Calcif Tissue Int (1992) 50:300–305

Calcified Tissue International

Clinical Investigation

Cigarette Smoking, Steroid Hormones, and Bone Mineral Density in Young Women

Mark Daniel,[1] Alan D. Martin,[2] and Donald T. Drinkwater[3]

[1]The Sport & Exercise Sciences Research Institute, The University of Manitoba, Winnipeg, Manitoba, Canada; [2]The School of Physical Education, The University of British Columbia, 6081 University Boulevard, Vancouver, B.C., Canada V6T 1Z1; and [3]The College of Physical Education, The University of Saskatchewan, Saskatoon, Canada.

Received April 15, 1991, and in revised form October 3, 1991

Summary. There are few studies of the effect of smoking on bone density in young women. The reported antiestrogenic effect of smoking could be a mechanism for a possible effect of smoking on bone. We measured bone mineral density (BMD) by dual-energy X-ray absorptiometry (whole body, proximal femur, lumbar spine), and serum levels (mid-follicular phase) of testosterone (T), estradiol (E_2), sex hormone-binding globulin (SHBG), and cortisol in 52 women (25 smokers, 27 nonsmokers) aged 20–35 years. The two groups did not differ significantly in age, height, weight, or the sum of eight skinfold thicknesses. The mean number of cigarettes smoked per day and the number of years of smoking were 16.9 and 12.9, respectively. There were no significant differences in BMD between smokers and nonsmokers at any site. For both smokers and nonsmokers, SHBG and the free androgen index (T/SHBG) made significant contributions ($P < 0.005$) to the variance in BMD at all sites except the lumbar spine. The free estradiol index (E_2/SHBG) contributed to whole body BMD ($P < 0.05$). For all subjects, there were significant inverse relationships between SHBG and BMD ($P < 0.002$), and positive relationships between T/SHBG and BMD ($P < 0.02$) for all sites except the lumbar spine. These data suggest that moderate smoking in young women is not associated with low BMD at any site. However, smokers had lower free estradiol and higher SHBG, both of which have been related to increased bone loss in older women.

Key words: Female–Smoking–Estradiol–Testosterone–Sex hormone-binding globulin–Cortisol–Bone density.

Cigarette smoking is often cited as a risk factor for osteoporosis and associated fractures [1]. For example, peri- and postmenopausal women who smoke cigarettes have greater risk and incidence of hip, vertebral, and forearm fractures [2–6] than nonsmokers. Two recent studies have reported lower bone mineral density (BMD) in women who smoke [7, 8]. But many investigations have found no relationship between cigarette smoking and osteoporosis or fracture risk in women [9–13]. Thus, the influence of smoking is at best, unclear. It is possible that associations of cigarette smoking with fracture risk and incidence are spurious; the low body weight by which smokers are characterized [14] may be a factor more relevant than smoking itself. Jensen [9] has demonstrated identical variation in bone mineral content in smokers and nonsmokers, influenced only by degree of overweight, and Slemenda et al. [15] have reported that the rate of bone loss at menopause is not influenced by smoking. An independent effect of smoking on bone has not been established.

Virtually all investigations of the influence of smoking on bone mineral content or density, or incidence of fracture, have been conducted in postmenopausal women. Little is known about the effect of smoking on bone in younger women. Daniell [2], observing significantly lower percent cortical area in postmenopausal smokers relative to nonsmokers, nonetheless did not observe a similar relationship in younger, premenopausal women. In a sample of premenopausal women, Stevenson et al. [16] found no relationship between cigarette smoking and femoral BMD, but did observe significantly lower BMD at the lumbar spine in smokers. McCulloch et al. [17] initially reported no significant difference in trabecular BMD at the os calcis in young women aged 20–35 years who either smoked daily or did not. These researchers subsequently restructured the same sample of young women into nonsmoking, moderate, and heavy smoking categories. They found that the heavy smokers had significantly lower BMD at the os calcis than the moderate smokers and nonsmokers [18].

A hypoestrogenic state is a well-accepted risk factor for osteoporosis [19]. Serum concentrations of estrogens are inversely related to rate of bone loss in peri- and postmenopausal women [20]. Estrogen replacement therapy (ERT) preserves bone mass in postmenopausal women [21] and has recently been demonstrated to increase BMD in women with established postmenopausal osteoporosis [22]. Though response in bone mineral content in postmenopausal smokers and nonsmokers undergoing ERT is similar, postmenopausal smokers have lower estrogen levels than nonsmokers [23], supporting other circumstantial evidence suggesting estrogen deficiency in women who smoke cigarettes. Smoking is associated with early natural menopause and menopausal symptoms [24], greater risk of oligomenorrhea, and greater prevalence of hirsutism [25]. Both pre- and postmenopausal smokers have a lower incidence of endometrial [26] and breast [27] cancers, which are known to be estrogen-dependent. In premenopausal women, smoking is associated

Offprint requests to: A. D. Martin

with infertility [28]; there is a direct relationship between the number of cigarettes smoked per day and time required to become pregnant [29]. Moreover, pregnant women who smoke have lower serum estrogen levels than nonsmokers [30]. But whether an antiestrogenic effect of smoking is due to decreased estrogen production, as is suggested by direct inhibition by cigarette smoke and nicotinic alkaloids of human granulosa cell aromatase activity and the consequent conversion of androgens to estrogens [31], or increased degradation of estrogens via enhanced 2-hydroxylation of estrone [32], remains open to question.

