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Effect of smoking and smoking cessation on bone mass, bone remodeling, vitamin D, PTH and sex hormones

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Abstract

Objective: To assess the effect of smoking and smoking cessation on bone density, bone remodeling markers, sex hormones, and vitamin D-PTH axis in healthy young subjects. *Materials and methods:* We studied 74 healthy people (31 men, 43 women; mean age 32.2 (7) years) divided into 52 never smokers and 22 smokers, 15 of which stopped smoking for one month. *Results:* Male smokers compared with never smokers showed lower BMD (0.971 (0.11) g/cm^2 vs. 1.069 (0.09) g/cm^2, P=0.042); higher plasma estrone levels (32.37 (10.13) pg/mL vs. 20.91 (5.46) pg/mL, P=0.001); and lower serum iPTH levels (16.2 (3.5) pg/mL vs. 28.8 (2.0) pg/mL, P=0.008). In women, BMD values were similar in smokers than in never smokers, but 25-hydroxyvitamin D levels were lower in smokers (31.9 (15.1) ng/mL vs. 16.8 (9.9) ng/mL, P=0.002). After adjusting by age and coffee consumption, female smokers had higher urinary-NTX levels than never smokers. After smoking cessation, statistically significant decreases of 25-hydroxyvitamin D and SHBG plasma levels were observed in men and women, respectively. *Conclusions:* Tobacco increases bone resorption and affects bone mass by some alterations in sex hormone metabolism, but also importantly by alterations on the vitamin D-PTH axis.

Keywords: Smoking, Bone Mass, Bone Remodeling, Vitamin D, Sex Hormones

Introduction

Smoking is an important determinant of osteoporosis[1,2]. There are a wide variety of mechanisms by which smoking induces bone toxic effects. A decrease in intestinal calcium absorption[3-6], low body weight[3,7,8] and earlier menopausal age[9-13] have been described. There is also a direct toxic effect on bone[14] and alterations in blood supply of the femoral head[15]. However, the most prominent mechanism seems to be their action on estrogen metabolism. In women, there is a decrease in estrogen urinary excretion[16], accelerated metabolic degradation of exogenous estrogens[7], increased 2-hydroxylation of estradiol to 2-hydroxyestradiol, a less active metabolite[17] and increased hepatic cytochrome P450 activity[18]. In men, the sex hormones derangement induced by tobacco smoking is more complex. Enzymatic inhibition of the androgen metabolism with subsequent decrease in local estrogen levels on bone has been suggested[19-22]. In addition, smoking has been associated with vitamin D metabolism derangement[23,24]. However, most studies of the relationship between smoking and osteoporosis have been carried out in postmenopausal women or elderly men after a long-time exposure to the toxic agent, and there is little information regarding the effect of smoking on bone in young people.

Therefore, the objective of this study was to assess the effects of smoking on bone mass and bone turnover in a group of healthy young volunteers, that is, at an early stage of the exposure to the toxic agent, as well as on the gonadal and vitamin D hormone systems. The effect of smoking cessation on these variables was also examined.

Methods

A cross-sectional study followed by a longitudinal analysis was conducted in non-consecutive healthy volunteers. Participants, both current smokers and never smokers, were

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	Smokers n = 22	Non-smokers n = 52	*P*
Age	**35.7 (8.6)**	**30.8 (5.7)**	**0.021**
Weight	67.0 (12.0)	65.6 (11.9)	Ns
Height	1.6 (0.7)	1.7 (0.8)	Ns
BMI	24.5 (3.7)	23.2 (3.1)	Ns
Calcium intake	1091.7 (558.6)	1019.2 (39.8)	Ns
Alcohol intake	3.3 (5.8)	1.7 (5.5)	Ns
Coffee intake	**3.8 (2.8)**	**2.0 (1.2)**	**0.001**
Physical exercise	7/22	21/52	Ns
Solar exposure	10/22	23/52	Ns

BMI: Body mass index. Values of age, weight, height, BMI, calcium intake, alcohol intake and coffee intake were expressed in mean (SD), referred to 75 percentile in the last two. Physical exercise and solar exposure were expressed in percentage. Age (years); Weight (Kg); Height (metres); BMI (Kg/m^2); Calcium intake (mg/day); Alcohol intake (g/day): Coffee intake (coffee cups/day). Physical exercise was considered positive when the volunteer made sport or walking during one hour or more daily. Solar exposure was defined when the volunteer made an active exposure. Differences between age, weight, height, BMI, and calcium intake were analyzed by Student's t test. Alcohol intake and coffee intake by U-Mann Whitney test. Physical exercise and solar exposure by chi square test.

