Calcif Tissue Int (1997) 60:496–500

Calcified
Tissue
International

Effect of Tobacco Consumption on Bone Mineral Density in Healthy Young Males

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Received: 30 July 1996 / Accepted: 31 December 1996

Abstract. Smoking is related to a decreased bone mass and increased risk of osteoporotic fractures. Nevertheless, the effect of smoking in males is poorly understood. The purpose of this study was to assess the repercussion of smoking on bone mass in otherwise healthy male smokers and its relationship with markers of mineral metabolism and hormone profile. We measured bone mineral density (BMD) in 57 healthy males (26 nonsmokers, 31 smokers; aged 20–45 years) by dual X-ray absorptiometry (DXA, Hologic QDR_{1000}) in the lumbar spine and proximal femur. In a subset we measured biochemical markers of bone metabolism and hormonal profile. We found significant differences in BMD between heavy smokers (more than 20 cigarettes/day) and nonsmokers in all skeletal sites. Serum levels of dehydroepiandrosterone sulfate (S-DHEAS) were lower in smokers and correlated with femoral BMD measurements. No significant differences in bone turnover markers were found. Our findings show that smoking by healthy young males is associated with decreased bone mass.

Key words: Bone mineral density — Smokers — Healthy men — Mineral metabolism — Sex steroids.

Although most studies have been undertaken in women, cigarette smoking is a frequently cited risk factor for osteoporosis and associated fractures [1–3]. Tobacco was linked to an increased prevalence of vertebral fractures in men in the cohort studies of Seeman and Melton [4] in which the relative risk of vertebral fracture in smokers was 2.3 and was independent of alcohol consumption. We have previously reported a decreased bone mass in premenopausal smokers associated with characteristic changes in the hormonal profile [5]. However, the mechanism by which smoking affects bone in men is unclear. The aim of this study was to assess the repercussions of smoking on bone mass in young males, and to investigate the possible alterations in mineral metabolism and hormone profile associated with cigarette smoking.

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Subjects and Methods

Fifty-seven males from the staff of the University of Granada Hospital and the student body of the University of Granada Medical School and Nursing School participated voluntarily in the study. We excluded those men who were receiving or had received, during the previous 3 years, medication that may have altered phosphorus or calcium metabolism or bone mass (e.g., thyroid hormone, corticosteroids, androgens, diuretics, antiacids, antiepileptics, or anticoagulants). All of them consumed a normal diet that included at least 500 mg of calcium/day, and drank less than 40 g of alcohol/day. All participants were informed about the nature of the study and gave their consent to participate. The study was approved by our center's ethical committee.

Each participant was assigned to one of two groups depending on whether he was a smoker or nonsmoker. Smokers were considered to have consumed more than eight cigarettes per day for at least 2 years previously. There were 31 smokers and 26 nonsmokers; of the former, 20 consumed fewer than 20 cigarettes/day, and 11 smoked 20 or more/day.

In a subpopulation of the sample consisting of 15 smokers and 17 nonsmokers, we assessed mineral metabolism parameters and hormone profile. Starting 1 week before the test, all subjects received a normocaloric, gelatin-free diet supplying known amounts of calcium (800–1000 mg/day) and phosphorus (1000 mg/day). The men were studied over a 3-day period. Fasting blood samples were collected to determine serum levels of calcium (S-Ca), phosphorus (S-P), creatinine (S-Cr), and alkaline phosphatase (S-AP), and two 24-hour urine collection were used to determine urinary (U)-Ca, U-P, and U-Cr. Renal threshold phosphate (TmP) was calculated from these data. Serum concentrations of midregion parathyroid hormone (S-PTH), calcitonin (S-CT), osteocalcin (S-BGP), estradiol ($S\text{-}E_2$), testosterone (S-T), progesterone (S-Prog), dehydroepiandrosterone sulfate (S-DHEAS), and sex hormone binding globulin (S-SHBG) were measured. Free estradiol index (FEI) and free testosterone index (FTI) were calculated from the following formula: total hormone × 1000/SHBG. On the third day, a fasting urine sample was collected to determine urinary hydroxyproline (U-OHPr) and U-Cr, and the U-OHPr/U-Cr and U-Ca/U-Cr ratios were calculated. Assays were performed as previously described [5].

