Effects of Smoking on Tibial and Radial Bone Mass and Strength May Diminish with Age

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Purpose: The purpose of the study was to assess the effect of cigarette smoking on indicators of bone strength across a wide age range, controlling for physical activity and neuromuscular performance.

Methods: We conducted a cross-sectional study with 41 smokers (mean age ± SD, 41.0 ± 16.1 yr) and 53 nonsmokers (47.5 ± 18.2 yr) of both sexes. Bone strength indicators (BSI) were assessed in the lower leg and forearm by peripheral quantitative computed tomography along with physical activity, muscle cross-sectional area, and maximal voluntary muscle force.

Results: Physical activity level and muscle cross-sectional area of the leg and arm were similar in smokers and nonsmokers. Although trabecular volumetric bone mineral density and epiphyseal bone mineral content, both indicators of BSI, decreased with age in the nonsmokers' tibia ($P < 0.001$), this was not observed in the smokers (interaction age × smoking: $P = 0.014$ and $P = 0.032$ for density and content, respectively). Regression coefficients were nonsignificant in nonsmokers, whereas coefficients in smokers were -1.24 mg/cm · yr [95% confidence interval (CI) = -2.16–0.33; $P = 0.01$] for content and -1.20 mg/cm^3 · yr (95% CI = -1.76–0.62; $P < 0.001$) for trabecular density. The BSI values in the smokers were independent of their smoking history ($r^2 = 0.000$–0.021), and no effects of sex were observed in the smoking-related differences in BSI.

Conclusions: Smoking compromises bone strength by diaphyseal marrow cavity expansion and epiphyseal trabecular bone content reductions. These effects seem to wane with age. The causes of the attenuated effect of smoking on bone at old age remain enigmatic but might be linked to an interaction between the smoke-related factors and senescence processes affecting bone. **(*J Clin Endocrinol Metab* 95: 0000–0000, 2010)**

Half a century ago, Doll and Hill proposed (1) and then demonstrated (2) that cigarette smoking elicits lung cancer. Nowadays, a host of disorders is known that are either caused or aggravated by smoking, such as coronary heart disease, peripheral vascular disease (3), and chronic obstructive pulmonary disease (COPD) (4).

A possible link between smoking and osteoporosis was first proposed by Daniell in 1976 (5), and there is now ample evidence to suggest that smoking does indeed increase the risk of fractures (6–8). This elevated fracture risk may be attributable to a reduction in bone strength, because areal bone mineral density (aBMD), as assessed by

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Abbreviations: aBMD, Areal bone mineral density; BSI, bone strength indicators; CI, confidence interval; COPD, chronic obstructive pulmonary disease; FEV_{1pred}, predicted forced expiratory volume in 1 sec; pQCT, peripheral quantitative computed tomography; vBMD, volumetric bone mineral density.

dual x-ray absorptiometry, is reduced in smokers at the hip (9–11), the radius (12, 13), and the lumbar spine (10, 11).

It has to be considered, however, that dual x-ray absorptiometry does not allow for a detailed analysis of bone geometry. It is therefore unclear from the above studies whether smoking affects the material properties of bone (*e.g.* degree of mineralization) and/or the morphology of the bone (*e.g.* geometrical measures). Peripheral quantitative computed tomography (pQCT) (14), which measures true volumetric bone mineral density (vBMD) and thus allows the assessment of several bone strength indicators (BSI), has recently been applied by Lorentzon *et al.* (15) to address the question of how smoking affects bone. They observed in young healthy male smokers a 4–5% reduction in lumbar spine and femoral neck aBMD and in distal tibia vBMD and cortical tibial area compared with nonsmokers, confirming that smoking does affect bone at the tissue level. Similar results have been obtained for the radius of middle-aged (approximately 40 yr) men (16). It is well known that the risk for osteoporotic fractures increases with age. A metaanalysis by Law and Hackshaw (17) suggests that "the effect of smoking on [areal] bone mineral density increases cumulatively with age." Yet, Kanis *et al.* (8) found that the increased risk of hip fracture related to smoking decreased with age. Hence, it is equivocal whether the effects of smoking on bone are aggravated with age or not. Part of the discrepancies between studies might be related to sex, as reflected by the reduced radial cortical area in smoking women but not in men (18), or different levels of physical activity (19). Thus, a better understanding of the interplay between aging and the effects of smoking upon bone is required. To this purpose, we have organized a case-control study to compare the effects of cigarette smoking upon forearm and lower leg bone measures, as assessed by pQCT, in healthy smokers and nonsmokers across the age range controlling for the level of physical activity. We hypothesized that the effects of smoking would be aggravated with increasing age and that women would be affected to a greater extent compared with men.