Table 1. Baseline characteristics of active smokers and controls.

recruited by announcement charts or electronic mail from the health care employees of our hospital. The duration of the recruitment period was two years. Inclusion criteria for smokers were daily consumption of at least 15 cigarettes, less than 45 years of age in men, and regular menstrual cycles in women. The cumulative exposure to smoking was of 15.64 (7.8) packages*year. Never smokers were matched by age and sex. Exclusion criteria were as follows: alcohol consumption greater than 60 g of ethanol per day, and evidence of any disease that may affect bone and mineral metabolism, particularly chronic renal failure, chronic liver disease, malabsorption, and endocrine disorders.

The study was approved by the Institutional Review Board and all participants gave written informed consent.

At the beginning of the study, all smokers were requested to stop smoking for one month, without the use of any drug or nicotine-containing substances. Those who agreed to quit were interviewed by the one of the investigators to assess abstinence status. A simple questionnaire was used to collect anthropometric data, nutritional habits, including calcium intake[25], coffee and alcohol use, physical activity, and sun exposure, which was assessed on a semi-quantitative scale.

Bone mineral density (BMD) at the lumbar spine, femoral neck, and total femur was measured by dual-energy X-ray absorptiometry using a Hologic 4500SL® bone densitometer (Hologic, Waltham, MA) and results were expressed in g/cm^2. The co-efficient of variation is 1.0% for the lumbar spine and 1.65% for total femur.

Fasting blood samples and 2-hour urine samples (second void) after at least 7 hours of night bed rest were collected. In female volunteers, analysis was performed between the 3rd and 7th day of the last menstrual period to minimize cycle variability. The analysis was carried out at baseline in both groups. In the group that stopped smoking, the second sampling was obtained after one month of abstinence with a mean of 33 (2.4) days in men and 3 to 7 days after the next menstrual period with a mean of 28.6 (9.1) days, in women. Blood samples were frozen and preserved at –80ºC until the end of the study when the hormonal analysis was made for the whole study population. Similarly, urine samples were also frozen at –80ºC for the NTx measurements at the end of the study.

Plasma calcium, phosphorus, and magnesium levels were measured by an autoanalyzer (Olympus AU 510, Merck, Madrid, Spain). Serum calcium and magnesium concentrations were determined by complexometric assay and serum phosphorus by direct colorimetric assay. Plasma hormone assays by radioimmunoassay for testosterone (Diagnostic Products Corporation-DIPESA, Madrid, Spain), estrone, androstendione, dihydrotestosterone (Diagnostic Systems Laboratories INC, Nuclear Iberica, Madrid, Spain), 25-hydroxyvitamin D_3 (25 D), 1,25-dihydroxyvitamin D_3 (1,25 D), and osteocalcin (INCSTAR, DiaSORIN, Madrid, Spain). Chemiluminescent immunoassay were used to measure intact parathyroid hormone (iPTH), dehydroepiandrosterone (DHEAS), and serum hormone binding globulin (SHBG) (INMULITE-ONE, DPC-DIPESA, Madrid, Spain), 17-β estradiol, follicle stimulating hormone (FSH), and luteinizing hormone (LH) (Centaur® assays, Bayer Diagnostics, Barcelona, Spain). Free testosterone index and free estrogens index were calculated as: free testosterone index = testosterone/SHBG, and free estrogens index = (17-β estradiol + estrone)/SHBG. Urinary N-telopeptide (NTx) concentration was measured by enzyme-linked immunosorbent assay (ELISA) (OSTEOMARK®, Ostex International, Seattle, USA). Tartrate-resistant acid phosphatase (TRAP) levels were measured by ELISA (Merck, SA, Madrid, Spain). Interassay reproducibility was 8.6% for iPTH, 15.6% for 25 D, 6.9% for 1,25 D, 3.9% for TRAP and 11.3% for osteocalcin.

Analyses were performed with SPSS software version 10.0 for Windows. A two-sided *P* value of less than 0.05 was considered to indicate statistical significance. Data are expressed as mean (SD) or median (interquartile [25th-75th] range). Comparisons between groups were made using the Student's t-test or the Mann-Whitney *U* test. For categorical data the chi-square (χ^2) test or the Fisher's exact test were used. Analyses pre- and post- abstinence were tested with the use of analysis of variance (ANOVA) for repeated measures.