BMD expressed as g/cm^2 was measured with dual-energy-X-ray absorptiometry (DXA) (Hologic QDR-1000, Walthmam, MA) at the lumbar spine (L2–L4) and at four sites in the proximal femur: femoral neck, trochanter, intertrocanter region, and Ward's triangle. During spine measurements, the legs were bent at the hip and the lower legs were elevated to minimize lumbar lordosis. For measurements in the femur, a foot support was used so that the leg was positioned at 20°C inward rotation. Our laboratory's *in vivo* precision has a long-term coefficient of variation (CV) of 2.2% in the spine, 1.8% in the femoral neck, and 2.3% in Ward's triangle. Stability of the instrument with time was checked by daily scan of a spine phantom of known composition (Hologic Inc.).

Table 1. Characteristics of smokers and nonsmokers

		Smokers (n = 26)				
	Nonsmokers (n = 26)	Total (n = 31)	*P* value[a]	<20 cig./day (n = 20)	>20 cig/day (n = 11)	*P* value[b]
Age (years)	27.8 ± 6.9	28.7 ± 7.0	ns	28.0 ± 6.6	29.6 ± 7.6	ns
Weight (kg)	73.3 ± 10.5	75.2 ± 9.9	ns	73.9 ± 9.6	77.1 ± 10.5	ns
Height (cm)	175.5 ± 6.9	174.4 ± 6.4	ns	174.5 ± 7.3	174.3 ± 5.2	ns
BMI (kg/m^2)	23.6 ± 3.2	24.7 ± 3.0	ns	24.3 ± 2.8	25.4 ± 3.2	ns

Values are mean ± SD
[a] Differences between smokers and nonsmokers
[b] Differences between heavy smokers (>20 cig/day) and those smoking (<20 cig/day)

All values were expressed as mean ± standard deviation (SD). Log transformation of the hormone assay results was done when they failed to show normal distribution. The significance of the differences between groups was determined with Student's *t* test. A linear correlation test was used to determine the relationship between BMD values and other variables. A probability ($P \leq 0.05$) was taken to indicate significant differences. In addition, a multiple regression analysis was performed to calculate the effects of age, weight, and number of cigarettes consumed per day on the BMD.

Results

Mean age of the men in this study was 28.2 ± 6.9 years. Smokers consumed a mean of 17.6 ± 8.3 cigarettes/day, and had been smoking for a mean of 10.5 ± 5.1 years at the time of the study. Men who consumed 20 cigarettes/day or more had smoked a mean of 24.6 ± 8.3 for a mean period of 11.7 ± 5.6 years, and daily consumption among those who consumed fewer than 20 cigarettes/day was 13.1 ± 2.5 cigarettes/day for a mean period of 8.0 ± 3.6 years. There were no significant differences in age or body weight between smokers and nonsmokers, or between smokers of more or fewer than 20 cigarettes/day (Table 1).

Bone Mineral Density Measurements

Table 2 shows' the values of bone mineral density (BMD) in smokers and nonsmokers. There were no statistical differences between smokers (less than 20 cigarettes/day) and nonsmokers in any skeletal region. However, we found significant differences between heavy smokers (more than 20 cigarettes/day) and nonsmokers in all sites.

Multiple regression analyses are shown in Table 3A&B. The age was the more predictive single determination of BMD in femoral neck and trochanter; in the lumbar spine it was the weight. Smoking was not a significant determinant of BMD elsewhere.

Endocrine Profiles

There were no differences between smokers and nonsmokers in concentrations of S-T, S-E_2, S-P, S-SHBG, FTI, and FEI. The only significant difference was the serum concentration of S-DHEAS ($P < 0.05$) (Table 4).

Mineral Metabolism Parameters

No significant differences were found in serum or urinary concentrations of Ca and P, TmP, AP, PTH, BGP, and urinary hydroxyproline/creatinine and Ca/creatinine ratios (Table 5).

Correlation Studies

Weight showed significant correlation with BMD just in the lumbar spine ($P < 0.04$). Moreover, S-DHEAS and BMD were correlated in the trochanter area ($P < 0.05$), and near significant in the intertrochanter area ($P = 0.05$). Finally, a direct correlation between concentration of S-PTH and BMD in the femoral neck ($P < 0.02$) and the intertrochanter region ($P < 0.02$) were found (Table 6).

Discussion

Osteoporosis has traditionally been a disorder almost synonymously associated with postmenopausal women. Nevertheless, in the last few years, it has been acknowledged that the problem of osteoporosis in men represents an important public health issue [6–9]. Factors implicated in the pathogenesis of bone loss in men are not well understood and environmental risk factors probably do not differ greatly between women and men.