Participants and Methods

Participants

In total, 94 Caucasian adults volunteered for the study. Participants were recruited from the local community. Nonsmokers had never smoked in their lives. Participants were excluded if they had been diagnosed with cardiovascular diseases (such as stroke and chronic heart failure), arthritis, or used medications known to affect bone metabolism (*e.g.* bisphosphonate therapy, calcium supplementation, and hormone replacement therapy). Ex-smokers, potentially pregnant women, and people suffering from COPD were also excluded. To ensure the latter, all participants underwent standard spirometry, and participants with a predicted forced expiratory volume in 1 sec (FEV_{1pred}) of less than70% were excluded from further study. For pQCT, the right lower leg and the right forearm were tested, unless they had been fractured in the past, in which case the left limb was tested (n = 7).

All participants gave written informed consent, and procedures were approved by the Local Ethics Committee and were in accordance with the Helsinki Declaration.

Assessment of smoking history

Smoking history was assessed by questionnaire. Smoking volume was given as cigarette pack-years, defined as the number of cigarette packs smoked per day multiplied by the number of years smoked. Changes in smoking habits between the past and current dose were taken into account.

Assessment of physical activity

Because exercise is nowadays believed to have important beneficial effects on bone health (19), and muscle contractions seem to play an important role in skeletal integrity (20, 21), we have matched smokers (n = 41) and nonsmokers (n = 53) for both their physical activity level and muscle force-generating capacity. The level of physical activity was assessed by the Baecke questionnaire (22). Values less than 7 represent a low physical activity level.

Muscle strength

A subgroup of participants (n = 62) also underwent measurements of quadriceps muscle function by standard dynamometry (Cybex norm dynamometer; Ronkonkoma, New York, NY). The results of these experiments have been published elsewhere (23). In short, quadriceps muscle cross-sectional area, maximal voluntary force-generating capacity, and contractile properties were similar in smokers and age- and physical activity-matched nonsmokers. In smokers, however, the skeletal muscle resistance to fatigue was significantly lower compared with nonsmokers, irrespective of the duration and intensity of smoking.

Scanning procedures

Scans by pQCT were obtained from the right forearm and lower leg with an XCT2000 (Stratec Medizintechnik, Pforzheim, Germany) as described elsewhere (21, 24). Sectional images with a pixel size of 0.5 mm were obtained from the tibia at 4% (distal epiphysis), 14% (metaphysis), and 38 and 66% (diaphysis) of its length and from the radius at 4 and 60% of the ulna's length. The scans taken at 66% of the tibia length and at 60% of the ulna length were also used to assess gross anatomical muscle cross-sectional area (Ar.Mus).

Image analysis and data processing

The image analysis and data processing are essentially similar to previous studies (21). All scans were visually inspected before further processing. Image analysis was performed according to the manufacturer's recommendations with the integrated XCT software (version 6.00B). The XCT device produces pQCT images where the pixels are representative of vBMD. It is calibrated to hydroxyl apatite, and the zero point is set to the apparent vBMD of olive oil. All XCT analyses were performed with the automated loop facility of the software package. Data were then further processed with Microsoft Access.

TABLE 1. List of pQCT variables and their acronyms

Acronym	Variable
Ar.Bo	Bone cross-sectional area
Ar.Ct	Cortical cross-sectional area
Ar.Epi	Epiphyseal cross-sectional area
Ar.Fat	Subcutaneous fat cross-sectional area
Ar.MB	Combined muscle and bone cross-sectional area
Ar.Mus	Gross muscle cross-sectional area
Ar.tot	Limb's total cross-sectional area
EsC	Endocortical circumference
M:B ratio	Muscle to bone ratio
PsC	Periosteal circumference
RPol	Polar moment of resistance (adjusted for vBMD)
T.Fat	Thickness of subcutaneous fat layer
vBMC	Volumetric bone mineral content
vBMC.tot	Total vBMC normalized for joint size
vBMD	Volumetric bone mineral density
vBMD.Ct	Cortical vBMD
vBMD.tb	Trabecular vBMD

Acronyms are in accordance with the recommendations for high-resolution pQCT by American Society for Bone and Mineral Research (http://nomenclature.bb.asbmr.org).

