

Lean Body Mass, Not Estrogen or Progesterone, Predicts Peak Bone Mineral Density in Premenopausal Women^{1–2}

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Abstract

Estrogen and body fat content are important predictors of bone mineral density (BMD) in postmenopausal women, but their association with BMD in premenopausal women is less clear. Mounting evidence suggests that dietary fats can have detrimental effects on bone health. In a cross-sectional sample of healthy 30- to 40-y-old women ($n = 242$), we investigated the predictors of BMD at the hip and spine by multilevel multiple regression analyses. Predictor variables in the models included dietary intake of various fats, serum concentrations of sex steroids, blood chemistries and markers of metabolic syndrome, anthropometric variables, and ethnicity. Among these premenopausal women, lean body mass was the strongest independent predictor ($P < 0.0001$) and African-American ethnicity ($P < 0.05$) was another positive independent predictor of BMD at the hip and spine. Dietary fats were not independent predictors of BMD of hip and spine. Lean body mass and being African-American explained 33% of the variance in hip BMD. Lean body mass, African-American ethnicity, and serum concentrations of triglycerides (a negative predictor, $P = 0.0001$) explained 28% of the variance in spine BMD. In contrast, luteal phase serum concentrations of estradiol, progesterone, and testosterone were not predictors of BMD. It remains to be determined whether efforts to increase lean body mass in premenopausal women with normal levels of endogenous estrogen may be an effective preventive strategy to preserve bone health. *J. Nutr.* 139: 250–256, 2009.

Introduction

Bone mineral density (BMD)⁵ is influenced by both genetic and environmental factors. Maximum adult BMD (known as peak BMD) for different skeletal sites is achieved between the late teens and ~30 y of age (1,2). Low peak BMD is a significant risk factor for osteoporotic fractures later in life (3).

Heritable factors account for 50–80% of the individual variance in BMD at different skeletal sites, as indicated by twin and family studies (4,5). Predictors of BMD and bone loss have been widely studied in postmenopausal women in whom factors such as age, body weight, years since menopause, levels of estradiol and sex hormone-binding globulin (SHBG), smoking,

and calcium intake were associated with differences in BMD (6–10). Estradiol levels and body weight are considered the most important predictors of BMD after menopause (11).

Although loss of BMD accelerates during and after menopause, it can begin as early as in the 3rd decade of life (12,13). Therefore, optimizing and preserving BMD before menopause may prevent bone loss and decrease future fracture risk. Animal and epidemiological studies suggest that PUFA and SFA intake influence bone growth and modeling (14–17), but the role of fatty acids in predicting peak BMD in premenopausal women is less clear. In this cross-sectional sample of 242 premenopausal women, we examined the association of dietary fatty acids and sex steroids with BMD at the hip and lumbar spine. In addition, we evaluated the interaction of nutrients and hormones with metabolic, demographic, and anthropometric variables.

Materials and Methods

Study design. Healthy premenopausal women of all major ethnicities were recruited from within an 80-km radius of Galveston, Texas, using Web-mail, posted advertisements, and postal mail. The study protocol was approved by the Institutional Review Board of the University of Texas Medical Branch and the Human Research Protection Office of the U.S. Army Medical Research and Materiel Command. Written informed consent was obtained from each subject.

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⁵ Abbreviations used: BMD, bone mineral density; CRP, C-reactive protein; DXA, dual-energy X-ray absorptiometry; HDL-C, HDL-cholesterol; MUFA, monounsaturated fatty acid; SHBG, sex hormone-binding globulin.

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Only 30- to 40-y-old subjects were included to target near peak bone mass and to avoid including peri- or postmenopausal women. Subjects were selected for having 1 menstrual cycle per month so that blood could be collected only during the luteal phase to study the association of endogenous estradiol and progesterone with BMD. Subjects who were pregnant, breast-feeding, or had used any contraceptive medication (oral, injection, or patch) during the preceding 6 mo were excluded.