In our study, males who smoked heavily showed significantly lower BMD than nonsmokers at all sites measured. Moreover, the reduction in BMD was independent of age and body mass index. This negative effect of tobacco use has been previously described in older men [6] and could explain the increased prevalence of osteoporotic fractures in subjects smoking for many years [4]. Thus, the characteristics of our study population, young males without other risk factors, highlight the significance of these findings. The mechanism by which smoking affects bone in men is unclear, although some factors such as the level of physical activity could be implicated. Previous reports suggest that tobacco consumption alters the osteoblastic activity [10, 11]. However, we were unable to demonstrate any differences between smokers and nonsmokers in biochemical markers of bone metabolism.

Studies of the hormone profile in smokers have yielded variable results. We were unable to show any significant difference between smokers and nonsmokers in serum SHBG, testosterone, estradiol and their free indexes. However, an unexpected finding was the higher levels of S-DHEAS observed in nonsmokers. Moreover, the levels of S-DHEAS correlated with the BMD at trochanteric and intertrochanteric areas as reported in perimenopausal women [12].

Table 2. Differences in bone mineral density between smokers and nonsmokers

BMD (g/cm^2)	Nonsmokers (n = 26)	Smokers				
		Total (n = 31)	*P* value[a]	<20 cig/day (n = 20)	>20 cig/day (n = 11)	*P* value[b]
Femoral neck	0.877 ± 0.121	0.880 ± 0.131	ns	0.900 ± 0.120	0.826 ± 0.153	<0.05
Trochanter	0.783 ± 0.119	0.743 ± 0.129	ns	0.765 ± 0.126	0.687 ± 0.128	<0.05
Intertrochanter	1.191 ± 0.156	1.188 ± 0.243	ns	1.236 ± 0.237	1.067 ± 0.231	<0.05
Ward's triangle	0.746 ± 0.159	0.737 ± 0.155	ns	0.758 ± 0.147	0.682 ± 0.173	<0.05
Lumbar spine	1.050 ± 0.154	1.009 ± 0.107	ns	1.022 ± 0.119	0.980 ± 0.103	<0.05

Values are mean ± SD
[a] Differences between smokers and nonsmokers
[b] Differences between heavy smokers (>20 cig/day) and nonsmokers

Table 3A. Multiple regression analysis between the BMD at the five sites and the variables age, weight, and smoking (number of cigarettes/day)

BMD (g/cm^2)	Regression coefficients				
	Intercept	Age	Weight	Cigarettes/day	R^2
Femoral neck	0.738	−0.0056[a]	0.0041[a]	−0.0009	0.16
Trochanter	0.766	−0.0058[a]	0.0025	−0.0003	0.17
Intertrochanter	1.016	−0.0074	0.0055	−0.0032	0.12
Ward triangle	0.878	−0.0066	0.0046	−0.0024	0.15
Lumbar spine	0.817	−0.0038	0.0047[a]	−0.0031	0.18

[a] $P < 0.05$

Table 3B. Multiple regression analysis between the BMD at the five sites and the variables age, weight, and smoking (yes or no)

BMD (g/cm^2)	Regression coefficients				
	Intercept	Age	Weight	Smoking	R^2
Femoral neck	0.743	−0.0058[a]	0.0041[a]	−0.0034	0.15
Trochanter	0.696	−0.0062[a]	0.0025	−0.0364	0.15
Intertrochanter	1.005	−0.0080	0.0055	−0.0008	0.10
Ward triangle	0.869	−0.0070[b]	0.0024[c]	−0.0004	0.13
Lumbar spine	0.737	−0.0038	0.0047[a]	−0.0427	0.16

[a] $P < 0.05$; [b] $P = 0.05$; [c] $P = 0.06$

S-DHEA and its sulfate are quantitatively the largest products of the adrenal cortex and the most abundant steroids in peripheral blood, but their functions are uncertain. Recently, S-DHEAS has been associated with a decreased risk of cardiovascular events [13, 14], gastric cancer [15], and with the aging process [16]. Moreover, because S-DHEAS levels decrease with age in parallel with decreasing BMD, it has been related with senile osteoporosis [17] and also with the protective effect of overweight in postmenopausal osteoporosis [18]. Thus, the higher levels of S-DHEAS found in male nonsmokers could be related to their greater values of BMD compared with heavy smokers.