Using the XCT software, the outer bone contours were identified with the separation threshold set to 650 mg/cm^3 for the metaphysis and diaphysis and to 180 mg/cm^3 for the epiphysis (software option contour mode 1). A list of the pQCT variables is given in Table 1. The following BSI were analyzed. Total volumetric bone mineral content (vBMC.tot) was taken as the TOT_CNT value. For the diaphyseal sites, the polar moment of resistance (RPol) was assessed as the RP_TOT_W value, and cortical vBMD (vBMD.Ct) values were assessed with adjustment for the partial volume effect as previously described (25). Cortical area (Ar.Ct) was taken as the variable CRT_A, and periosteal and endocortical circumferences (PsC and EsC, respectively) were taken as the variables PERI_C and ENDO_C, respectively. For the epiphyseal sites, trabecular vBMD (vBMD.tb) within the central 45% of the epiphysis was also assessed, and the total epiphyseal area (Ar.Epi) was assessed as an estimate of joint size. All values of vBMC.tot were normalized for Ar.Epi unless otherwise stated.

The Ar.Mus was assessed as follows. A region of interest was set to cover the entire image. From this, the total limb cross-sectional area (Ar.tot) and the combined muscle and bone cross-sectional area (Ar.MB) was then assessed with an iterative contour detection algorithm (software option contour mode 3) with the threshold set to −52 mg/cm^3 for Ar.tot and to 41 mg/cm^3 for Ar.MB. In addition, a 5 × 5 smoothing filter was applied for Ar.MB (software option F03F05). The combined bone area (Ar.Bo; radius and ulna for the arm and tibia and fibula for the leg) was segmented with a separation threshold of 650 mg/cm^3, and software option peel mode 2 with the inner threshold set to 100 mg/cm^3 to yield Ar.Bo. Ar.Mus was subsequently computed as Ar.MB − Ar.Bo.

Statistical analyses

All variables were normally distributed. Group means were compared using a univariate ANOVA with sex and smoking classification as independent variables and age and Ar.Mus as covariates. To evaluate the relationship between BSI and smoking as a function of age, multiple linear regression analyses were carried out with age, smoke × age, sex, sex × age, and Ar.Mus (21) as independent predictors. To specifically investigate the effects of aging on BSI, a similar multiple linear regression analysis was carried out but with only the nonsmokers included. Physical activity as a covariate in the model did not improve the regression model and was therefore excluded.

To investigate the effects of smoking history on BSI, cigarettes smoked per week, years smoked, and cigarette pack-years were fed into the model with only the smokers selected. Unstandardized β-values were used. Differences were considered significant if $P < 0.05$. All statistical testing was performed using SPSS version 16.0 (SPSS Inc., Chicago, IL). Results are presented as mean ± SD or mean (95% confidence interval) when accounting for covariates.

Results

Participant characteristics

Table 2 shows the characteristics of the nonsmokers (25 men and 28 women) and smokers (20 men and 21 women). Male and female smokers had smoked for 21.3 ± 20.1 and 27.0 ± 13.0 yr, respectively, and were currently consuming 17 ± 12 and 15 ± 6 cigarettes per day, respectively. Smoking history was similar between men and women. Age, weight, height, physical activity levels, and FEV_{1pred} were comparable in smokers and nonsmokers. However, all smokers had a percent FEV_{1pred} higher than 70%, suggesting that our cohort did not encompass patients with COPD.

Muscle scans by pQCT

For both the forearm and the lower leg, Ar.Mus did not differ between smokers and nonsmokers (Table 2). However, Ar.Mus was smaller in women than men ($P < 0.001$ for both sites). No difference was found in muscle to bone ratio between smokers and nonsmokers. There was no sex-related difference in the muscle to bone ratio in the lower leg, but the ratio was lower in the arm of women than men ($P < 0.001$).

Interactions between physical activity, muscle size, and bone measures

The level of physical activity did not significantly improve the prediction of BSI and was excluded from the statistical analysis. However, participants with a higher Ar.Mus (while controlling for physical activity level) also had a higher vBMD.tb (r = 0.514; $P < 0.001$) and vBMC.tot at the epiphysis (r = 0.760; $P < 0.001$) and diaphysis (r = 0.679; $P < 0.001$). Similar results were obtained for the radius.

Sex differences in bone measures in nonsmokers

Taking into account age- and sex-related differences in Ar.Mus, we found in the nonsmokers group that men had

TABLE 2. Participant's anthropometric characteristics (mean ± SD)