		Spine	Total femur	Femoral neck
Men	Smokers (n = 9)	0.971 (0.11)*	0.977 (0.12)	0.818 (0.12)
	Non-smokers (n = 22)	1.069 (0.09)*	1.034 (0.12)	0.894 (0.12)
Women	Smokers (n = 13)	0.990 (0.08)	0.936 (0.08)	0.830 (0.07)
	Non-smokers (n = 30)	1.036 (0.09)	0.943 (0.10)	0.842 (0.11)

Values expressed in mean (SD). BMD (g/cm^2).
*$p = 0.042$. Student t-test, unadjusted.

Table 2. BMD. Differences between smokers and non-smokers.

	Men		Women	
	Smokers n = 9 (7 in NTX)	Non-smokers n = 22 (14 in NTX)	Smokers n = 13 (8 in NTX)	Non-smokers n = 30 (19 in NTX)
Calcium	9.2 (0.6)	9.3 (0.4)	9.2 (0.5)	9.2 (0.4)
Phosphorus	3.7 (0.4)	3.7 (0.6)	3.7 (0.6)	3.7 (0.5)
Magnesium	1.5 (0.6)	1.7 (0.7)	2.0 (1.5)	2.2 (1.3)
Osteocalcin	2.4 (0.6)	2.2 (0.9)	2.1 (0.8)	1.9 (1.0)
TRAP	8.8 (2.8)	8.06 (1.8)	7.68 (2.7)	7.1 (1.7)
Urinary-NTX	9.1 (2.4)	10.1 (1.6)	**12.1 (1.2)***	**7.3 (0.7)***

TRAP: tartrate-resistant acid phosphatase. Urinary-NTX: Urinary N-telopeptide. Values expressed in mean (SD). Calcium, phosphorus, and magnesium: mg/dl. Osteocalcin: ng/ml; TRAP: UI/l; Urinary-NTx: nM BCE*/mM creatinine. *BCE: Bone Collagen Equivalents. Normal values: Calcium: 8.5-10.5 mg/dl; Phosphorus: 2.5-4.8 mg/dl; Magnesium: 1.8-2.5 mg/dl; Osteocalcin: 0.7-6.9 ng/ml; TRAP: 5-8.6 UI/l; Urinary-NTX: men (3-51 nM BCE/mM creatinine), premenopausal women (5-65 nM BCE/mM creatinine).
*$p = 0.004$. ANOVA test.

Table 3. Biochemical and bone remodeling markers in smoker and non-smoker volunteers after adjusting by age and coffee consumption.

Results

A total of 78 agreed to take part in the study but 4 subjects (2 men, 2 women) were excluded because of thyroid disorders in 2 and current treatment with carbamazepine in 2. The study population included 74 subjects, with a mean age of 32.2 (7). Twenty-two people were current smokers (9 men, 13 women) and 52 never smokers (22 men, 30 women) (controls). Fifteen people (7 men, 8 women) stopped smoking for one month. In a sub-group of these volunteers, urine NTx was determined. This was formed by all 15 smokers that stopped smoking, and 33 never smokers (14 men, 19 women), randomly selected from the control group.

Baseline data of the study groups are shown in Table 1. Smokers were older than never smokers and consumed a greater daily amount of coffee. The same differences were observed in the sub-set of subjects undergoing urine NTx testing. Therefore, results were adjusted by age and coffee consumption.

Decreased lumbar BMD was detected in male smokers compared with controls (0.971 (0.11) g/cm^2 vs. 1.069 (0.09) g/cm^2, $P=0.042$) but this difference was no longer significant when adjusting by age and coffee consumption (Table 2). In women, no differences were found.

No differences were found in serum calcium, phosphorus, and magnesium concentrations between smokers and controls. A tendency to a decrease in serum calcium concentrations was shown after one month of smoking abstinence, but differences were not statistically significant. No differences were found in serum levels of osteocalcin, tartrate-resistant acid phosphatase, and urine NTx between smokers and controls. However, after adjusting by age and coffee consumption, female smokers had higher urine NTx than never smokers (12.1 (1.2) nM BCE/mM creatinine vs. 7.3 (0.7) nM BCE/mM creatinine, $P=0.004$) (Table 3). In the second phase of the study no differences were found. All these results are shown in Table 3.