However, elevated S-DHEAS is the more consistent finding in male smokers [14, 19–21]. This discrepancy may be partly methodological, it is often difficult to differentiate between an effect of smoking from an effect of a factor that covariates with smoking. In this sense S-DHEAS has shown to be related with age [19–21], BMI [21], the use of multivitamins [20], and probably with alcohol use, physical activity, and psychological stimulation [22]. Moreover, Salvini et al. [20] showed only a moderate direct association with cigarette smoking and a powerful influence of age on decreasing levels of S-DHEAS in men aged 40–84 years. Finally, our population sample is significantly younger than others (range 20–45 years). In this subpopulation S-DHEAS is likely to reach its maximum levels after increasing during the early decades of life [23], making smoking a lesser influence.

Nevertheless, the lack of predictive value of S-DHEAS levels in the multiple regression analysis suggests that its effects on bone, if any, are subtle and minimized by the effects of age and weight. The same is valid for smoking; as stated before, it showed a deleterious effect on bone mass only when comparing heavy smokers and nonsmokers. Again, the youthfulness of our male population may explain the differences in the relative importance of age and weight in lumbar and femoral sites.

In conclusion, our study addresses the deleterious effect

Table 4. Comparison of hormone parameters between smokers and nonsmokers

	Smokers (n = 15)	Nonsmokers (n = 17)	*P* value
S-T (ng/ml)	5.1 ± 1.7	5.4 ± 1.7	ns
S-E_2 (pg/ml)	30.8 ± 11.6	37.2 ± 21.8	ns
S-P (ng/ml)	0.76 ± 0.31	0.70 ± 0.25	ns
S-SHBG (nmol/liter)	22.2 ± 20.2	19.5 ± 8.9	ns
FTI	37.6 ± 34.6	30.2 ± 11.5	ns
FEI	240 ± 226	174 ± 58	ns
S-DHEAS[g] (μg/ml)	2301 ± 1127	3107 ± 993	<0.05

Values are mean ± SD
S-T = serum testosterone; S-E_2 = serum estradiol; S-P = serum progesterone; S-SHBG = serum sex hormone binding-globulin; FTI = free testosterone index; FEI = free estradiol index; S-DHEAS = serum dehydroepiandrosterone sulfate

Table 5. Comparison of mineral metabolism parameters between smokers and nonsmokers

	Smokers (n = 15)	Nonsmokers (n = 17)	*P* value
Serum			
S-Ca (mg/dl)	9.7 ± 0.3	9.6 ± 0.3	ns
S-P (mg/dl)	3.6 ± 0.5	3.5 ± 0.6	ns
S-AP (U/liter)	154 ± 41	121 ± 32	ns
S-BGP (ng/ml)	2.8 ± 1.8	3.4 ± 0.4	ns
S-PTH (pmol/liter)	61.6 ± 15.6	52.1 ± 6.5	ns
Urine			
U-Ca (mg/24/hour)	217 ± 89	188 ± 83	ns
U-P (mg/24/hour)	892 ± 281	583 ± 212	ns
U-OHPr/Cr (mg/g)	0.025 ± 0.011	0.023 ± 0.010	ns
U-Ca/Cr (mg/dl)	0.12 ± 0.07	0.14 ± 0.09	ns
TmP (mg/dl)	3.2 ± 0.5	3.4 ± 0.4	ns

Values are mean ± SD
S-Ca = serum calcium; S-P = serum phosphorus; S-AF = serum alkaline phosphatase; S-BGP = serum osteocalcin; S-PTH = serum midregion parathyroid hormone; U-Ca = urinary calcium; U-P = urinary phosphorus; U-OHPr/Cr = urinary hydroxyproline/creatinine ratio; U-Ca/Cr = urinary calcium/creatinine ratio; TmP = renal threshold phosphate

Table 6. Correlation between BMD and weight, S-DHEAS, and S-PTH

BMD (g/cm^2)	Weight		S-DHEAS		S-PTH	
	r	*P* value	r	*P* value	r	*P* value
Femoral neck	0.25	ns	0.21	ns	0.52	<0.02
Trochanter	0.12	ns	0.50	<0.05	0.28	ns
Intertrochanter	0.20	ns	0.47	=0.05	0.51	<0.02
Ward triangle	0.11	ns	0.09	ns	0.39	=0.07
Lumbar spine	0.30	<0.04	0.37	ns	0.35	ns

Values are mean ± SD
S-DHEAS = serum dehydroepiandrosterone sulfate; S-PTH = serum midregion PTH

of tobacco consumption on bone mass, even in otherwise healthy young male subjects. Further studies are warranted to clarify the extent and the underlying mechanism of bone damage in smokers.

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