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The influence of smoking on vitamin D status and calcium metabolism

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Objective: To assess the influence of smoking on serum parathyroid hormone (PTH), serum vitamin D metabolites, serum ionized calcium, serum phosphate, and biochemical markers of bone turnover in a cohort of 510 healthy Danish perimenopausal women.
Design: A cross-sectional study.
Setting: Copenhagen, Denmark.
Subjects: Five-hundred-and-ten healthy women aged 45–58 y, included 3–24 months after last menstrual bleeding. None were using hormone replacement therapy.
Methods: The women were grouped according to their current smoking status. The two groups were compared with regard to serum levels of 25-hydroxyvitamin D (25OHD) and 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D), intact PTH, ionized calcium and phosphate, osteocalcin, as well as urine pyridinolines. Bone mineral density (BMD) was measured with DEXA-scans. Multiple regression analyses were performed to detect the effect of potentially confounding lifestyle factors, such as calcium and vitamin D intakes, alcohol and coffee consumption, sunbathing, and physical exercise.
Results: Fifty percent were current smokers. Smokers had significantly reduced levels of serum 25OHD ($P = 0.02$), 1,25$(OH)_2$D ($P = 0.001$), and PTH ($P < 0.001$). There was no difference in serum ionized calcium between smokers and non-smokers. We found a negative effect of smoking on serum osteocalcin ($P = 0.01$), while urinary pyridinolines were similar in the two groups. The small differences in lifestyle between the two groups could not explain these findings. Smokers had small but significant reductions in bone mineral density.
Conclusions: Smoking has a significant effect on calcium and vitamin D metabolism, which is not likely to be explained by other confounding lifestyle factors. The depression of the vitamin D-PTH system seen among smokers may represent another potential mechanism for the deleterious effects of smoking on the skeleton, and may contribute to the reported risk of osteoporosis among smokers.
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Descriptors: smoking; 25-hydroxyvitamin D; calcitriol; parathyroid hormone; osteocalcin; bone mineral density

Introduction

Tobacco smoking is in most studies found to be associated with a low bone mass and an increased risk of osteoporotic fracture (Law & Hackshaw, 1997). An increased bone loss has been registered in smokers (Krall & Dawson-Hughes, 1991; Vogel *et al*, 1997).

Several hypotheses have been put forward concerning the mechanisms by which smoking affects bone, the main focus being on the antiestrogenic effect. Smokers are lean (Wack & Rodin, 1982), have an early menopause (Jick *et al*, 1977), and have reduced levels of circulating oestrogens due to an increased hepatic turnover (Daniel *et al*, 1992). All these factors contribute to a reduced exposure to estrogen, resulting in an increased early bone loss. Other lifestyle factors are regarded as more prevalent among smokers compared to nonsmokers such as less physical activity, increased alcohol intake, or associated nutritional deficiencies, all of which might play a role. A direct toxic effect of tobacco smoking on bone cells is also a possibility (Fang *et al*, 1991).

Other hormonal systems, glucocorticoids, pituitary, and thyroid hormones, may be affected by smoking (Fisher *et al*, 1997; Salvini *et al*, 1992; Kirschbaum *et al*, 1994). Parathyroid hormone (PTH) and vitamin D metabolites are crucial in the regulation of calcium homeostasis and bone metabolism. An effect of smoking on PTH or 25-hydroxyvitamin D (25OHD) levels has only been investigated in few studies (Scragg *et al*, 1992, 1995; Mellström *et al*, 1993; Gudmondsson *et al*, 1987; Landin-Wilhelmsen *et al*, 1995; Ortego-Centeno *et al*, 1994, 1997). This cross-sectional study was performed to assess an influence of tobacco smoking on circulating vitamin D levels and calcium metabolism in a cohort of healthy Danish women who had recently undergone menopause.