As shown in Table 4, male smokers compared with never smokers had higher plasma estrone levels (32.37 (10.13) pg/mL vs. 20.91 (5.46) pg/mL, $P=0.001$); plasma free testosterone index (0.76 (0.09) vs. 0.48 (0.05), $P=0.019$); and free estrogens index (2.97 (0.59) vs. 1.18 (0.35), $P=0.022$).

	Men		Women	
	Smokers (n = 9)	Non-smokers (n = 22)	Smokers (n = 13)	Non-smokers (n = 30)
FSH	5.13 (4.02)	4.15 (1.87)	6.88 (1.84)	7.81 (3.92)
LH	5.26 (1.48)	4.58 (2.06)	7.09 (3.14)	6.36 (3.56)
17-β	30.67 (19.95)	24.11 (12.39)	63.08 (28.15)	47.87 (30.30)
Estrone	**32.37 (10.13)***	**20.91 (5.46)***	30.31 (9.39)	27.80 (12.16)
Free EI	**2.97 (0.59)****	**1.18 (0.35)****	1.44 (0.26)	1.29 (0.16)
T	19.80 (8.27)	17.11 (4.66)	1.12 (0.56)	1.34 (2.87)
Free TI	**0.76 (0.09)*****	**0.48 (0.05)*****	0.02 (0.03)	0.04 (0.02)
DHT	0.78 (0.85)	0.57 (0.36)	0.29 (0.12)	0.23 (0.12)
D4AND	1.68 (0.98)	2.01 (0.74)	2.29 (0.94)	2.09 (0.74)
SHBG	32.02 (18.57)	38.02 (13.82)	71.55 (22.97)	75.72 (44.67)
DHEAS	260.50 (115.84)	256.04 (103.82)	166.00 (79.16)	186.63 (81.36)
IPTH	**16.2 (3.5)******	**28.8 (2.0)******	20.6 (2.9)	22.6 (1.7)
25 D	26.8 (13.4)	27.5 (13.8)	**16.77 (9.9)*******	**31.94 (15.1)*******
1,25 D	40.7 (14.00)	37.1 (11.5)	33.0 (9.0)	39.6 (13.8)

FSH: follicle stimulating hormone. LH: luteinizing hormone. 17-β: 17-betaestradiol. Free EI: free estrogens index. T: testosterone. Free TI: free testosterone index. DHT: dihydrotestosterone. D4AND: androstendione. SHBG: serum hormone binding glogulin. DHEAS: dihydroepiandrostendione sulfate. Values expressed in mean (SD). FSH, and LH (mUI/ml); 17-β, estrone, and T (pg/ml); DHT, and D4AND (ng/ml); SHBG (nmol/l); DHEAS (mcg/dl). iPTH: intact parathyroid hormone. 25 D: 25 hydroxyvitamin D. 1,25 D: 1,25 dihydroxyvitamin D. iPTH and 1,25 D (pg/ml); 25 D (ng/ml).
*$p = 0.001$. **$p = 0.019$. ***$p = 0.022$. ****$p = 0.008$. *****$p = 0.002$. ANOVA test.

Normal values: FSH: men (1-16 mUI/ml), women in follicular fase (1.1-7.6 mUI/ml); LH; men (2-18 mUI/ml), women in follicular fase (0.8-9.8 mUI/ml); 17-β: men (0-44 pg/ml), women in follicular fase (<12-266 pg/ml); Estrone: men (30-90 pg/ml), women in follicular fase (30-130 pg/ml); Testosterone: men (9-41 pg/ml), women (0.2-3.2 pg/ml); DHT: men (0.5-2.1 ng/ml), women (0.2-0.5 ng/ml); D4AND: men (0.5-2.1 ng/ml), women (0.1-2.99 ng/ml); SHBG; men (10-70 nmol/l), women (18-114 nmol/l); DHEAS: men (80-560 mcg/dl), women (35-430 mcg/dl). iPTH: 9-51 pg/ml; 25 D: 18-54 ng/ml; 1,25 D: 8-80 pg/ml.

Table 4. Hormonal baseline values in smoker and non-smoker volunteers adjusting by age and coffee consumption.

In women, smoking cessation was associated with a decrease in SHBG only (67.44 (21.36) nmol/L vs. 56.35 (17.02) nmol/L, $P=0.036$) whereas no changes were detected in men in gonadal hormones.

