausal outpatient women. Our hypothesis was that current smoking habits may adversely affect phosphocalcic homeostasis by inhibiting the vitamin D-PTH axis, and, overall, have adverse effects on bone health and fracture risk.

METHODS

Study population

This observational cross-sectional - prospective study was conducted from the outpatient clinic for the Diagnosis and Prevention of Osteoporosis at the Geriatric Clinic of *[blinded for review]*. The data refers to the patients who accessed the outpatient clinic for the first assessment until November 2016 (for the flow-chart of the sample selection, please see Online Resource 1). From an initial sample of 2113 post-menopausal women, 693 were excluded from our analyses due to incomplete biochemical data, 28 because information was missing on reproductive age, and 325 because they reported being under treatment with bone-modifying drugs at the time of our examination (bisphosphonates, strontium ranelate, teriparatide or denosumab). The final sample of our cross-sectional analysis included 1067 patients. After a mean of two years, we prospectively evaluated BMD changes in a subsample of 357 women (treated or non-treated with osteoporosis treatment) who had complete data on

bone mineral density (BMD) even at the first follow-up visit. No differences in age (64.6±9.9 vs 65.2±9.9 years), body mass index (BMI), reproductive period duration, family history of osteoporosis, dietary calcium intake, smoking habits and prevalence of vertebral fractures at baseline were observed between the women of the subsample (n=357) and participants in the main analytical sample (n=1067).

The study complies with the ethical standards of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All participants were fully informed about the nature, purpose and procedures of the study, and gave their written informed consent.

Data collection

Patient characteristics. Clinical and pharmacological data were collected for each patient during the medical interview. The following information was assessed: age, dietary calcium intake (defined as scarce with a calcium intake <1000 mg/day, estimated on the basis of patients' reports of their dietary calcium intake[27]), reproductive history (number of years of fertility, the number of full-term pregnancies and non-term pregnancies, total months of breastfeeding), presence of diabetes, nephrolithiasis, chronic gastrointestinal or liver disease, connective tissue diseases, organ transplantation and cancer; prior fragility fracture; chronic drug use affecting bone metabolism (e.g. prolonged steroid therapy, defined as the use of > 5 mg/day of prednisolone or equivalent for a period that is greater or equal to 3 months; thyroid hormones; estroprogestinic drugs; aromatase inhibitors; immunosuppressants; anticonvulsants, calcium and vitamin D supplements). The body weight and height of all participants were measured, and BMI (kg/m^2) was calculated.

Smoking habits. Smoking habits were classified as never smokers, former smokers (for at least one year in the past, and with smoking cessation of more than one year) and current smokers. Data on the dose and duration of smoking habits were also collected for former and current smokers, and the number of pack-years was calculated using the following formula:

(number of cigarettes smoked per day*number of years smoked)/20. Smoking intensity was classified as < or ≥8 pack-years, considering as a cut-off the median pack-years of former and current smokers. Smoking duration was categorized as < or ≥20 years, 20 years being the median duration of the habit of former and current smokers in our sample.

Biochemical data. Serum calcium, phosphate, parathyroid hormone (PTH) and 25-hydroxyvitamin D (25OHD), and 24-hour urinary calcium and phosphate levels were assessed in all patients following standard quality-control procedures. Levels of 25OHD were measured by radioimmunoassay (RIA kit; DiaSorin), and intra- and inter-assay coefficients of variation were 8.1% and 10.2%, respectively. Measurements below 25OHD concentrations were categorized as severe deficiency (≤25.0 nmol/l), deficiency (25.1-50.0 nmol/l), insufficiency (50.1-75.0 nmol/l) and sufficiency (>75.0 nmol/l)[28]. Serum intact PTH was measured by two-site immunoradiometric assay, with intra-assay and inter-assay coefficients of variation around 3.0% and 5.5%, respectively. Serum calcium and phosphate were measured by standard colorimetric assays.