An antiestrogenic effect of smoking could mediate effects on BMD in young women, a possibility not yet investigated. To test the hypothesis that relationships between endocrine factors and BMD differ between premenopausal smokers and nonsmokers and, furthermore, to clarify the nature of the relationship between smoking and BMD in young women, we undertook a cross-sectional study of levels of endogenous steroids, sex hormone-binding globulin (SHBG), and BMD in relation to cigarette smoking in women aged 20–35 years.

Methods

Subject Selection

Subjects were recruited by means of a poster campaign and public service announcements on local television and radio stations; others were recommended by those already participating in the study. Posters and announcements enabled self-selection by specification of eligible gender (female), age range (20–35 years), and smoking status (smoker/nonsmoker).

Of 187 respondents, 130 completed a screening questionnaire after which 52 were excluded due to (1) history of use of oral contraceptives or any other hormonal medication (estrogen, progestin, glucocorticoid, or thyroid) within 4 months prior to participation in the study; (2) prescription or nonprescription drug use within 1 month of participation in the study; (3) menstrual cycles less than 21 or greater than 36 days; (4) competitive athletics or 8 hours or more of planned physical activity per week; (5) fluctuations in weight of 5 kg or more within the 6 months preceding the study; (6) history of any endocrine abnormality; (7) pregnancy; and (8) poor general health. The major reason for exclusion was oral contraceptive use; no measurements were made on those excluded. All women provided their informed, written consent. The research protocol was approved by the Human Ethics Committee of the Faculty of Medicine at the University of Manitoba.

The sample was then grouped according to self-reported smoking status. Of those remaining, 44 were nonsmokers and 34 were regular cigarette smokers. In view of the lack of effect of smoking fewer than eight cigarettes per day on body weight [33], respondents reporting seven cigarettes per day or less (n = 9) for the 5-year period preceding the study were excluded. Nonsmokers were defined as those who had never smoked ("never" smokers) and former smokers who had (1) not smoked at all for at least the 5-year period preceding participation in the study and (2) not smoked for a period of time equal to or greater than the duration of the period for which they smoked. Former smokers who did not meet these criteria (n = 17) were dropped from the sample, leaving the nonsmoking group composed of 16 never smokers and 11 former smokers. Therefore, final sample size was 52 (25 smokers, 27 nonsmokers).

Physical Activity

The nature of any noncompetitive planned physical activity of less than 8 hours per week was assessed by questionnaire; respondents reporting competitive athletics or 8 hours or more of planned activity per week were excluded from the study. Subjects were asked to define the types of activities they were involved in and to estimate the frequency and amount of time spent pursuing various activities over the course of an average week. On the basis of responses to these questions, numerical weights were assigned according to degree of activity; all subjects were then grouped into one of three blocks (none, low, or moderate), each corresponding to a numerically defined range of activity.

Anthropometry

Weight, height, and eight skinfold thicknesses were measured. A Harpenden caliper (British Indicators Ltd.) was used to obtain skinfold thickness measurements on the right side at the biceps, triceps, subscapular, iliac crest, supraspinale, abdominal, front thigh, and medial calf sites according to established protocol [34]. Subjects wore swimwear, no shoes, and were in a postabsorptive state. All measures were performed in triplicate and the median value was used.

Blood Samples and Analytical Methodology for Hormones

Ten milliliters of blood were drawn into a glass serum separator tube by venipuncture of the antecubital vein between 0800 and 1200 hours following an overnight fast of at least 10 hours and abstinence from alcohol for at least 5 days. Blood was drawn in the midfollicular phase (on the 7th or 8th day of the menstrual cycle). Serum was obtained by centrifugation at 3300 rpm for 15 minutes (room temperature); aliquots of serum were stored at −20°C until analyzed (1–4 months).

All blood analyses were performed on serum specimens. Total serum testosterone (T) and cortisol concentrations were determined by commercially purchased solid phase ^{125}I-radioimmunoassay kits (Coat-a-Count; DPC Diagnostic Products Corp., Los Angeles, CA). Total serum SHBG and estradiol (E_2) concentrations were determined by commercially purchased solid phase fluoroimmunoassay kits (DELFIA, Wallac Oy, Turku, Finland SF). There was one assay series each for testosterone and cortisol, and two each for SHBG and estradiol; an equal number of samples from each of the smoking and nonsmoking groups was included for each of the latter two assay series. All assays were performed in duplicate, and individual assays were monitored by quality control samples provided with each kit. Intraassay coefficients of variation for testosterone and cortisol were less than 4%. Intraassay coefficients of variation were less than 6% for SHBG and less than 3% for estradiol. Interassay coefficients of variation for SHBG and estradiol were both ~8%.