Smoker males had lower serum iPTH than controls (16.2 (3.5) pg/mL vs. 28.8 (2.0) pg/ml, $P=0.008$). A small non-significant decrease in iPTH was observed in women. No changes associated with smoking cessation were observed in women for vitamin D or iPTH. There were also lower 25 D serum levels in smoker women (16.8 (9.9) ng/ml vs. 31.9 (15.1) ng/ml; $p=0.002$) as compared with non-smokers (Table 4 and Figure 1a). No differences in 1,25 D serum levels were found. After smoking cessation the 25 D serum levels decreased significantly in men (29.5 (14.2) ng/mL vs. 22.5 (12.6) ng/mL, $P=0.037$) (Figure 1b).

Discussion

Our results suggest the deleterious effect of smoking in bone mass even in young smokers. Bone loss at the lumbar spine has already been described[24,26-32] in a dose-dependent manner[3,8,26,30], and when smoking ceases bone loss velocity decreases[26]. However, few studies in young people have been carried out[28,31,32]. In a recent study[33], young smoker women showed a lower BMD than non-smokers, although oral contraception use attenuated the negative effect of smoking. In a Caucasian population tobacco was not associated with any effect on BMD[34]. We found a trend towards decreased BMD values in all areas in smokers of both genders. Although after adjusting by age and coffee consumption, the statistical significance was only marginal in men, we believe that the limited number of individuals limits our power and that the overall findings confirm the negative effect of smoking on bone mass.

In contrast to other studies[3,30], we did not find any differences in total serum calcium between smokers and non-smokers. Smoking abstinence for one month did not produce any change in serum levels of calcium, phosphorus or magnesium. These results reflect the relative insensitivity of these parameters to the changes in tobacco exposure, very likely because of their efficient regulating mechanisms. However, other studies have shown a wide range of serum calcium lev-