Radiographic examination. BMD, T-score of the lumbar spine (L1–L4) and total hip were measured with *dual-energy X-ray absorptiometry* (DXA) using standard methods. Osteoporosis was defined as a T-score ≤-2.5 in at least one of these two sites. The presence of vertebral fractures was investigated by reading *thoracic and lumbar spine X-ray pictures*, performed in anterior-posterior and lateral view within 12 months from the outpatient visit. The presence of a vertebral fracture was evaluated by measuring the anterior, middle and posterior heights of each vertebra with the aid of a caliper. It was defined as a reduction ≥20% in vertebral anterior, middle or posterior height, or as a loss in vertebral body height in relation to normal-looking adjacent vertebra according to the criteria proposed by Genant[29, 30]. The assessment of vertebral fractures took into account deformities linked to spinal curvatures with parallax distortion of vertebral borders, osteoarthritis, degenerative disk

disease or Schmorl's nodes. If available, sequential radiographs were compared to confirm the presence of incident vertebral fractures. The evaluation was performed by two trained medical practitioners who work in the field of geriatrics and osteoporosis, who through discussion reached a general consensus.

Data analysis

Participant characteristics were expressed as means ± standard deviations for quantitative-continuous variables normally distributed, and as frequencies for the categorical ones. Normal distributions for the continuous variables were tested using the Shapiro-Wilk test.

Baseline characteristics of patients on smoking habits were compared using ANOVA for continuous variables, and the Chi-square test for categorical ones. Serum PTH levels were compared among categories of 25OHD concentrations (<25, 25.1-50, 50.1-75, >75 nmol/l) through ANOVA. The linear association between serum 25OHD and PTH levels in never, former and current smokers was investigated by linear regression analysis and illustrated with regression curves. The strength of the association between these parameters was estimated by the slope of the regression curve, expressed as B coefficient and standard error (SE).

ANOVA was also used to compare the baseline lumbar and femoral BMD in patients categorized by smoking habits, smoking intensity and duration. Lumbar and femoral BMD changes over a 2-year follow-up period were compared by smoking habits using the general linear model (GLM), adjusted for potential confounders: age, family history of osteoporosis, scarce dietary calcium intake, breastfeeding duration, baseline BMD, BMI, serum 25OHD, and use of drugs acting on bone metabolism (antiresorptive or anabolic) during follow-up.

The association between smoking (which consider smoking habits, smoking intensity and duration) and the presence of baseline vertebral fractures was evaluated using binary logistic regression. Two models were fitted: the first was adjusted for age only, and the second for potential confounding factors that differed between women with different smoking habits, or

that could affect the association between smoking and fractures. The covariates included: reproductive age, BMI, chronic liver diseases, 25OHD levels, number of pregnancies, heavy alcohol consumption, use of aromatase inhibitors[31], use of estroprogestins[32], lumbar and total femoral BMD. The strength of the associations was expressed as odds ratios (ORs) and 95% confidence intervals (95%CIs). For all tests, statistical significance was assumed for a p-value <0.05. All analyses were performed using Statistical Package of Social Science version 21.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS

Our study population included 1067 women with a mean age of 65±10 years. The characteristics of the sample as a whole, stratified by smoking habits (76.1% never, 14.1% former, and 9.3% current smokers) are reported in Table 1.

As shown, current smokers were significantly younger than former and never smokers and had the lowest BMI values. They were also more likely to have had no pregnancies, earlier menopause and a shorter reproductive age compared with former and never smokers. Among the risk factors for osteoporosis, current smokers showed a higher rate of heavy alcohol consumption and of chronic liver disease. No significant differences between groups were observed concerning the prevalence of dietary calcium intake (33.8% in the sample as a whole), family history of osteoporosis, and other diseases or use of drugs affecting bone metabolism (for data not shown in Table 1, see Online Resource 2). The mean 25OHD levels in the sample as a whole were at the lower normal limit. Although no differences in the prevalence of vitamin D supplementation were observed between groups, the highest 25OHD concentrations were found among former smokers. Concerning the other biochemical

parameters, we found that only serum phosphate differed significantly among groups, showing a gradual increase from never to current smokers.