Methods

Subjects

The present group is a subgroup of a cohort participating in an ongoing multicentre investigation, testing the hypothesis that hormone replacement therapy initiated shortly after the menopause reduces the risk of later osteoporotic fractures (the Danish Osteoporosis Prevention Study, DOPS) Healthy Caucasian perimenopausal women from the gen-

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eral community were recruited by mailing as previously described (Mosekilde *et al*, 1999). The inclusion criteria were: (1) intact uterus: age 45–58 y *and* amenorrhea between 3 and 24 months, *or* perimenopausal symptoms (including menstrual irregularities) *and* elevated serum follicle stimulating hormone (FSH); (2) hysterectomized: age 45–52 y *and* elevated serum FSH. According to the exclusion criteria, none of them were taking hormone replacement therapy. The biochemical screening prior to inclusion ensured that all participants had serum levels of calcium, phosphate and alkaline phosphatase within the normal range. Two thousand women were included in four centres in Denmark. As some of the biochemical analyses have only been performed in one of the centres, the present paper deals with the baseline data of the 510 women participating in the centre of Copenhagen, Denmark (latitude 55° N).

Tobacco, diet, and other lifestyle factors

All participants were interviewed at baseline by the same investigator using an extensive questionnaire. The average number of cigarettes smoked per day was registered and participants were classified according to their current smoking status. Former smokers were included in the non-smoking group, as most of the variables were subject to day-to-day variations. Current caffeine consumption (coffee, tea) was reported as cups/d. We assessed the degree of sunlight exposure by asking the subjects to select which one of the following categories most accurately described their behaviour: 'Never' meant that the person preferred not to stay in direct sunlight, but casual sun exposure occurred in daily life: 'Occasionally' meant that the person sometimes spent time out-door in the sunshine; 'Regularly' meant that the person quite often intentionally exposed themselves to sunshine to get suntanned.

Current leisure-time physical activity was assessed by adding the average number of hours per weeks spent on the following activities: walking, jogging, dance, gymnastics, swimming, bicycling, and other sports.

Calcium, vitamin D, and alcohol intakes were assessed by using 4- or 7-d diet records in 488 participants. The participants recorded all foods and beverages they consumed daily, estimating quantities in household measures. They received oral and written instructions and the importance of keeping accurate records was explained. A trained dietitian reviewed the records with 50% of the participants as to obtain additional information to improve the estimation of household measures, using the method developed at the National Food Agency (Haraldsdottir *et al*, 1985). The alcohol intake consisted mostly of wine. Individual intakes of nutrients were calculated by using Dankost Software version 1.3b, a program based on the official Danish food tables (Møller, 1989). The subjects were asked to state whether or not they took vitamin D supplementation at least half of the year (in Denmark, the majority of dietary supplements that include vitamin D contains 200 IU, which equals 5 μg cholecalciferol).

Biochemical measurements

All blood samples were obtained from the subjects under fasting conditions between 0800 and 1100 h. Serum was stored at −80°C until analysis and had a single thaw at time of analysis. Serum levels of 25OHD and 1,25-$(OH)_2$D were measured by radioimmunoassays. These methods involve specific extraction procedures with ether, preliminary chromatography and a competitive binding assay using rat kidney (25OHD) and calf thymus (1,25-$(OH)_2$D), respectively (Lund & Sorensen, 1979; Lund *et al*, 1979). The limits of detection were 5 ng/ml and 5 pg/ml, respectively. The intra- and inter-assay precision was 8.3% and 10.2% for 25OHD, respectively, and 8.4% and 12.9% for 1,25-$(OH)_2$D, respectively. Vitamin D_2 and D_3 metabolites were measured together by these methods.

Serum follicle-stimulating hormone (FSH) was measured in hysterectomized women and in those with regular bleedings. FSH, total alkaline phosphatase, phosphate, and ionized calcium values were determined using standard methods (FSH with Double Antibody from DPC (Diagnostic Products Corporation), Los Angeles; alkaline phosphatase and phosphate with Cobas Mira from Roche, Switzerland; and ionized calcium with ICA-1 and TNC-1 from Radiometer, Copenhagen).

Bone markers were measured in approximately half of the cohort, irrespective of smoking status. Serum osteocalcin was measured with DAKO osteocalcin ELISA kit (Copenhagen): limit of detection 1.0 μg/l, intra- and inter-assay precisions 3.1–4.6% and 4.7–7.8%, respectively. Urine pyridinium crosslinks, that is pyridinoline and deoxypyridinoline were measured by high-performance liquid chromatography and fluorescence detection after hydrolysis of the urine, as previously described (Kollerup *et al*, 1992). The intra- and inter-assay precisions were 5–7% and 12–14%, respectively.