	Male nonsmokers (n = 25)	Female nonsmokers (n = 28)	Male smokers (n = 20)	Female smokers (n = 21)
Age (yr)	42.9 ± 19.4	45.4 ± 17.5	37.8 ± 18.2	39.0 ± 15.3
Height (m)	1.79 ± 0.06	1.64 ± 0.08[b]	1.77 ± 0.06	1.64 ± 0.05[b]
Weight (kg)	77 ± 9	68 ± 14[b]	76 ± 11	70 ± 12[b]
BMI (kg/m^2)	23.9 ± 1.9	24.8 ± 4.8[b]	24.2 ± 2.7	25.6 ± 3.7[b]
Physical activity score	8.4 ± 1.5	8.3 ± 1.2	8.3 ± 1.5	8.1 ± 1.5
Cigarettes per day	0	0	17 ± 12[a]	15 ± 6[a]
Cigarette pack-years	0	0	16.7 ± 20.5[a]	15.0 ± 12.5[a]
FEV_{1pred} (%)	99 ± 13	102 ± 15	90 ± 11	97 ± 15
Calf				
Ar.Mus (cm^2)	83.1 ± 10.1	62.8 ± 9.5[b]	78.4 ± 10.1	61.9 ± 6.5[c]
M:B ratio	10.0 ± 2.0	9.5 ± 1.6	9.6 ± 1.5	10.3 ± 2.8
Forearm				
Ar.Mus (cm^2)	44.1 ± 6.5	27.8 ± 3.2[b]	41.0 ± 5.6	29.4 ± 2.9[c]
M:B ratio	14.0 ± 2.1	12.0 ± 1.7[b]	13.1 ± 1.8	12.7 ± 1.5[c]

[a] Different from nonsmokers ($P < 0.05$).

[b] Different from men ($P < 0.05$).

[c] Different from men ($P < 0.001$).

a similar vBMD.tb in their tibia epiphyses but greater epiphyseal vBMC.tot (unnormalized) ($P < 0.001$) than women (Table 3). The greater joint size in men (Ar.Epi; $P = 0.003$) did largely account for this difference, because vBMC.tot normalized for Ar.Epi was not different between the sexes ($P = 0.073$). Similar effects were found for the radius epiphysis (Table 3).

Group differences of bone measures are demonstrated in Table 3. For the tibia diaphysis, men had a significantly greater vBMC.Ct ($P < 0.001$) and PsC ($P = 0.001$, Table 3) than women. However, no sex differences were found for EsC and vBMD.Ct of the tibia and the radius (Table 3). These differences were associated with greater RPol in the tibia in men compared with

TABLE 3. BSI

	Male nonsmokers (n = 25)	Female nonsmokers (n = 28)	Male smokers (n = 20)	Female smokers (n = 21)
Tibia 4% (epiphysis)				
vBMC.tot (not normalized for Ar.Epi) (mg/mm)	472 ± 58	323 ± 50[a]	438 ± 52[b]	316 ± 60[a]
Ar.Mus (mm^2)	13.3 ± 1.4	11.1 ± 0.9[a]	13.5 ± 2.0	10.4 ± 1.3[a]
vBMC.tot (mg/mm^3)	35.6 ± 4.3	29.1 ± 3.7	32.9 ± 5.0[b]	30.5 ± 3.9
vBMD.tb (mg/cm^3)	277 ± 36	231 ± 33	264 ± 39	234 ± 38
Tibia 38% (diaphysis)				
vBMC.Ct (mg/mm)	462 ± 49	335 ± 56[a]	418 ± 50[b]	333 ± 36[a]
Ar.Ct (mm^2)	375 ± 41	271 ± 44[a]	339 ± 44[b]	267 ± 30[a]
vBMD.Ct (mg/cm^3)	1234 ± 16	1228 ± 32	1231 ± 23	1245 ± 33
RPol (mm^3)	2390 ± 421	1648 ± 225[a]	2189 ± 393	1574 ± 323[a]
EsC (mm)	35.8 ± 5.7	34.9 ± 5.3	36.9 ± 4.8	33.2 ± 5.6
PsC (mm)	77.5 ± 4.6	68.2 ± 3.1[a]	75.0 ± 4.5	66.8 ± 4.6[a]
Radius 4% (epiphysis)				
vBMC.tot (not normalized for Ar.Epi) (mg/mm)	172 ± 24	114 ± 20[a]	162 ± 39	114 ± 14[a]
Ar.Epi (mm^2)	4.5 ± 0.6	3.4 ± 0.5[a]	4.6 ± 1.1	3.5 ± 0.5[a]
vBMC.tot (mg/mm^3)	37.9 ± 4.6	33.7 ± 5.1	36.0 ± 5.4	33.4 ± 4.7
vBMD.tb (mg/cm^3)	236 ± 37	185 ± 31	236 ± 49	200 ± 32
Radius 60% (diaphysis)				
vBMC.tot (mg/mm)	135 ± 17	97 ± 14[a]	132 ± 18	101 ± 14[a]
Ar.Ct (mm^2)	111 ± 14	79 ± 10[a]	109 ± 14	82 ± 12[a]
vBMD.Ct (mg/cm^3)	1262 ± 18	1267 ± 31	1246 ± 25	1271 ± 14
RPol (mm^3)	416 ± 95	270 ± 50	426 ± 100	270 ± 57
EsC (mm)	21.0 ± 3.8	19.8 ± 2.3	21.7 ± 4.8	18.4 ± 3.3
PsC (mm)	42.8 ± 3.1	37.3 ± 2.3[a]	43.0 ± 3.3	37.1 ± 2.3[a]

For explanation of acronyms see Table 1.