Measurement of BMD

BMD (g/cm^2) was measured at the lumbar spine (L2–L4) and the right proximal femur (neck, trochanter, and Ward's triangle) using dual energy X-ray absorptiometry (Model DPX, Lunar Corp., Madison, WI). Accuracy and precision of this model have been previously reported [35, 36]. Whole body BMD was also determined. The same technologist performed all analyses.

Statistical Analysis

For each subject, the sum of all eight skinfolds (SSF) was determined. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). The following endocrine indicators were derived: T/SHBG (free androgen index), E_2/SHBG (free estradiol index), and the ratio T/E_2.

To improve kurtosis and positive skewness, all hormonal variables were transformed to logarithmic (base 10) values for analysis (T, E_2, T/E_2 ratio, SHBG, T/SHBG, E_2/SHBG, and cortisol). Parametric techniques were used for statistical analysis of the transformed values. Means and confidence intervals (95%) of back-transformed values are reported; the confidence limits were determined from standard errors of the transformed values, and are therefore slightly asymmetric. Nonparametric comparisons (not reported) of the original, untransformed, variables using the Mann-Whitney U-test, yielded conclusions identical to those of the parametric tests. BMD measurements were normally distributed and did

Table 1. Characteristics of premenopausal smokers and nonsmokers

	Nonsmokers (n = 27)	Smokers (n = 25)	
	Mean ± SD	Mean ± SD	*P* value[a]
Age (yr)	28.7 ± 5.2	29.5 ± 3.6	0.373
Weight (kg)	58.8 ± 7.4	59.7 ± 8.9	0.634
Height (cm)	164.3 ± 6.3	166.0 ± 6.3	0.233
Body mass index (kg/m^2)	21.8 ± 2.1	21.7 ± 3.2	0.903
Sum of skinfolds (mm)	107.8 ± 41.8	114.9 ± 46.7	0.569
Age at menarche (yr)	13.1 ± 1.2	13.4 ± 1.4	0.487

[a] Two-tailed independent *t* test

not require transformation; means and standard errors are reported. SuperANOVA (©1989 Abacus Concepts Inc.) and StatView SE + Graphics (©1987 Abacus Concepts Inc.) software was used for statistical analysis of the data on a Macintosh® SE/30 microcomputer (Apple Computer, Inc.). Statistical significance was set at the 0.05 level of probability.

Descriptive characteristics were tested for differences between groups by Student's *t* test. Between-groups differences in BMD and endocrine variables (considered dependent) for smokers and nonsmokers were tested first by two-tailed independent *t* test and then by two-way analysis of covariance (ANCOVA). The covariate for analyses of BMD was body weight; BMD at the lumbar spine and proximal femur is significantly correlated with body weight in men [37], and preliminary analysis of the present sample also showed significant correlations at the same sites ($P < 0.01$). For endocrine variables, the covariate was SSF; adipose tissue is the dominant site for the peripheral aromatization of androgens to estrogens [38], serum levels of estrogens and aromatization rates of androgens to estrogens correlate with adiposity in women [39], and cortisol production rates increase as a function of overweight [40]. As an indicator of overall adiposity, we considered SSF a covariate superior to the BMI [41]. Body weight and SSF values were transformed to logarithmic (base 10) values for use as covariates; the untransformed values were skewed to the right. Linearity of dependence relationships was established by evaluating plots of residuals. A first-order smoking by covariate interaction term was included for analysis of variances (ANCOVAs).

The relationship of smoking with endocrine variables and BMD, between the smoking and nonsmoking groups, was tested for interaction by assessing homogeneity of slopes, where hormonal variables were considered independent and BMD dependent. A factorial ANOVA model was constructed which allowed testing of smoking status and a hormonal parameter plus an interaction term containing these main effects against BMD at each specified region. Main effects were adjusted for body weight, and dependent group means were contrasted at the mean value for body weight.

Results

The mean age of participants was 29.1 ± 4.5 years (mean age ± SD) (range 20–35). For smokers, the mean number of cigarettes smoked per day was 16.9 ± 6.3 (range 8–30); the mean smoking duration was 12.9 ± 4.5 years (range 5–21); and the mean age of initiation of smoking was 16.9 ± 2.9 years (range 13–28). Smokers did not differ significantly from nonsmokers for any level of planned physical activity. There were no significant differences between groups for age, weight, height, BMI, SSF, or age at menarche (Table 1).