Serum intact PTH (PTH) was measured with Immulite intact PTH immunoassay kit (DPC, Los Angeles): limit of detection 0.3 pmol/l, intra- and inter-assay precisions 5.4–7.0% and 5.0–5.5%, respectively.

Bone measurements

Bone mineral density (BMD) of the whole body, spine, and the hip was measured using a Hologic QDR-1000 W DXA scanner (Hologic Inc., Waltham, MA) for the first 294 scans and a Hologic QDR-2000 W scanner for the rest. Pencil beam measurements were used for all whole body scans, as well as the regional scans performed on the QDR-1000W. The spine and hip scans on the QDR-2000 were performed with fan beam. The densitometers were cross calibrated and the calibration factors on the QDR-2000 were set to obtain phantom results identical to those obtained on the QDR-1000W. The precision error for BMD assessments in vivo, as determined by the coefficient of variation, was 1.4% for the lumbar spine, and 0.8% for the hip and whole body, respectively.

Statistical analysis

Differences between smokers and non-smokers were evaluated by using Students *t*-test for independent data or Mann-Whitney U-test as appropriate. A test for trend was used to determine whether smokers were sunbathing more frequently than non-smokers. Relations among variables in the whole population or within each group were explored using Pearsons correlation and multiple regression analysis. In order to determine the influence of life-style factors on PTH, 25OHD and 1,25-$(OH)_2$D, three multiple linear regression analyses were performed, with log PTH, log(25OHD) and 1,25-$(OH)_2$D, respectively, as the dependent variable, and the following lifestyle factors and BMI as predictors: smoking (yes/no), calcium and vitamin D intake, alcohol, coffee, and tea consumption, weekly hours of leisure-time physical activity, and frequency of sunlight exposure ('never' = 1; 'occasionally' = 2; 'regularly' = 3) All *P*-values are two-tailed. Significance limits was $P \leq 0.05$. The analyses were performed with the Fastat Statistics Package for Macintosh.

The study was approved by the Ethics Committee of Copenhagen, and written informed consent was obtained from all subjects.

Results

Fifty percent were current smokers, of whom 50.4% ($n=128$) smoked 1–19 cigarettes per day and 49.6% ($n=126$) 20 cigarettes or more.

Demographic and biological characteristics of the study sample and differences between smokers and non-smokers are shown in Table 1.

Smokers entered menopause at an earlier age than non-smokers. However, age was not a predictor of 25OHD, $1,25(OH)_2D$, or PTH. The age span in this study was quite narrow (45–58).

Smokers had on average a 9% decrease of serum levels of 25OHD, and $1,25(OH)_2D$ corresponding to 0.3 SD, and a 22% decrease of serum PTH (Table 1). There were no differences in serum ionized calcium between smokers and non-smokers. A trend toward an increased serum phosphate level among smokers was found (Table 1). Hypovitaminosis D, defined as serum level of 25OHD below 15 ng/ml, was seen among 20.9% of the smokers versus 13.7% of the non-smokers.

No linear relationships were observed between number of cigarettes per day and serum 25OHD, serum $1,25(OH)_2D$ or PTH. A non-significant decrease of mean serum 25OHD (s.d.) levels was observed when smokers were categorized: 1–10 cigarettes per day: 24.9 (11.5); 11–20 cigarettes per day: 24.1 (11.6); more than 20 cigarettes per day: 22.5 (9.9) ng/ml. Serum $1,25(OH)_2D$ was not correlated to serum PTH or 25OHD values. Serum PTH and serum 25OHD were negatively related to each other in non-smokers ($P=0.04$), but not in smokers.

There was a positive association between PTH and BMI ($P<0.001$) in both groups. Smokers were leaner (Table 1) than non-smokers, but cigarette smoking was still an independent predictor of PTH in multiple regression analysis considering these 2 variables ($P<0.0005$). Smokers had lower levels of osteocalcin than non-smokers. Osteocalcin and serum $1,25(OH)_2D$ were highly correlated, but in multiple regression analysis including serum $1,25(OH)_2D$, smoking still was an independent predictor of osteocalcin ($P=0.01$). We found no differences in alkaline phosphatase and pyridinoline levels between smokers and non-smokers (Table 1).