[a] $P < 0.05$ compared with men.

[b] $P < 0.05$ compared with nonsmokers, both accounting for age and muscle size.

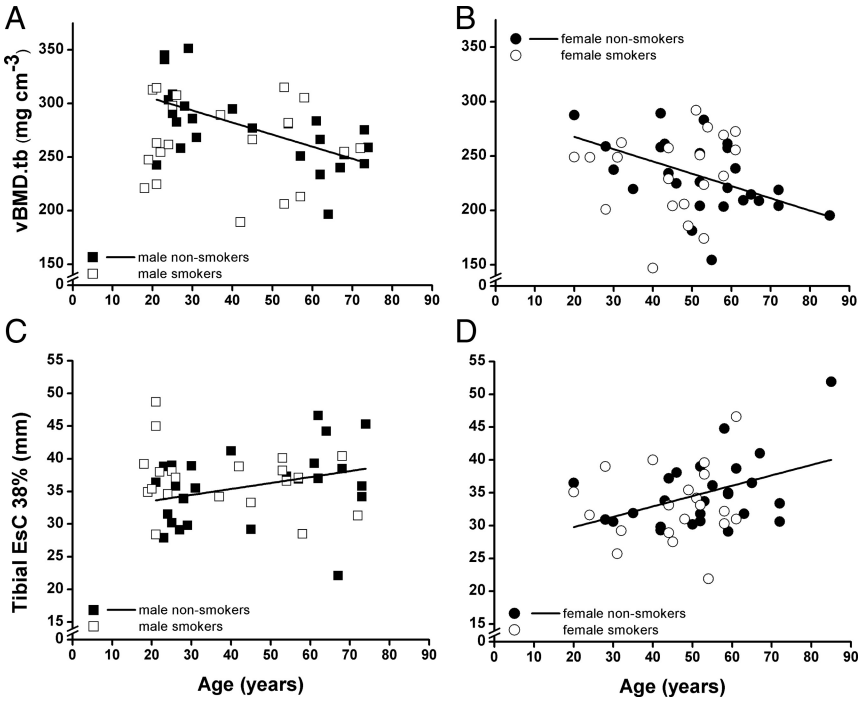

FIG. 1. Trabecular density of the epiphysis (A and B) and endocortical circumference of the diaphysis (C and D) of the tibia for smokers and nonsmokers of men (A and B) and women (B and D). Although trabecular density declined ($P < 0.001$; A and B) and endocortical circumference increased ($P < 0.011$; C and D) with age in the nonsmokers, no such relationship was observed in the smokers. For slopes and 95% CI, see *Results*.

women ($P < 0.001$). In the radius, unadjusted analyses suggested sex-related differences in vBMC.Ct and RPol, which were no longer significant after adjustment for forearm Ar.Mus.

Effects of age in nonsmokers

In nonsmokers, vBMD.tb of the tibia was negatively related to age (slope, -1.12 mg/cm^3 · yr; 95% CI = -1.60 to -0.63; $P < 0.001$; Fig. 1A), and so was vBMC.tot (-0.14 mg/cm · yr; 95% CI = -0.19 to -0.09; $P = 0.001$). Tibia EsC increased with age by 0.11 (95% CI = 0.03 to -0.20) mm · yr ($P = 0.012$; Fig. 1B), whereas Ar.Ct tended to decline with age ($P = 0.068$). Neither PsC nor RPol were found to be correlated with age.

Similar results were obtained for the radius. vBMD.tb was negatively related to age (slope, -0.78 mg/cm^3 · yr; 95% CI = -1.38 to -0.17; $P = 0.013$; Fig. 2A), and a trend was found for vBMC.tot (slope, -0.08 mg/cm · yr; 95% CI = -0.16–0.10; $P = 0.082$). However, no age-related differences in EsC or Ar.Ct were observed in the diaphysis of the radius (Fig. 2B). Similarly, age did not significantly affect PsC of both the tibia and the radius, nor did it affect RPol of both bones. Only after taking into account physical activity as a covariate, the age × sex interaction EsC of the radius was apparent as a significant increase with age in the women only ($P = 0.045$).