Endocrine Profiles

The contribution of smoking to variance in endocrine variables is summarized in Table 2. For unadjusted variables, there were no significant differences between groups for serum testosterone, estradiol, the ratio T/E_2, SHBG, T/SHBG, or cortisol, but E_2/SHBG was significantly lower in smokers ($P < 0.02$). There was a trend toward lower serum estradiol in the smokers ($P = 0.077$). For adjusted variables, there were no significant differences between groups for serum testosterone, estradiol, the ratio T/E_2, T/SHBG, E_2/SHBG, or cortisol. However, adjusted SHBG was significantly greater in smokers ($P < 0.01$). No significant contribution to the variance in any endocrine variable was made by SSF (the covariate), although significance was approached when testosterone was dependent ($P = 0.07$), but a significant smoking by SSF interaction was observed with SHBG ($P < 0.01$) (not shown in Table 2). Simple regression analysis, for the sample group overall, indicated a significant relationship between estradiol (independent) and SHBG (dependent) ($P < 0.002$); estradiol explained 17.3% of the variance in SHBG.

BMD Measurements

Table 3 summarizes the contribution of smoking to variance in BMD by region. For unadjusted variables, there were no significant differences between groups for whole body BMD; differences at the lumbar spine (L2–L4) and right proximal femur (neck, trochanter, and Ward's triangle regions) were not significant. Adjustment for body weight showed a trend to higher BMD at the femoral neck in smokers ($P = 0.074$), but there were no significant differences between groups at any region. Significant portions of the variance in BMD were explained by body weight for the whole body ($P < 0.01$) and lumbar spine (L2–L4) ($P < 0.02$). None of the smoking by body weight interaction terms were significant (not shown in Table 2).

Relationship of Smoking and Endocrine Variables to BMD

Main effects contributions (by factor) to variance in BMD were as follows. Smoking, as a single main effect, was not found to contribute significantly to variance in BMD, when endocrine variables were also considered as factors. However, several endocrine variables made significant contributions to variance in BMD: (1) SHBG levels contributed significantly to the whole body ($P < 0.005$), femoral neck ($P < 0.002$), trochanter ($P < 0.005$), and Ward's triangle ($P < 0.005$) regions; (2) the ratio T/SHBG contributed significantly to the whole body ($P < 0.005$), femoral neck ($P < 0.005$), trochanter ($P < 0.002$), and Ward's triangle ($P < 0.005$) regions; and (3) the ratio E_2/SHBG contributed significantly to the whole body region ($P < 0.05$). There were no significant interactions of smoking with any endocrine variable for any bone region. Body weight consistently explained significant portions of the variance in BMD for the whole body and lumbar spine (L2–L4) regions, but not any other.

As there was no significant influence of smoking on BMD, or any significant smoking by endocrine variable interaction at any region, the results were pooled, and the nature of the relationships of SHBG, T/SHBG, and E_2/SHBG with BMD were explored for the entire sample by linear regression. Endocrine variables were considered independent, and BMD dependent. There were significant ($P < 0.002$) inverse relationships between SHBG and BMD at every region of interest except the lumbar spine; the slope of the regression line for the lumbar spine was negative, but the relationship of BMD with SHBG was insignificant. Correlation coefficients for relationships between SHBG and BMD for the whole body and proximal femur sites ranged from −0.42 to −0.48. There were positive relationships of T/SHBG with BMD at all regions of interest; except for the

Table 2. Serum levels of steroid hormones and sex hormone-binding globulin in 52 premenopausal women according to smoking status[a]

	Mean (95%) confidence intervals)		
	Nonsmokers (n = 27)	Smokers (n = 25)	*P* value
Testosterone (nmol/liter)			
Unadjusted[b]	1.589 (1.463, 1.724)	1.530 (1.409, 1.661)	0.649
Adjusted for sum of skinfolds[c]	1.608 (1.487, 1.740)	1.528 (1.413, 1.652)	0.159
Estradiol (pmol/liter)			
Unadjusted[b]	307.3 (274.6, 343.9)	250.9 (224.2, 280.7)	0.077
Adjusted for sum of skinfolds[c]	310.7 (277.9, 347.3)	252.0 (225.4, 281.6)	0.206
Testosterone/Estradiol ratio[d]			
Unadjusted[b]	5.169 (4.593, 5.817)	6.098 (5.419, 6.863)	0.168
Adjusted for sum of skinfolds[c]	5.175 (4.590, 5.835)	6.064 (5.378, 6.837)	0.780
SHBG (nmol/liter)[e]			
Adjusted[b]	57.11 (49.06, 66.48)	62.44 (53.65, 72.68)	0.409
Adjusted for sum of skinfolds[c]	57.85 (52.29, 64.00)	63.38 (57.28, 70.11)	0.006
Testosterone/SHBG ratio (×1000)[d]			
Unadjusted[b]	27.81 (24.75, 31.26)	24.50 (21.80, 27.53)	0.283
Adjusted for sum of skinfolds[c]	27.80 (24.77, 31.18)	24.12 (21.49, 27.05)	0.123
Estradiol/SHBG ratio (×1000)[d]			
Unadjusted[b]	5.381 (4.802, 6.030)	4.018 (3.585, 4.503)	0.013
Adjusted for sum of skinfolds[c]	5.371 (4.790, 6.022)	3.976 (3.546, 4.458)	0.196
Cortisol (nmol/liter)			
Unadjusted[b]	381.5 (342.7, 424.7)	383.3 (342.9, 428.6)	0.951
Adjusted for sum of skinfolds[c]	379.8 (341.0, 422.9)	385.5 (344.6, 431.3)	0.921