The FSH levels did not differ between the two groups.

There were small differences in life-style between smokers and non-smokers. Smokers had decreased intakes of

Table 1 Biological characteristics of the study sample

	Smokers $n=254$	*Non-smokers* $n=256$	*P*
Antropometric data			
Age (y)	50.1 (2.7)	51.1 (2.9)	< 0.0005
Weight (kg)	67.3 (11.6)	70.1 (13.3)	0.01
BMI (kg/m^2)	24.9 (3.9)	25.8 (4.8)	0.03
Biochemical parameters			
S-25(OH)D (ng/ml)	22.1 (7–48)	25.0 (6–58)	0.02
S-$1,25(OH)_2D$ (pg/ml)	26.1 (9.7)	29.0 (9.5)	0.001
PTH (pmol/l)	2.3 (0.4–5.7)	2.8 (0.8–6.8)	< 0.001
S-Osteocalcin (μg/l)*	7.6 (5.5–10.6)	8.1 (5.7–11.8)	0.1
P-phosphat (mmol/l)	1.24 (0.18)	1.21 (0.18)	0.06
P-ionized calcium (mmol/l)	1.27 (0.04)	1.27 (0.03)	0.30
Total alkaline phosphatase (U/l)	143 (82–233)	142 (77–255)	0.72
FSH (IU/l)**	18 (8–58)	21 (8–64)	0.46
U-Pyridinoline* (nmol/mmol creatinine)	42.6 (26.8–70.2)	44.8 (28.0–85.3)	0.16
U-Deoxypyridinoline* (nmol/mmol creatinine)	12.3 (6.6–29.0)	12.3 (5.9–28.7)	0.99
Dietary data			
Calcium intake (mg/d)	737 (274–1573)	818 (339–1448)	0.02
Vitamin D intake (μg/d)	2.2 (0.9–10.8)	2.3 (0.7–16.9)	0.31
Percentage of the population taking Vitamin D supplementation	46.1%	51.2%	0.46
Bone measurements			
BMD spine (g/cm^2)	1.009 (0.14)	1.034 (0.14)	0.05
BMD total hip (g/cm^2)	0.894 (0.11)	0.916 (0.11)	0.03
BMD whole-body (g/cm^2)	1.074 (0.08)	1.092 (0.08)	0.01

Values are mean (s.d.) or median (2.5th and 97.5th percentiles).
*$n=134$ smokers and 130 non-smokers; **$n=125$ smokers and 112 non-smokers.
P-values are for differences between smokers and non-smokers (two-sample t-test or Mann-Whitney test as appropriate).
BMD values are age-adjusted.
FSH = follicle stimulating hormone; BMD = bone mineral density; PTH = parathyroid hormone.

Table 2 The influence of sun exposure frequency on serum 25OHD levels

			Mean serum 25OHD (ng/ml)		
Frequency of sun exposure	*N*	*Percentage of smokers #*	*Smokers*	*Non-smokers*	*P**
'Never'	90	45.5%	17.4	22.0	0.02
'Occasionally'	181	44.2%	24.6	25.6	0.60
'Regularly'	235	55.3%	25.9	29.2	0.02

Test for trend (Mann-Whitney U test)$P=0.03$.
**P*-values refer to T-test between smokers and non-smokers.

calcium, while dietary vitamin D intakes were similar in the two groups (Table 1). Smokers sunbathed more frequently than non-smokers (Table 2), and were less inclined to take vitamin supplementation, although the latter was not significant. A trend towards fewer hours of leisure-time physical activity among smokers was seen ($P = 0.08$). There were no significant differences in alcohol consumption and solarium use, while smokers were drinking more coffee ($P < 0.0005$). To assess the influence of possible confounding life-style factors on PTH, 25OHD and 1,25-$(OH)_2$D, respectively, we performed multiple regression analyses with BMI and the above mentioned lifestyle variables including smoking on the whole population. Neither caffeine intake, alcohol consumption, or leisure-time physical activity were significant predictors of log PTH, log 25OHD and 1,25-$(OH)_2$D. Besides smoking, vitamin D intake and sun exposure were positively associated with 25OHD, and body mass index (BMI) negatively, while vitamin D intake was positively and calcium intake negatively related to 1,25-$(OH)_2$D (Table 3).