Table 2 shows PTH values in never, former and current smokers, categorized according to 25OHD levels. As reported, for each 25OHD category, current smokers had lower mean PTH than former and never smokers. Considering the differences in PTH values across 25OHD categories, we found that PTH decreased significantly with increasing 25OHD in former ($p=0.03$) and never smokers ($p<0.001$), while PTH levels did not vary according to 25OHD categories in current smokers. The linear regression curves between serum 25OHD and PTH concentrations in never, former and current smokers are illustrated in Figure 1. As shown, the inverse association between 25OHD and PTH was stronger for never smokers women (B=-0.20, SE=0.003, $p<0.001$), compared to former (B=-0.14, SE=0.01, $p=0.006$) and current smokers (B=-0.11, SE=0.004, $p=0.01$).

The prevalence of osteoporosis in our sample was 77.1% (n=823) (Table 1). No significant differences were observed in lumbar and femoral BMD, nor when comparing the never, former and current smokers, or participants by smoking intensity or duration (for details see Online Resource 3). However, when evaluating BMD changes over the 2-year follow-up period in the subsample of 357 patients (Table 3), and after adjusting for potential confounders, we found that femoral BMD markedly worsened among current smokers compared with never smokers ($p=0.04$).

Table 4 shows the results of a logistic regression analysis on the association between smoking habits and the presence of vertebral fractures at the first outpatient visit (n=293 patients with at least one vertebral fracture in the sample as a whole). After adjustment for potential confounders, the risk of having sustained vertebral fractures, compared to never smokers, increased by 74% (95%CI 1.07-2.83) for current smokers, and by 83% (95%CI 1.01-3.32) for current smokers with the highest smoking intensity (≥8 pack-years).

DISCUSSION

Our study confirms that smoking has negative effects on bone metabolism, bone mineral density and may increase the risk of vertebral fractures in women referred to an osteoporosis outpatient clinic. In particular, our findings highlight that compared with never smokers, current smokers had a weaker inverse association between serum vitamin D and PTH levels, a greater BMD loss over time at the femoral site, and a higher chance of sustaining vertebral fractures, particularly among those with higher intensity of smoking. From these findings possible underlying factors that may strengthen the association between smoking and poor bone health include risky health behaviors, reduced estrogen exposure, and vitamin D-PTH axis inhibition.

The study sample included a population at high fracture risk composed of post-menopausal women who, most likely, were selected through a first screening by general practitioners. This was confirmed by the low baseline BMD observed at both lumbar and femoral sites. Even if no significant differences were found between participants classified by smoking habits, smoking intensity or duration, in the subsample considered in the longitudinal evaluation two years later, only current smokers were found to have a decrease in femoral BMD. These results corroborate previous studies which found an association between cigarette smoking exposure and BMD reduction in multiple skeletal sites[15, 33], especially the femoral one[22].

With regard to vertebral fractures, the chance of reporting clinical or subclinical vertebral fractures at the first assessment increased by 74% in current compared to never smokers, but was similar between former and never smokers. Consistently with our findings, previous studies[34] suggest a reversibility of the detrimental effects of tobacco exposure on bone, and

support the hypothesis that quitting smoking may represent an osteoprotective event[16]. Moreover, in evaluating the association between smoking intensity and duration with vertebral fractures, current smokers with the highest smoking intensity had an 83% higher risk of having sustained vertebral fracture than never smokers at our first outpatient visit. Several mechanisms may explain the influence of smoking, especially high intensity, on BMD and fracture risk.

Firstly, smoking may indirectly affect bone health because smoking habits tend to coexist with other risky behaviors such as limited physical activity or imbalanced diet with insufficient calories and calcium intake, or excessive coffee and alcohol consumption. In keeping with this hypothesis, in our sample, women who were current smokers showed lower BMI values and reported a higher prevalence of heavy drinking compared with former and never smokers. These factors therefore expose them to increased bone resorption and fracture risk[15, 35].