Effects of smoking on BSI

There were no significant main effects of smoking on the mean values of BSI (Table 3). However, an interaction effect of smoke × sex was discovered for epiphyseal vBMC.tot ($P = 0.036$) and diaphyseal vBMC.tot ($P = 0.005$), implying lower values in smoking than in nonsmoking men, whereas values were similar between smoking and nonsmoking women (Table 3). Similarly, diaphyseal Ar.Ct in the tibia was lower only in the smoking men compared with their nonsmoking counterparts (smoke × sex interaction, $P = 0.013$). EsC in the male smokers only tended to be higher (smoke × sex interaction, $P = 0.097$), and PsC and RPol were similar in smokers and nonsmokers of both sexes. In the radius, Ar.Ct ($P = 0.093$), EsC, PsC, and RPol were all similar in smokers and nonsmokers, and no smoke × sex interactions were observed.

Interestingly, however, although vBMC.tot and vBMD.tb of the tibial epiphysis decreased with age in the nonsmokers (both $P < 0.001$), this effect was not observed in the smokers (interaction age × smoking, $P = 0.004$ and $P = 0.022$ for v.BMC.tot and vBMD.tb, respectively; Fig. 1A and Table 4). The slope was significant in nonsmokers for vBMC.tot (-0.14 mg/cm · yr; 95% CI = -0.19 to -0.09; $P < 0.001$) and for vBMD.tb (-1.12 mg/cm^3 · yr; 95% CI = -1.60 to -0.63; $P < 0.001$) but not in the smokers [partial correlation, -0.037 ($P = 0.835$) and -0.111 ($P = 0.524$) for vBMC.tot and vBMD.tb, respectively], suggesting that young, but not older, smokers have lower epiphyseal bone strength compared with nonsmokers. Using these values for a sample size estimation, it turned out that over 150 smokers would have had to be tested to find a significant aging effect in the smokers as observed in the nonsmokers in this study.

Likewise for the tibia diaphysis, the observed increase in EsC with age in the nonsmoking participants was not present in smokers (interaction smoke × age, $P = 0.038$). In the smokers, but not in the nonsmokers, the regression coefficient for EsC over age was significant: -0.14 mm/yr (95% CI = -0.52–0.23; $P = 0.436$; Fig. 1B), and the intercept for smokers was 6.85 mm (95% CI = 0.13–13.58; $P = 0.046$) greater than for nonsmokers. No differences in age-related changes were observed in the PsC between smokers and nonsmokers (Table 4). The differ-

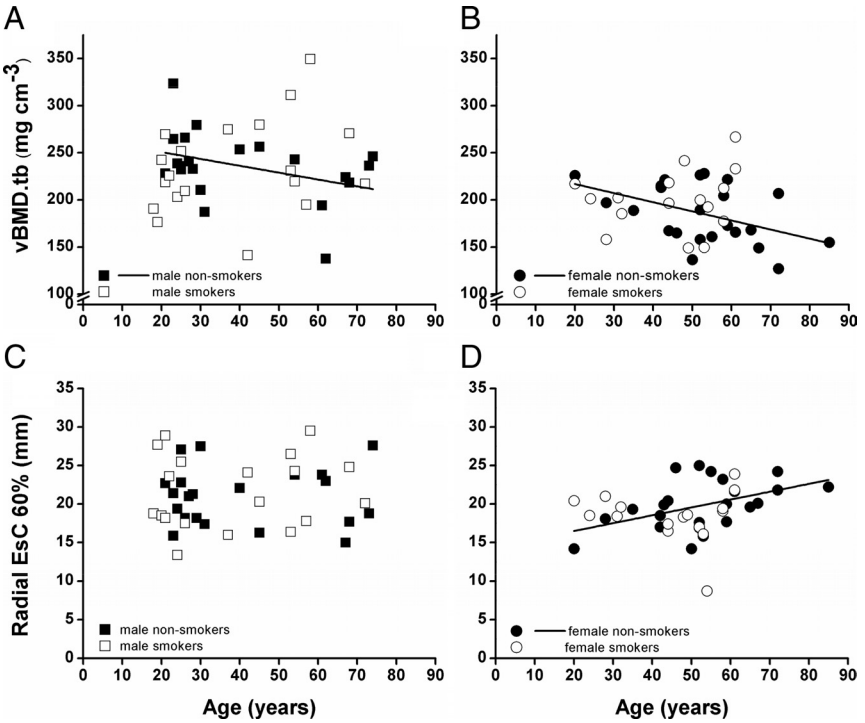

FIG. 2. Trabecular density of the epiphysis (A and B) and endocortical circumference of the diaphysis (C and D) of the radius for smokers and nonsmokers of men (A and C) and women (B and D). Although trabecular density declined ($P = 0.013$; A and B) in the nonsmokers, no such relationships were observed in the smokers. Endocortical circumference (C and D) did not change with age in smokers or nonsmokers. For slopes and 95% CI, see *Results*.

ences in the slopes between smokers and nonsmokers were similar in both sexes.