Smokers had decreased age-adjusted BMD as compared to non-smokers in all measurements (Table 1). We investigated the relationship between vitamin D metabolites and bone density among smokers and non-smokers. In both groups we found the same inverse linear relationship between serum 1,25$(OH)_2$D and bone density, though it did not reach significance in the hip among non-smokers. In each region, the intercepts (I) were significantly different from each other between the smoking and non-smoking group, whereas the regression coefficients (β) did not differ. At the spine, the values (s.e.) were I = 1078 (25.4) and $\beta = -2.4$ (0.9) in smokers, and I = 1106 (28.3) and $\beta = -2.7$ (0.9) in non-smokers. For the whole body, I = 1113 (14.8) and $\beta = -1.4$ (0.5) in smokers, and I = 1132 (17.1) and $\beta = -1.5$ (0.6) in non-smokers (the unit of BMD being mg/cm^2). Thus, the regression lines describing the relation between BMD and 1,25$(OH)_2$D for smokers, and non-smokers, respectively, were parallel with each other. For a given 1,25$(OH)_2$D value, smokers had reduced BMD compared to non-smokers. Serum 25OHD and PTH were not associated with bone assessments in univariate analysis, neither in the whole population nor after splitting up the population according to their smoking status.

Discussion

In Denmark, we unfortunately have a very high prevalence of smoking among women: the overall prevalence is 40–51% for women between 45 and 64 y old (Bunnage, 1992). Thus, our study group is not unusual, and the population is very suitable to study the effects of tobacco.

In this cohort of normal perimenopausal women, we have found a significant negative association between

Table 3 Multiple regression analysis of log 25OHD, 1,25(OH)2D and log PTH against smoking, BMI and a number of lifestyle factors

Dependent variable *Predictors*	*Coefficient*	*Standard error*	*P-value*
Log serum 25OHD (ng/ml)			
Smoking (no = 0, yes = 1)	− 0.121	0.047	0.01
Sun exposure*	0.142	0.030	< 0.001
Vitamin D intake (μg/d)	0.014	0.007	0.04
Vitamin supplementation (no = 0, yes = 1)	0.097	0.045	0.03
BMI (kg/m^2)	− 0.014	0.005	0.006
Alcohol intake (g/d)	− 0.001	0.001	0.47
Coffee (cups per d)	0.007	0.006	0.24
Tea (cups per d)	0.008	0.006	0.22
Calcium intake (mg/d)	− 0.000	0.000	0.42
Physical activity (hours per week)	0.010	0.005	0.07
$R^2 = 0.12$			
Serum 1,25(OH)2D (pg/ml)			
Smoking (no = 0, yes = 1)	− 3.264	0.942	0.001
Sun exposure*	0.055	0.611	0.93
Vitamin D intake (μg/d)	0.290	0.134	0.03
Vitamin supplementation (no = 0, yes = 1)	0.744	0.914	0.42
BMI (kg/m^2)	− 0.063	0.106	0.55
Alcohol intake (g/d)	0.007	0.027	0.81
Coffee (cups per d)	0.081	0.124	0.51
Tea (cups per d)	− 0.091	0.126	0.47
Calcium intake (mg/d)	− 0.004	0.001	0.01
Physical activity (hours per week)	0.045	0.107	0.68
$R^2 = 0.05$			
Log serum PTH (pmol/l)			
Smoking (no = 0, yes = 1)	− 0.202	0.056	< 0.001
Sun exposure*	− 0.059	0.036	0.11
Vitamin D intake (μg/d)	0.000	0.008	0.98
Vitamin supplementation (no = 0, yes = 1)	− 0.108	0.055	0.048
BMI (kg/m^2)	0.021	0.006	0.001
Alcohol intake (g/d)	− 0.002	0.002	0.26
Coffee (cups per d)	0.003	0.007	0.72
Tea (cups per d)	− 0.004	0.008	0.57
Calcium intake (mg/d)	− 0.000	0.000	0.23
Physical activity (hours per week)	0.004	0.006	0.49
$R^2 = 0.09$			