A second indirect mechanism that may mediate the adverse effects of smoking on bone, concerns bone metabolism. As observed by previous studies[16, 18, 33, 36, 37], our current smokers showed lower mean PTH values compared with former and never smokers. Interestingly, such concentrations did not change significantly in response to different serum 25OHD levels and we found a weaker inverse association between 25OHD and PTH in current and former smokers compared with never smokers. To our knowledge, these are the first findings suggesting that smoking may inhibit vitamin D-PTH axis in post-menopausal women. Moreover, this effect can be particularly marked with advanced age since there seems to be a stronger association between serum PTH and 25-OHD levels in older people[38]. Such mechanism may play a role in reducing intestinal absorption of calcium[16–18] therefore limiting the usefulness of calcium supplementation in individuals who smoke. The impact of smoking on PTH seems reversible. In fact, a study shows that one year after smoking

cessation, serum PTH concentrations were similar to those of never smokers[16]. Despite the apparent suppression of vitamin D-PTH axis, our sample showed no differences in serum and urinary calcium by smoking habits. There are contrasting results on this issue, as some authors observed reduced serum calcium values in smokers compared to non-smokers[15, 17], while others report no significant differences[17, 33]. Conversely, the higher serum phosphate levels observed in smokers compared to the never smokers in our sample may confirm the effect of smoking on PTH suppression[18, 20, 39].

A third mechanism that may mediate the effect of smoking on bone health and on the vitamin D-PTH axis inhibition concerns estrogens. A previous study, in particular, found reduced plasma levels of estradiol and estrone and lower concentrations of their urinary metabolites in smokers compared with never smokers[40]. Estrogens play a key role in reducing monocyte-to-osteoclast differentiation and in modulating osteoclast recruitment, resorption activity and apoptosis[41]. By limiting the exposure of bone tissue to estrogens, cigarette smoking could thus increase bone demineralization processes and the risk of fractures[42]. Although we did not measure estrogen concentrations, an earlier menopause and shorter fertility period observed in our current smokers compared with never smokers may corroborate the potential role of smoking in reducing estrogen exposure on bone. However, further investigation is needed to clarify this point.

The association between current smoking habits and the presence of vertebral fractures in our sample was significant, even after body mass index adjustments for alcohol consumption, fertile age, and 25OHD levels. This finding supports the hypothesis of a direct toxic effect of smoking on bone tissue. Smoking has adverse effects on bone, especially higher smoking intensity. It tends to cause an alteration of collagen fibers which can lead to subverted bone architecture and enhanced recruitment and proliferation of osteoclasts[34]. These factors may

be responsible for progressive demineralization and bone frailty which lead to an increased risk of fracture.

It is worth noting that this study has some limitations. First of all, our sample is representative of a population at high risk and prevalence of osteoporosis, since the women who accessed our outpatient clinic had probably been selected by their family doctor or other specialist, or after a densitometric examination, for a specialist visit. Secondly, missing data on nutrition and on physical activity levels represents a further limitation to our study since it hindered a complete evaluation of unhealthy behaviours which, in addition to smoking, may affect bone health and increase fracture risk (e.g. low calcium, vitamin D, protein and energy intake; and physical inactivity). Thirdly, since only a subsample of patients at the follow-up visit provided complete data, prospective evaluation of the detrimental effects of smoking on BMD could therefore be limited by the small sample at a short follow-up. On the contrary, the strengths of our study include the availability of information on smoking habits, fertility period, biochemical data on bone metabolism, and densitometric and radiological scans to assess BMD and the presence of clinical or subclinical vertebral fractures. Moreover, the large sample in a real outpatient setting enhances the potential value of our study and its implications in clinical practice.