Smoking effects upon vBMC.tot and vBMD.tb (Fig. 2A) in the radius were similar to the tibia, albeit less pronounced. In the nonsmokers, the vBMC.tot demonstrated a trend to decrease with age by −0.08 mg/cm · yr (95% CI = −0.16–0.10; $P = 0.082$), whereas it did not change in the smokers (interaction, $P = 0.034$ and $P = 0.459$ for slope in smokers only). Similar values were obtained for vBMD.tb, for which the slope was −0.78 mg/cm^3 · yr (95% CI = −1.38 to −0.17) in nonsmokers and 1.33 mg/cm^3 · yr (95% CI = −1.38–4.04) in the smokers (interaction, $P = 0.004$; Fig. 2A). No age or age × smoking effects were observed in EsC (Fig. 2B) or PsC of the radius. Similarly to the tibia, the differences in the slopes between smokers and nonsmokers in the radius were similar in both sexes.

Relation between smoking exposure and BSI

When years smoked, cigarette smoked per week, and cigarette pack-years were fed into the multiple linear regression model with only the smokers selected, no relationships were observed between smoking exposure and BSI (P values ranged from 0.110–0.999).

Discussion

This study has shown that in young smokers of either sex, the epiphyseal bone mineral content and trabecular vBMD are reduced, whereas there is no reduction in cortical vBMD. In addition, in male, but not female, smokers, tibial diaphyseal cortical area is reduced compared with nonsmokers. This study therefore supports the view that reduced aBMD in smokers (9, 10, 12, 13) is caused by reductions in bone mass (15, 16, 18) as well as by marrow cavity expansion. In addition, we observed that the bones of men appear to be more affected by smoking than those of women. The principle hypothesis of the present study was that the effects of smoking on BSI, such as trabecular density and bone mineral content, would be aggravated with increasing age. Quite clearly, this does not seem to be the case, and the opposite appears to be true, because the detrimental effects of cigarette smoking were most pronounced at a young age and were mitigated at an older age. These results are not explicable by differences in physical activity level or parameters of neuromuscular functioning between groups because these parameters were carefully controlled in the present study.

Our data are in good agreement with a number of studies that have reported detrimental effects of smoking on bone (9–13, 15, 16, 18, 26). This effect appeared to be independent of the current or cumulative dose of smoking in both men and women and extends similar observations by Lorentzon *et al.* (15) in young men.

Interestingly, the effects of smoking appeared more severe in men than in women. Although in men the cortical area of the diaphysis and the vBMC.tot were reduced in the tibia as a result of smoking, no such effect was observed in women. Our data are in some contrast to those of Kaji *et al.* (18). We have no explanation for this discrepancy, but it is possible that part of it is related to differences in the study population (Asians *vs.* Caucasians), and probably also to the fact that groups in our study were matched for physical activity and that there was no difference in muscle function between smokers and nonsmokers (23).

It was concluded from a metaanalysis (17) that the fracture risk attributable to smoking is aggravated with increasing age and that this aggravation is mediated by a reduction in aBMD. In these studies, however, the data were obtained

TABLE 4. Results for the linear regression modeling of BSI with the following factors entered into the model: smoking, age, smoke × age, sex, age × sex, and muscle size

	Slope (β)	95% CI	*P* value, $\beta \neq 0$
Tibia			
vBMD.tb tibia (epiphysis)			
Age (nonsmokers only)	−1.12	−1.60 to −0.63	<0.001
Smoke	−51.5	−93.60 to −9.50	0.014
Smoke × age	1.04	0.15, 1.93	0.022
vBMC.tot tibia 4% (epiphysis)			
Age (nonsmokers only)	−0.14	−0.19 to −0.09	<0.001
Smoke	−7.45	−12.34 to −2.57	0.030
Smoke × age	0.15	0.05–0.26	0.004
Ar.Ct tibia 38% (diaphysis)			
Age (nonsmokers only)	−0.63	−1.31–0.05	0.068
Smoke	−29.35	−78.18–19.48	0.235
Smoke × age	0.19	−0.84–1.22	0.709
EsC tibia 38% (diaphysis)			
Age (nonsmokers only)	0.11	0.03–0.20	0.012
Smoke	6.85	0.13–13.58	0.046
Smoke × age	−0.15	−0.29 to −0.01	0.038
EsC tibia 38% (diaphysis)			
Age (nonsmokers only)	0.00	−0.06–0.07	0.902
Smoke	0.86	−4.45–6.16	0.749
Smoke × age	−0.06	−0.17–0.05	0.311
Radius			
vBMD.tb radius 4% (epiphysis)			
Age (nonsmokers only)	−0.78	−1.38 to −0.17	0.013
Smoke	−52.07	−98.93 to −5.21	0.030
Smoke × age	1.36	0.36–2.35	0.009
vBMC.tot radius 4% (epiphysis)			
Age (nonsmokers only)	−0.08	−0.16–0.10	0.082
Smoke	−7.67	−13.90 to −1.43	0.017
Smoke × age	0.14	0.01–0.28	0.034
Ar.Ct radius 60% (diaphysis)			
Age (nonsmokers only)	−0.04	−0.25–0.16	0.672
Smoke	−7.04	−20.78–6.70	0.310
Smoke × age	0.19	−0.10–0.49	0.190
EsC radius 60% (diaphysis)			
Age (nonsmokers only)	0.04	−0.02–0.11	0.187
Smoke	2.09	−2.88–7.05	0.405
Smoke × age	−0.05	−0.15–0.06	0.308
PsC radius 60% (diaphysis)			
Age (nonsmokers only)	−0.01	−0.04–0.06	0.584
Smoke	−0.05	−3.36–3.25	0.974
Smoke × age	0.01	−0.06, 0.08	0.826