*Frequency of sun exposure: Never = 1, Occasionally = 2, regularly = 3.
PTH = parathyroid hormone; BMI = body mass index.

smoking and serum levels of 25OHD, 1,25$(OH)_2$D, and PTH. A negative effect of smoking on serum 25OHD and PTH levels was found in a Swedish case-control study of elderly men who had undergone gastrectomy (Mellström *et al*, 1993). A 10% decrease of serum 25OHD was seen among smokers in the control group (mean difference 2.8 ng/ml), which is in accordance with our findings. Slightly reduced concentrations of 25OHD and 1,25$(OH)_2$D, albeit non-significantly, has been described in healthy American postmenopausal female smokers (Krall *et al*, 1991). In two Swedish studies, reduced levels of intact PTH among smokers have been reported in young women (Gudmundsson *et al*, 1987) and in a population-based study of men and women aged 25–64 years (Landin-Wilhelmsen *et al*, 1995). In contrast, Orgego-Centeno *et al* (1994, 1997) could not demonstrate such differences in small populations of young men and women from the south of Spain. In two other studies, no association was found between smoking and serum levels of 25OHD in younger men from New Zealand and elderly English women (Scragg *et al*, 1992, 1995). However, the studies with negative findings included only small numbers of smokers.

The reduced PTH level seen among smokers could not be explained by differences in serum ionized calcium or serum phosphate. Smokers may differ from non-smokers in weight, caffeine intake, calcium and vitamin D intakes, alcohol consumption and physical exercise habits, all of which can potentially confound an association between smoking and endogenous vitamin D and PTH levels (Kiel *et al*, 1996). In our study we found small differences in lifestyle between smokers and non-smokers. Smokers were sunbathing more frequently, which would increase serum 25OHD. No significant difference in vitamin D intakes was observed. Vitamin D intake was estimated using 4–7 d diet records. As vitamin D is only present in substantial amounts in few natural foods, like fat fish, a potential misclassification of subjects eating fat fish infrequently may occur. However, we are comparing two large groups and the misclassification error will be expected to be of the same magnitude in each group. The decreased calcium intake among smokers, if it has any effect, would tend to increase serum 1,25-$(OH)_2$D, whereas we found decreased levels among smokers. None of the other lifestyle factors had any appreciable influence on 25OHD, 1,25-$(OH)_2$D, and PTH. Furthermore, after correcting for these potential confounders by multiple regression analysis, smoking was still significantly associated with serum levels of 25OHD, 1,25-$(OH)_2$D, and PTH. BMI is another potential confounder, as PTH was positively associated with BMI and smokers were leaner than non-smokers. However, the effect of smoking on PTH could not entirely be explained by a decreased BMI among smokers as smoking was still an independent predictor of PTH, when corrected for BMI in multiple regression analysis. An effect of smoking on serum PTH and 1,25-$(OH)_2$D mediated through an effect on estrogen levels could not be excluded in this investigation as estrogens were not measured. However, there were no differences in FSH levels between smokers and non-smokers. In addition, though some studies of treatment with estrogen suggest a stimulatory effect of estrogen on calcitriol level (Van Hoof *et al*, 1994), cross-sectional and prospective studies have shown that the first years of the natural menopause are not accompanied by changes in circulating levels of PTH or 1,25-$(OH)_2$D (Prince *et al*, 1995; Falch *et al*, 1987). It therefore seems unlikely that the suppression of PTH and 1,25-$(OH)_2$D seen among smokers should be caused by a suppression of the endogenous estrogen level.

In this study, we could not demonstrate dose-response relationships between number of cigarettes per day and the investigated parameters. However, we do not have information on brand of cigarettes and ways of smoking (depth of inhalation, frequency of puff drawing, filter tips). The smoke composition varies greatly in various cigarette brands, and the daily number of cigarettes may not reflect accurately the degree of exposure.