[a] Statistical analysis of $\log_{10}$ transformed values; means and confidence limits are back-transformed to the natural scale
[b] Two-tailed independent *t* test
[c] Data adjusted for sum of skinfolds by analysis of covariance
[d] Ratios are unitless (nmol × liter^{-1}/nmol × liter^{-1})
[e] SHBG denotes sex hormone-binding globulin

Table 3. Bone mineral density in 52 premenopausal women according to smoking status

	Nonsmokers (n = 27) Mean ± SE	Smokers (n = 25) Mean ± SE	*P* value
Whole body (g/cm^2)			
Unadjusted[a]	1.150 ± 0.014	1.162 ± 0.017	0.599
Adjusted for weight[b]	1.151 ± 0.014	1.160 ± 0.014	0.140
Lumbar spine (g/cm^2)			
Unadjusted[a]	1.197 ± 0.018	1.225 ± 0.025	0.361
Adjusted for weight[b]	1.199 ± 0.020	1.223 ± 0.021	0.700
Femoral neck (g/cm^2)			
Adjusted[a]	0.995 ± 0.022	1.033 ± 0.021	0.224
Adjusted for weight[b]	0.994 ± 0.021	1.030 ± 0.022	0.074
Trochanter (g/cm^2)			
Unadjusted[a]	0.839 ± 0.023	0.866 ± 0.028	0.465
Adjusted for weight[b]	0.839 ± 0.025	0.864 ± 0.026	0.306
Ward's triangle (g/cm^2)			
Unadjusted[a]	0.950 ± 0.027	0.981 ± 0.027	0.416
Adjusted for weight[b]	0.949 ± 0.027	0.979 ± 0.028	0.173

[a] Two-tailed independent *t* test
[b] Data adjusted for weight by analysis of covariance

lumbar spine, all relationships were significant ($P < 0.02$), and correlation coefficients ranged from 0.33 to 0.46. The slopes of the regression lines for E_2/SHBG with all BMD regions were positive, but the only significant relationship observed was with whole body BMD ($P < 0.05$, r = 0.32).

Discussion

The data demonstrate no significant effect of smoking on BMD at any region in young, premenopausal women. This lack of an effect of cigarette smoking on BMD or content agrees with the results of several other investigations involving premenopausal women [2, 11–13, 17]. However, the extent to which the severity of smoking (e.g., duration of habit, mean number of cigarettes smoked per day) influences BMD remains to be resolved. There might be a dose-response relationship between cigarette usage and BMD: those investigators reporting effects of smoking on BMD have typically observed them in "heavy," as opposed to "moderate" or "light," smokers [7, 15, 18]. Our results and those of other studies observing a lack of effect of smoking have generally concerned moderate (less than 20 cigarettes per day) smokers.

We observed no significant differences in BMD between smokers and nonsmokers whether the results were unadjusted or adjusted for body weight (by analysis of covariance). However, as there was no group difference in body weight such as is usually seen with smoking, it is possible that the effect of smoking on BMD may be mediated through its effect on body weight. Also, the groups were not different in overall body fatness, though the smokers had a more android distribution (reported elsewhere) [42]. We ruled out the influence of physical activity by screening highly active women from the study, and checking for possible differences between smokers and nonsmokers in moderate or lower levels of physical activity (there were none).

Another unexpected observation was higher serum concentration of SHBG in smokers ($P < 0.01$). High serum levels of SHBG are normally associated with estrogenic dominance, and low levels are associated with androgenic dominance [43]. Thus, observations of elevated SHBG in female smokers imply greater serum levels of estrogens and/or lower serum levels of androgens, yet we observed a trend to lower estrogen relative to androgen levels. Unadjusted, serum testosterone levels were about 4% lower in smokers, estradiol was lower by almost 18%, and the ratio T/E_2 was greater by almost 18% (Table 2). Adjustment for fatness had little impact (1%) on these values. Several other studies have

observed significantly greater serum concentrations of androgens in female smokers [44–47], but none observed a significant difference in serum levels of estradiol or estrone. However, all samples were composed of postmenopausal women. Young women have not been studied sufficiently: a study of older premenopausal women (mean age of about 50 years) found no effect of smoking on serum estrone or estradiol, but a nonsignificant trend toward greater serum testosterone in smokers was observed [46]. Two investigations of pregnant smokers and nonsmokers [30, 48] found smokers to have significantly lower serum estrone and estradiol concentrations.