CONCLUSIONS

In conclusion, our study confirms that smoking has negative health effects on bone mineral density in post-menopausal women. The mechanisms involved can include the inhibition of vitamin D-PTH axis, changes of calcium and phosphate metabolism and of estrogenic activity. In current smokers, the risk of sustaining vertebral fractures seems to be influenced more by the intensity than by the duration of smoke exposure. Conversely, previous smoking habits do not seem to substantially influence bone mass and fracture risk, suggesting a

possible partial reversibility of the detrimental effects of smoking on bone after quitting. Overall, our findings support interventions aimed at smoking cessation and those aimed at detecting vitamin D deficiency in post-menopausal age.

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Conflict of Interest: Caterina Trevisan, Agnese Alessi, Gaia Girotti, Bruno Micael Zanforlini, Anna Bertocco, Mattia Mazzochin, Francesca Zoccarato, Francesca Piovesan, Marta Dianin, Sandro Giannini, Enzo Manzato, and Giuseppe Sergi declare that they have no conflict of interest.

Contributors: CT, AA, GG and GS designed the study, CT, AA, and GG prepared the first draft of the paper. CT is the guarantor who performed the statistical analyses of the data. CT, AA, GG, BMZ, AB, FZ, MM, FP, MD and GS contributed to data collection and quality control. All authors revised the paper critically for intellectual content and approved the final version. All authors agree to be accountable for all aspects of the work and ensure that any questions related to the accuracy or integrity of the paper are properly investigated and resolved.

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TABLES

Table 1. Baseline characteristics of the sample as a whole and by smoking habits

Baseline characteristics	All (n=1067)	Smoking habits		
		Never (n=812)	Former (n=156)	Current (n=99)
Age (years)	65±10	66±10	65±10	61±10***
Body mass index (kg/m^2)	24.5±4.2	24.7±4.3	24.5±3.9	23.1±3.4**
Years of smoking	_	_	17±15	26±18
Cigarettes/day (number)	_	_	9±8	8±7
Pack-year	_	_	10.2±12.7	14.3±15.0
Menarche age (years)	13±2	13±2	13±2	13±1
Menopause age (years)	49±5	50±5	49±6	48±5*
Fertile age (years)	36±5	36±5	36±6	35±5*
Breastfeeding >18 months, n (%)	150 (14.1)	126 (15.5)	15 (9.6)	9 (9.1)
Pregnancies ≥1, n (%)	734 (68.8)	569 (70.1)	108 (69.2)	57 (57.6)*
Scarce calcium dietary intake	361 (33.8)	266 (32.8)	52 (33.3)	43 (43.4)
Chronic liver diseases	18 (1.7)	11 (1.4)	1 (0.6)	6 (6.1)**
Heavy alcohol consumption (>7 UA/day)	4 (0.4)	1 (0.1)	0 (0.0)	3 (3.0)***
Previous use of bisphosphonate, n (%)	223 (20.9)	162 (20.0)	34 (21.8)	27 (27.3)
Previous use of estroprogestins, n (%)	171 (16.0)	119 (14.7)	32 (20.5)	20 (20.2)
Current/previous use of aromatase inhibitors, n (%)	91 (8.5)	75 (9.2)	10 (6.4)	6 (6.1)

Use of vitamin D supplements, n (%)	399 (37.4)	310 (38.2)	55 (35.3)	34 (34.3)
Use of calcium supplements, n (%)	153 (14.3)	118 (14.5)	20 (12.8)	15 (15.2)
25OHD (nmol/l)	74.6±47.6	72.5±45.3	86.6±49.7	72.9±58.8**
PTH (pmol/l)	5.9±3.6	6.0±3.7	5.7±3.2	5.4±2.6
Serum creatinine (μmol/l)	68.8±14.4	68.7±15.3	70.6±11.6	67.0±10.6
Serum calcium (mmol/l)	2.4±0.1	2.4±0.1	2.4±0.1	2.4±0.1
Serum phosphate (mmol/l)	1.11±0.16	1.10±0.15	1.13±0.16	1.14±0.16*
24-hour urinary calcium (mmol)	4.5±2.9	4.5±2.9	4.4±3.0	4.8±2.8
24-hour urinary phosphate (mmol)	19.8±9.0	19.6±8.6	20.3±10.5	20.3±9.4
Osteoporosis, n (%)	823 (77.1)	630 (77.6)	117 (75.0)	76 (76.8)
Lumbar T-score (SD)	-2.6±1.0	-2.6±1.0	-2.5±1.2	-2.7±1.0
Lumbar BMD (g/cm^2)	0.77±0.11	0.77±0.11	0.78±0.13	0.76±0.11
Total hip T-score (SD)	-1.7±0.9	-1.7±0.9	-1.7±0.8	-1.8±0.9
Total hip BMD (SD)	0.74±0.10	0.74±0.10	0.74±0.10	0.73±0.11