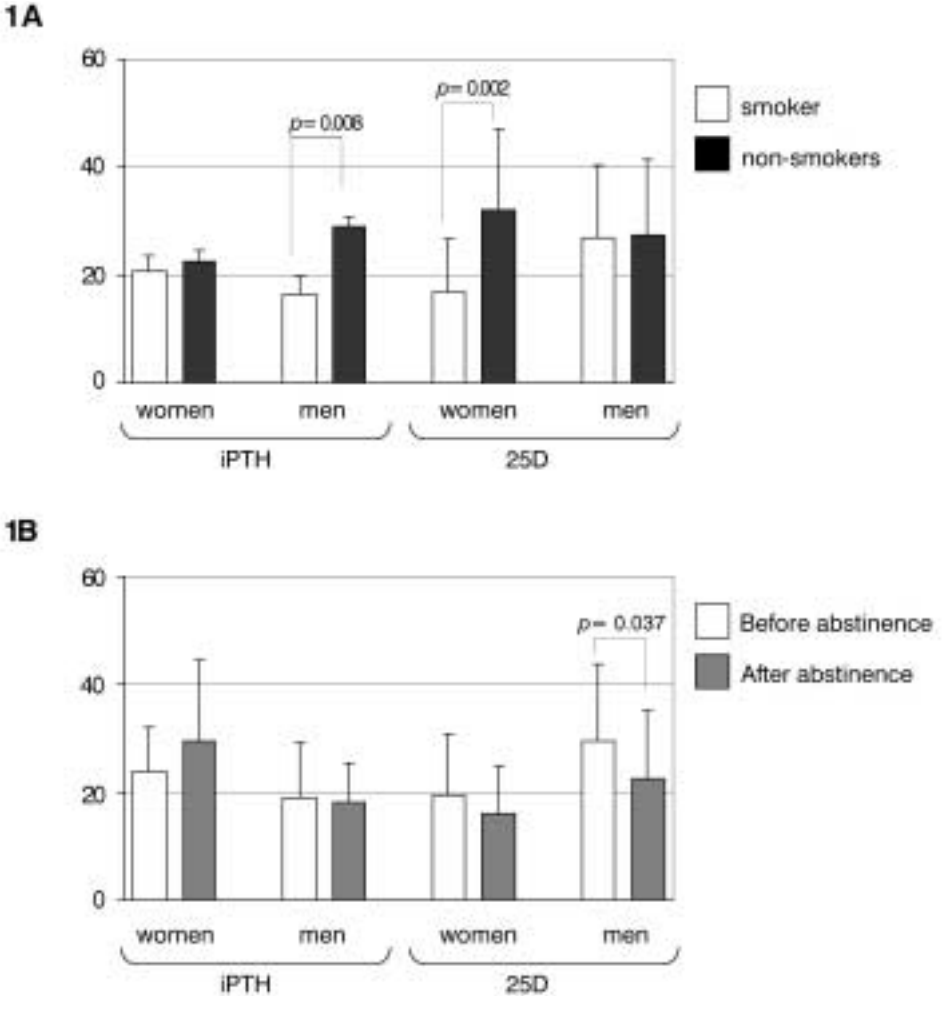

Figure 1A. iPTH-Vitamin D levels. Differences between smoker and non-smoker. Smoker men had significantly lower serum levels of iPTH (ANOVA test). All results were adjusted by age and coffee consumption. **1B.** iPTH-Vitamin D levels. Values in smoker volunteers at baseline and after one month of abstinence (ANOVA test). All results were adjusted by age and coffee consumption.

els in smokers[3,23,30], again underlying the limited the ability to detect changes in a relatively small number of cases.

Similar variability has been found in bone turnover markers related with smoking. Many authors described increased[35], decreased[23,24,36] or normal levels[30-32] as we found in bone formation markers. After adjustment, smoking women showed significantly higher urine values of NTx than non-smokers as previously described[37]. In men, however, marker levels were within normal range as also described in younger populations[31,32]. Oncken et al.[38] also reported increased urine NTx levels in postmenopausal women that decreased after smoking cessation. However, in our study we did not find such a decline after stopping tobacco consumption possibly because the abstinence period before the second measurement was shorter than on the study of Oncken et al.[38]. The increased bone resorption in young women during active smoking may be related with the negative effects of smoking on local estrogen metabolism in bone[19-22].

Sex hormones seem to be influenced by smoking according with previous studies[31,36,39-42]. In agreement with some authors we found that male smokers had higher levels of estrone[39] as well as higher levels of free testosterone index and free estrogens index. A possible explanation for the increased estrone levels proposed by Szulc et al.[39], would be that high levels of androgens might increase estrogens conversion in fat men because of the increased aromatization produced in adipose tissue. In our study smokers had a higher BMI than non-smokers and consequently this might be a contributing factor to our finding although our data do not directly support this hypothesis. On the other hand, female smokers showed no significant changes in sex hormones at baseline. There are conflicting published results showing either increased, normal or decreased levels of estrogens in smoking women[32,43-45]. Although in peri- and post-

menopausal women, smoking increases gonadotrophin levels[30], in younger premenopausal women, a more similar population to our group, no changes have been found[44]. According to SHBG levels, we found a significant decrease in their levels in our series after stopping smoking. This is in agreement with increased SHBG levels described in premenopausal smokers[32] and decreased SHBG after smoking cessation in postmenopausal women[38].

In our study, smoker women showed decreased 25 D levels at baseline. Similar results have been previously described in perimenopausal women[23,24] and men over the age of 50 years[39] possibly reflecting that smoking induces an increased activity of liver enzymes[24]. After smoking cessation, 25 D values decreased in men. These findings may indicate that smoking influences the vitamin D system and this can be a relevant contributing factor to their bone toxicity than previously considered. In association with the decreased 25 D levels, an increase in iPTH levels could be expected. However, iPTH levels were decreased in our volunteers, reaching significance in men. A similar picture of simultaneous decreased levels of both vitamin D and iPTH has been described[6,23]. Other authors also reported decreased[30,46] or normal[31,32] iPTH values. There is no clear explanation for the observed decrease in iPTH levels by these others authors and our own group. Slight differences in the plasma calcium concentrations together with a negative effect of tobacco on estrogen metabolism have been suggested[30]. Need et al.[6], speculated that PTH suppression may be produced by a small unmeasurable change in serum ionized calcium, and smoking would be the reason for this decrease.

The present results, however, should be interpreted according to the following limitations: firstly, the relatively small sample size, mainly caused by the difficulties in finding smokers volunteering to participate and especially to quit smoking; and secondly, because one-month abstinence period may be too short for detecting changes after smoking cessation. Although shorter periods of time, even a few days, have been useful for detecting significant changes in other circumstances[47], reversibility of the tobacco effects on bone physiology can be more subtle and require a longer period of observation.

Conclusions

Smoking seems to be associated with a decrease in lumbar BMD and an increased NTx biochemical marker. The gonadal axis changes were scarce and may not account for these changes. However, the effects of smoking on vitamin D metabolism are probably more relevant and need to be investigated in further studies.

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