There is only one other report in the literature on SHBG levels in premenopausal smokers and nonsmokers. Moore et al. [49] found smokers to be characterized by greater serum levels of SHBG ($P = 0.051$), as we did, but did not investigate serum levels of sex steroids. In postmenopausal women, two studies have failed to find differences in serum SHBG levels between smokers and nonsmokers [45, 50], but the latter observed a trend toward higher SHBG and lower estimated free estradiol (E_2/SHBG) in smokers [45]. The available evidence suggests that in female smokers (1) androgen/estrogen balance is shifted in favor of androgens, (2) smoking has differential effects on levels of SHBG and sex steroids, and (3) factors other than reproductive hormones regulate the concentration of SHBG.

We observed no significant difference in serum cortisol level between smokers and nonsmokers. This is in keeping with the lack of a significant difference in BMD between groups, as hypercortisolism may be connected to risk of osteoporosis [44, 51]. Other investigators have reported significantly elevated plasma cortisol precursor levels [44] in habitual smokers, and that smoking-induced secretion of adrenocorticotropin (ACTH) [52] results in significant increases in plasma cortisol levels in habitual smokers [53], but an effect of smoking on bone via direct effects on serum cortisol levels remains to be demonstrated.

In view of the differential effect of smoking on SHBG and sex steroid levels, it is interesting that the only significant endocrine relationships with BMD concerned SHBG (i.e., serum levels of SHBG, or the free hormone estimates T/SHBG and E_2/SHBG). Smoking did not interact with any endocrine variable in effects on BMD, indicating that the relationships we observed did not differ significantly between the smokers and nonsmokers. Our observations of strong inverse relationships between SHBG and all BMD regions save for L2–L4 arise perhaps as a result of the broad range of SHBG levels characterizing the entire sample. That smoking contributed to this broad range of values cannot be ignored, reflecting most certainly characteristics of the sample, but neither can the highly significant inverse relationships between SHBG and BMD be ignored, especially as the relationship between these two variables was the same for both groups. Similar findings have been reported previously. Wild et al. [54], using computed tomography, found an inverse relationship between vertebral BMD and SHBG binding capacity in postmenopausal women. Van Hemert et al. [55] measured relative metacarpal cortical area and its change over 9 years in 746 postmenopausal women, finding significant inverse relationships with SHBG and positive relationships with estradiol, and concluded that SHBG had a "bone wasting" effect.

Though serum levels of estradiol and testosterone were not related to BMD at any region in the present study, estimated free levels of these hormones were related to BMD at some regions. The ratio T/SHBG was positively related to BMD at the whole body and proximal femur regions, suggesting that free androgens have a favorable effect on BMD; other investigations have also observed apparently protective effects of testosterone on bone [4, 56]. Surprisingly, the ratio E_2/SHBG was related (positively) only to whole body BMD. This may be due to the inverse relationship between SHBG and BMD, in that an effect of SHBG on bone metabolism could be mediated indirectly by the negative influence of SHBG on bioavailable estrogen; SHBG could also interfere with estrogen recognition at the target tissue level. However, different regions appeared to be influenced by different factors. Excluding the generic whole body region, BMD at the proximal femur was related to the endocrine factors SHBG and T/SHBG, not body weight, whereas BMD at the lumbar spine was consistently related to body weight, not endocrine factors. These observations suggest that the factors regulating BMD differ throughout the body.

In summary, we found no significant differences in BMD between smokers and nonsmokers for any region of the body. This may be due to the moderate level of smoking or to the lack of a difference in body weight between the groups. Smokers had significantly lower serum levels of estimated free estradiol (unadjusted for fatness) and significantly greater serum SHBG levels (adjusted for fatness). There were no differences between groups in relationships between endocrine factors and BMD. For the overall sample, we observed an inverse relationship between SHBG and BMD.

Acknowledgments. The authors express their gratitude to Thomas H. Hassard, Ph.D., Director, Biostatistical Consulting Unit, Faculty of Medicine, University of Manitoba, for statistical assistance. We are also indebted to Charles Faiman, M.D., Section of Endocrinology and Metabolism, Faculty of Medicine, University of Manitoba, for advice regarding sex steroid assays, and to Mr. Derek Grant, Endocrinology and Metabolism Laboratory, Winnipeg Health Sciences Centre, for technical assistance.