Notes: numbers are means ± standard deviations, or n (percentages), as appropriate. *Abbreviations:* PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; BMD, bone mineral density. *p<0.05, **p<0.01, ***p<0.001 for the between-groups comparison performed with ANOVA or Chi-square test, as appropriate.

Table 2. Serum PTH levels in patients categorized by 25-hydroxyvitamin D concentrations and by smoking habits

25(OH)D	Never smokers		Former smokers		Current smokers	
(nmol/l)	**Mean±SD**	***p-value***	**Mean±SD**	***p-value***	**Mean±SD**	***p-value***
≤25.0	8.4±5.4		7.6±4.1		5.7±2.2	
25.1-50.0	6.8±3.9	<0.001	6.3±2.8	0.03	5.9±2.9	0.06
50.1-75.0	5.6±3.3		6.0±4.3		5.9±2.8	
>75.0	4.9±2.6		5.1±2.5		4.4±2.2	

Abbreviations: SD, standard deviation; 25(OH)D, 25-hydroxyvitamin D.

Table 3. Bone mineral density changes over the 2-year follow-up at lumbar and femoral site

		Smoking habits		
Changes from baseline to 2-year follow-up	**All (n=357)**	**Never smokers (n=264)**	**Former smokers (n=57)**	**Current smokers (n=36)**
Lumbar T-score	0.18±0.51	0.19±0.50	0.08±0.50	0.22±0.57
Lumbar BMD	0.02±0.06	0.02±0.06	0.01±0.06	0.03±0.06
Total hip T-score	0.13±0.49	0.15±0.51	0.09±0.42	-0.01±0.44*
Total hip BMD	0.01±0.06	0.02±0.06	0.01±0.05	-0.004±0.06*

Numbers are means ± standard deviations. GLM model adjusted for: age, osteoporosis family history, scarce dietary calcium intake, fertile age, body mass index (< vs ≥25 kg/m^2), serum 25-hydroxyvitamin D, baseline lumbar/total femoral BMD, use of drugs for the treatment of osteoporosis during the follow-up. *Abbreviations:* BMD, bone mineral density. *p<0.05, for the difference between never and current smokers.

Table 4. Logistic regression analysis of the association between smoking habits and the presence of vertebral fractures at the first outpatient visit

	≥1 vertebral fractures	Odds Ratio (95% Confidence Intervals)	
		Model 1	Model 2
Smoking habits			
Never smokers	219 (27.0%)	[ref]	[ref]
Former smokers	43 (27.6%)	1.08 (0.73-1.61)	0.99 (0.66-1.50)
Current smokers	31 (31.3%)	1.67 (1.04-2.69)*	1.74 (1.07-2.83)*
Smoking intensity			
Never smokers	219 (27.0%)	[ref]	[ref]
<8 pack-years (in former or current smokers)	35 (28.0%)	1.24 (0.80-1.91)	1.18 (0.76-1.85)
≥8 pack-years (in former smokers)	19 (27.5%)	1.06 (0.60-1.89)	0.93 (0.52-1.67)
≥8 pack-years (in current smokers)	20 (32.8%)	1.67 (0.94-2.99)	1.83 (1.01-3.32)*
Smoking duration			
Never smokers	219 (27.0%)	[ref]	[ref]
<20 years	27 (25.2%)	1.14 (0.71-1.85)	1.06 (0.63-1.73)
≥20 years	47 (31.8%)	1.37 (0.93-2.04)	1.35 (0.90-2.03)