For explanation of acronyms see Table 1.

with a variety of methods and from different skeletal sites, and the inclusion and exclusion criteria varied widely among studies. In a more stringent metaanalysis of 10 prospectively studied large cohorts, it was shown that the fracture risk attributable to smoking was mitigated, rather than increased, with age (8). Our data confirm this observation and provide evidence that this mitigation of the detrimental effects of smoking on BSI is not an effect mediated by changes in physical activity and/or neuromuscular performance.

Mechanisms by which cigarette smoking affects bone strength

Nicotine has been reported to compromise bone mass and strength (27). Also, other toxic factors in cigarette smoke, such as carbon monoxide, (hydrogen) cyanide, formaldehyde, and benzene may have detrimental effects upon bone (28). It is, however, difficult to see how such toxic effects could become blunted with age. One possible explanation for such blunting might be mediated via the endocrine system. Kiel *et al.* (29), for instance, have reported that smoking compromises aBMD in postmenopausal women who took estrogen replacement therapy but not in those that did not, which was interpreted by the authors as a hint that smoking may affect bone metabolism via its action upon estrogen. However, a number of studies demonstrated detrimental effects of smoking on bone in the absence of differences in plasma estrogen levels (15, 30). Moreover, the age-related mitigation of smoking

effects in our study occurred in men but not in women alike. It would therefore appear that a decline in bone strength is probably not mediated via estrogen alone. Other hormones that play a role in bone metabolism and can be affected by smoking are cortisol (31), vitamin D (32), and PTH (32). Finally, it is possible that smoking interferes more or less directly with processes involved in senescence. Oxidative stress, for example, is thought to increase with age in most if not all parts of our body. Likewise, oxidative stress increases even after smoking a single cigarette (23, 33) and may stimulate osteocyte apoptosis (34) and remodeling (35) and underlie the enhanced bone turnover often reported in heavy smokers (36).

Limitations

This is a case-control study, and it can therefore not prove causality. In other words, the differences between the smokers' and the nonsmokers' bones outlined here could have been caused by a third factor that affects the decision to either smoke or not. For example, there is a likely bias to exclude the more unhealthy of the elderly smokers, due to the strict criteria for inclusion into this study. In theory, such an effect could have caused the mitigation of the negative effects on smoking upon bone at old age. However, the mechanisms required for this would have to be rather indirect. Moreover, and from a practical point of view, controlled interventional studies, where people are asked to initiate smoking are ethically inappropriate, given the known toxic effects of cigarette smoking. Cohort studies are quite resource intensive and as much prone to self-selection bias as the current case-control study. We therefore believe that the evidence presented here is as strong as practically possible.

The sample size might be considered small, and therefore we did some sample size calculations. Using the changes in vBMD.tb, we would have needed at least 150 smokers to obtain a power of 0.8. It should be noted that in the nonsmokers, 53 people were enough to show an age-related decline in this parameter, emphasizing that the effect of age is stronger in nonsmokers than smokers, if it exists in the latter group at all.

Finally, it would have been desirable to also study trabecular bone structure in this cohort, in particular to assess differential effects of smoking and age upon the cortical and trabecular compartments in the epiphysis (37, 38). This would have required a scanner with greater resolution, which was not available to us at the time that this study was conducted. Nevertheless, resolution issues are unlikely to have affected the results presented here.

Conclusions

In conclusion, the present data suggest that smoking compromises bone strength by diaphyseal marrow cavity expansion and epiphyseal trabecular bone reductions. These effects were more pronounced in men than in women and appear to wane with age. The cause of this remains enigmatic, but it is possible that the effects of smoking on bone interfere with the senescence processes.

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