References

1. Kelsey JL (1989) Risk factors for osteoporosis and associated fractures. Public Health Rep 104(suppl):14–20
2. Daniell HW (1976) Osteoporosis of the slender smoker: vertebral compression fractures and loss of metacarpal cortex in relation to postmenopausal cigarette smoking and lack of obesity. Arch Int Med 136:298–304
3. Williams AR, Weiss NS, Ure CL, Ballard J, Daling JR (1982) Effect of weight, smoking, and estrogen use on the risk of hip and forearm fractures in postmenopausal women. Obstet Gynecol 60:695–699
4. Aloia JF, Cohn SH, Vaswani A, Yeh JK, Yuen K, Ellis K (1985) Risk factors for postmenopausal osteoporosis. Am J Med 78:95–100
5. Wickham CA, Walsh K, Cooper C, Barker DJ, Margetts BM, Morris J, Bruce SA (1989) Dietary calcium, physical activity, and risk of hip fracture: a prospective study. BMJ 299:889–892
6. Slemenda CW, Hui S, Longcope C, Wellman H, Johnston CC (1990) Predictors of bone mass in perimenopausal women: a prospective study of clinical data using photon absorptiometry. Ann Int Med 112:96–101
7. Mazess RB, Barden HS (1991) Bone density in premenopausal women: effects of age, dietary intake, physical activity, smoking, and birth control pills. Am J Clin Nutr 53:132–142
8. Hooper JL, Seeman E (1990) Tobacco use and reduced bone density: a co-twin control study. In: Christiansen C, Overgaard K (eds) Osteoporosis 1990, Vol 2. Osteopress ApS, Copenhagen, pp 1117–1118
9. Jensen GF (1986) Osteoporosis of the slender smoker revisited by epidemiologic approach. Eur J Clin Invest 16:239–242
10. Holbrook TL, Barrett-Connor E, Wingard DL (1988) Dietary calcium and risk of hip fracture: 14-year prospective population study. Lancet 2:1046–1049