Model 1: is adjusted for age. Model 2 is adjusted also for fertile age, body mass index, chronic liver diseases, serum 25-hydroxyvitamin D, number of pregnancies, heavy alcohol consumption, scarce dietary calcium intake, use of aromatase inhibitors, use of estroprogestinic drugs, lumbar bone mineral density. *p<0.05.

FIGURE LEGEND

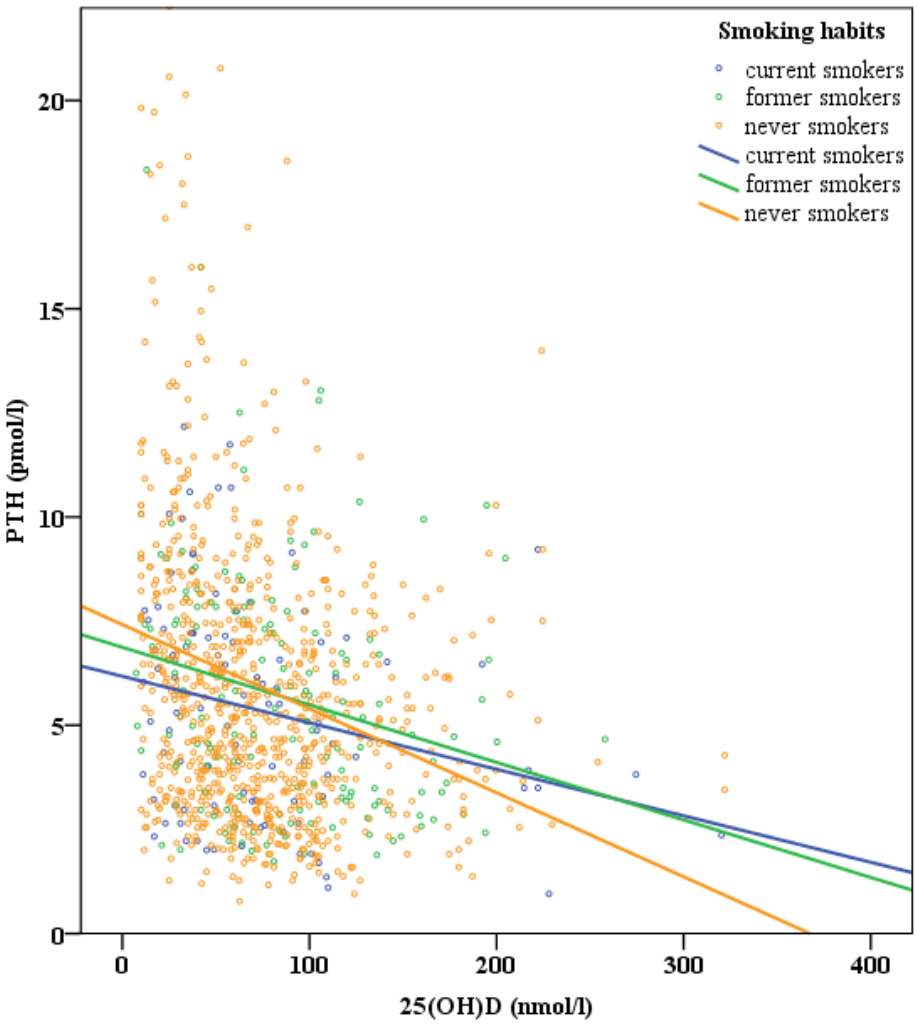

Fig. 1 Linear regression curves on the association between serum 25-hydroxyvitamin D and parathyroid hormone levels in the sample as a whole and by smoking habits

Abbreviations: PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.