The mechanisms whereby smoking could decrease circulating levels of PTH and vitamin D metabolites remain to be worked out. One of the difficulties of the research area is that tobacco smoke is composed of a large number of more or less potentially toxic chemical compounds, including 'tars' and nicotine, but also several heavy metals like cadmium, hydroxyquinones, thiocyanate, nitrosamines and others (Chiba *et al*, 1992; Smith *et al*, 1997; Hoffmann & Hoffmann, 1997). Several noxious mechanisms may be involved, but their importance is uncertain. Hydroxyquinones increase intracellular calcium and affect the liver (Orrenius *et al* 1989). An altered hepatic metabolism of 25OHD is possible, as smokers have enhanced hepatic degradation of another steroid, namely estrogen (Jensen *et al*, 1985). The serum calcium levels were identical in the two groups. The reduced serum PTH among smokers might therefore be explained by a decreased secretion or an increased degradation of the hormone. We found no correlation between intact PTH, which has a half-life of a few minutes, and serum 1,25-$(OH)_2$D, that has a half-life of a few hours. Other studies including healthy persons have also failed to detect such a correlation (Quesada *et al*, 1992; Orwoll *et al*, 1986). The lacking correlation does not rule out that there might be an impaired 1-alpha-hydroxylation among smokers. The decreased concentrations of serum 1,25-$(OH)_2$D seen in smokers might also be due to accumulation of cadmium in the kidney (Ewers *et al*, 1990; Kido *et al*, 1989).

Decreased calcium absorption may be the consequence of reduced serum 1,25-$(OH)_2$D levels (Krall and Dawson-Hugues, 1991). The unchanged plasma calcium among smokers in spite of lower levels of PTH and 1,25-$(OH)_2$D could be a result of a decreased calcium uptake in bone. The reduced serum osteocalcin levels in smokers might indicate a decreased osteoblastic activity. This is in agreement with in vitro studies that have shown a direct toxic effect of tobacco on osteoblasts (Fang *et al*, 1991). No differences were seen in total serum alkaline phosphatase. A recent population-based study has demonstrated a suppression of markers of bone turnover among female smokers (Woitge *et al*, 1998). In contrast to the changes in the formative bone marker, we found no differences between smokers and non-smokers in the urinary pyridinolines, indicating equal rates of bone resorption. Thus, our results might suggest that smoking has an inhibitory effect on bone formation, but more sensitive and specific bone markers on bone histomorphometry are needed to verify this theory.

In our material of perimenopausal women, smokers had a 2% decreased bone mineral density as compared with non-smokers. This is in accordance with a recent meta-analysis, where no effect of smoking could be detected until menopause, while deficits of 2% for every 10 year increase in age were seen after the menopause (Law &

Hackshaw, 1997). An association between circulating concentrations of 25OHD and bone density has been demonstrated in a number of studies (Khaw *et al*, 1992; Martinez *et al*, 1994). The relationships between bone density and vitamin D metabolites and PTH in the present study were modest or absent. It may seem paradoxical that, whereas $1,25(OH)_2D$ and BMD were inversely related to each other, smokers had at the same time decreased $1,25(OH)_2D$ levels and BMD, when compared to non-smokers. However, the same inverse relationship was maintained between serum $1,25(OH)_2D$ and bone density in both groups, but at a lower level among smokers. This inverse relationship has been described by others (Sowers *et al*, 1990; Prince *et al*, 1993) and has also been demonstrated earlier in this cohort (Brot *et al*, 1999). We have hypothesized that it was a compensatory mechanism for an underlying cause leading to decreased bone mineralization, like for instance low calcium intake. Thus, it seems that smokers compensate the same way, but not as well as non-smokers, for some underlying factors leading to decreased bone mass.

Conclusion

Smoking seems to depress the serum levels of 25OHD, $1,25(OH)_2D$, and PTH. Smokers had on average an approximately 10% decrease of circulating levels of 25OHD and $1,25(OH)_2D$, and a 20% reduced PTH secretion. These differences were not likely to be explained exclusively by other confounding lifestyle factors. The significance of these findings is still unsettled, but it is important to note that the proportion of subjects with suboptimal vitamin D status (that is, serum 25OHD below 15 ng/ml) was 50% greater among smokers. This figure is likely to increase as the population grows older. Even though the differences found may seem small, and probably would not have been detectable as significant had not the sample size been as large as it was, they may become clinically important if the exposure is sustained for decades, and may in part account for the decreased bone mass and increased fracture risk seen among smokers later in life.

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