11. Bilbrey GH, Weix J, Kaplan GK (1988) Value of single photon absorptiometry in osteoporosis screening. Clin Nucl Med 13:7–12
12. McDermott MT, Witte MC (1988) Bone mineral content in smokers. South J Med 81:477–480
13. Hall ML, Heavens J, Cullum ID, Ell PJ (1990) The range of bone density in normal British women. Br J Radiol 63:266–269
14. Garvey AJ, Bosse R, Seltzer CC (1974) Smoking, weight change, and age: a longitudinal analysis. Arch Environ Health 28:327–329
15. Slemenda CW, Hui SL, Longcope C, Johnston CC (1989) Cigarette smoking, obesity, and bone mass. J Bone Miner Res 4:737–741
16. Stevenson JC, Lees B, Devenport M, Cust MP, Ganger KF (1989) Determinants of bone density in normal women: risk factors for future osteoporosis? Br Med J 298:924–928
17. McCulloch RG, Bailey DA, Houston CS, Dodd BL (1990) Effects of physical activity, dietary calcium intake and selected lifestyle factors on bone density in young women. Can Med Assoc J 142:221–227
18. Whiting SJ, McCulloch RG, Bailey DA, Houston CS (1990) The effect of cigarette smoking on trabecular bone density in premenopausal women aged 20–35 years (abstract). J Bone Miner Res 5(suppl):S249
19. Cauley JA, Gutai JP, Sandler RB, LaPorte RE, Kuller LH, Sashin D (1986) The relationship of endogenous estrogen to bone density and bone area in normal postmenopausal women. Am J Epidemiol 124:752–761
20. Slemenda C, Hui SL, Longcope C, Johnston CC (1987) Sex steroids and bone mass. A study of changes about the time of menopause. J Clin Invest 80:1261–1269
21. Horsman A, Jones M, Francis R, Nordin C (1983) The effect of estrogen dose on postmenopausal bone loss. N Engl J Med 309:1404–1407
22. Lindsay R, Tohme JF (1990) Estrogen treatment of patients with established postmenopausal osteoporosis. Obstet Gynecol 76:290–295
23. Jensen J, Christiansen C, Rodbro P (1985) Cigarette smoking, serum estrogens, and bone loss during hormone-replacement therapy early after menopause. N Engl J Med 313:973–975
24. Kaufman DW, Slone D, Rosenberg L, Meittinen OS, Shapiro S (1980) Cigarette smoking and age at natural menopause. Am J Public Health 70:420–422
25. Hartz AJ, Kelber S, Borkowf H, Wild R, Gillis BL, Rimm AA (1987) The association of smoking with clinical indicators of altered sex steroids—a study of 50,145 women. Pub Health Rep 102:254–259
26. Lesko SM, Rosenberg L, Kaufman DW, Helmrich SP, Miller DR, Strom B, Schottenfeld D, Rosenshein NB, Knapp RC, Lewis J, Shapiro S (1985) Cigarette smoking and the risk of endometrial cancer. N Engl J Med 313:593–596
27. Vessey M, Baron J, Doll R, McPherson K, Yates D (1983) Oral contraceptives and breast cancer: final report of an epidemiological study. Br J Cancer 47:455–462
28. Tokuhata G (1968) Smoking in relation to infertility and fetal loss. Arch Environ Health 17:353–359
29. Garn SM (1968) Smoking and human biology. Hum Biol 57:505–523
30. Mochizuki M, Maruo T, Masuko K, Ohtsu T (1984) Effects of smoking on fetoplacental-maternal system during pregnancy. Am J Obstet Gynecol 149:413–420
31. Barbieri RL, McShane RM, Ryan KJ (1986) Constituents of cigarette smoke inhibit human granulosa cell aromatase. Fertil Steril 46:232–236
32. Michnovicz JJ, Herschcopf RJ, Naganuma H, Bradlow HL, Fishman J (1986) Increased 2-hydroxylation of estradiol as a possible mechanism for the anti-estrogenic effect of cigarette smoking. N Engl J Med 315:1305–1309
33. Albanes D, Jones Y, Micozzi MS, Mattson ME (1987) Associations between smoking and bodyweight in the US population: analysis on NHANES II. Am J Pub Health 77:439–444
34. Ross WD, Marfell-Jones MJ (1982) Kinanthropometry. In: McDougall SD, Wenger HA, Green HA (eds) Physiological testing of the elite athlete. Mutual, Ottawa, pp 75–115
35. Mazess RB, Trempe JA, Bisek JP, Hanson JA, Hans D (1991) Calibration of dual-energy X-ray absorptiometry for bone density. J Bone Miner Res 6:799–806
36. Mazess RB, Collick B, Trempe JA, Barden HS, Hanson JA (1989) Performance evaluation of a dual energy X-ray bone densitometer. Calibration of dual-energy X-ray absorptiometry for bone density. Calcif Tissue Int 44:228–232
37. Mazess RB, Barden HS, Drinka PJ, Bauwens SF, Orwoll ES, Bell NH (1990) Influence of age and body weight on spine and femur bone mineral density in U.S. white men. J Bone Miner Res 5:645–652
38. Perel E, Killinger DW (1979) The interconversion and aromatization of androgens by human adipose tissue. J Steroid Bioch 10:623–627
39. Vermeulen A, Verdonck L (1979) Factors affecting sex hormone levels in postmenopausal women. J Steroid Bioch 11:899–904
40. Mlynaryk P, Gillies RR, Murphy B, Pattee CJ (1962) Cortisol production rates in obesity. J Clin Endocrinol Metab 22:587–591
41. Garn SM, Leonard WR, Hawthorne VM (1986) Three limitations of the body mass index (letter). Am J Clin Nutr 44:996–997
42. Daniel M, Martin AD, Faiman C (1992) Sex hormones and adipose tissue distribution in pre-menopausal cigarette smokers. Int J Obesity (in press)
43. Vermeulen A, Verdonck L, Van Der Staten M, Orie N (1969) Capacity of the testosterone-binding globulin in human plasma and influence of specific binding of testosterone on its metabolic clearance rate. J Clin Endocrinol Metab 29:1470–1477
44. Friedman AJ, Ravnikar VA, Barbieri RL (1987) Serum steroid hormone profiles in postmenopausal smokers and nonsmokers. Fertil Steril 47:398–401
45. Khaw K-T, Tazuke S, Barret-Connor E (1988) Cigarette smoking and levels of adrenal androgens in postmenopausal women. N Engl J Med 318:1705–1709
46. Longcope C, Johnston CC (1988) Androgen and estrogen dynamics in pre- and postmenopausal women: a comparison between smokers and nonsmokers. J Clin Endocrinol Metab 67:379–383
47. Cauley JA, Gutai JP, Kuller LH, LeDonne D, Powell JG (1989) The epidemiology of serum sex hormones in postmenopausal women. Am J Epidemiol 129:1120–1131
48. Bernstein L, Pike MC, Lobo RA, Depue RH, Ross RK, Henderson BE (1989) Cigarette smoking in pregnancy results in marked decrease in maternal hCG and oestradiol levels. Br J Obstet Gynecol 96:92–96
49. Moore JW, Key TJA, Bulbrook RD, Clark GMG, Allen DS, Wang DY, Pike MC (1987) Sex hormone binding globulin and risk factors for breast cancer in a population of normal women who had never used exogenous sex hormones. Br J Cancer 56:661–666
50. Lapidus L, Lindstedt G, Lundberg P-A (1986) Concentrations of sex-hormone binding globulin and corticosteroid binding globulin in serum in relation to cardiovascular risk factors and to 12-year incidence of cardiovascular disease and overall mortality in postmenopausal women. Clin Chem 32:146–152
51. Baron JA (1984) Smoking and estrogen-related disease. Am J Epidemiol 119:9–22
52. Seyler LEJ, Pomerleau OF, Fertig JB, Hunt D, Parker K (1986) Pituitary hormone response to cigarette smoking. Pharmacol Biochem Behav 24:159–162
53. Gossain VV, Sherma NK, Srivastava L, Michelakis AM, Rovner DR (1986) Hormonal effects of smoking—II: effects on plasma cortisol, growth hormone, and prolactin. Am J Med Sci 291:325–327
54. Wild RA, Buchanan JR, Myers C, Lloyd T, Demers LM (1987) Adrenal androgens, sex hormone binding globulin and bone density in osteoporotic menopausal women. Maturitas 9:55–61
55. Van Hemert AM, Birkenhäger JC, De Jong FH, Vandenbroucke JP, Valkenburg HA (1989) Sex hormone binding globulin in postmenopausal women: a predictor of osteoporosis superior to endogenous oestrogens. Clin Endocrinol 31:499–509
56. Horsman A, Marshall DH, Nordin BEC (1981) The relation between bone loss and calcium balance in women. Clin Sci 59:137–142