These subjects participated in an extended dietary intervention study and their baseline data were the source of these analyses. Six baseline study visits were scheduled during the luteal phase of 2 separate menstrual cycles (3 visits per cycle), usually between cycle d 20 and 24. Sampling blood during the luteal phase, when progesterone synthesis is increased, allowed us to measure all 3 sex steroids (estradiol, progesterone, and testosterone) in 1 blood sample for assessing the association of sex steroids with BMD.

Anthropometrics, BMD, body composition, and reproductive factors. Body weight and height were measured at each study visit. Waist circumference was measured at the umbilicus and hip circumference at the widest point around the buttocks at each study visit. At 1 of the 6 study visits, BMD (expressed as g/cm²) was measured at left total hip and lumbar spine (lumbar spine 1–4) by dual-energy X-ray absorptiometry (DXA) in the supine anteroposterior position with a Hologic QDR 4500A densitometer (Hologic). The densitometer was calibrated daily using a spine phantom and adjusted to within 1% of the reference value provided by the manufacturer. Total, lean, and fat body mass and body fat as percent body mass were also measured by DXA. All DXA measurements were performed in duplicate (before and after repositioning) for all study participants (CV < 5%). Means of the 2 DXA measurements were used for statistical analyses. Reproductive histories were obtained using a self-administered, standard, gynecologic clinic questionnaire.

Nutrient intake. Study subjects were instructed to record total food intake (i.e. food items, brand names and amounts) for the 24-h period preceding each scheduled study visit. Three 24-h food records were analyzed using the Nutrient Data System for Research software, version v4.05/33 (Nutrition Coordinating Center, University of Minnesota) and averaged for statistical analyses.

Hormone assays and blood chemistries. Fasting venous blood samples were drawn between 0800 and 1000 during all study visits and serum and plasma were stored at –80°C until analysis. The plasma samples from the first 3 visits (all from 1 menstrual cycle) were analyzed for estradiol and testosterone by ELISA and for progesterone by direct RIA. Serum samples from study visits 1 and 5 were assayed for SHBG and C-reactive protein (CRP) by ELISA and for insulin by direct RIA. SHBG and CRP, though serum proteins, were grouped with and referred to as hormones in statistical analyses. All immunoassay kits were commercially available and were from Diagnostic System Laboratories. The intra- and inter-assay CV for all analytes were <10%. Means of serum hormone concentrations from different study visits were used for statistical analyses.

Serum concentrations of calcium, potassium, glucose, HDL-cholesterol (HDL-C), total cholesterol, triglycerides, total carbon dioxide, albumin, total proteins, and alkaline phosphatase were measured by a certified hospital clinical laboratory in samples from the first and 6th study visits using VITROS 5.1 FS (Ortho-Clinical Diagnostics) and means of the 2 measurements were used for statistical analyses.

Statistical analyses. Data are presented as means and 95% CI of the mean for continuous variables and as frequencies for categorical variables (ethnicity and parity) unless otherwise indicated. Outliers in the data were detected through frequency lists and scatter plots. Outliers from hormone and blood chemistry data ($n < 10$) were removed from the statistical analyses if they were >4 SD away from the mean. BMD, hormone, and blood chemistry data were checked for normal distribution. Values of CRP were log-transformed prior to analyses due to skewed distribution. Pearson correlation coefficients were computed to assess linear relationships between BMD at the hip and spine and the potential

predictor variables, i.e. fatty acid intakes, blood chemistries, and hormonal, reproductive, and anthropometric variables. We used ANOVA and *t* tests to compare group means across levels of categorical variables. Forward selection multiple regression analysis models were used to examine the independent association of predictor variables with BMD within each conceptual block. The conceptual blocks for our multilevel approach were: 1) nutrients, followed by; 2) blood chemistry; 3) hormones (including SHBG and CRP); 4) anthropometrics; and 5) ethnicity. Fatty acid intake was adjusted for energy intake by including energy intake into all models. Simple linear regression was used to identify variables associated with BMD at total hip or spine. A 2-sided α level of 0.05 was used to determine significance. Separate models were constructed to determine predictors of hip and spine BMD. Estradiol, because of its effects on BMD in postmenopausal women, and progesterone and testosterone were forced into both sets of multivariate regression models. All statistical analyses were performed using SAS (version 9.1, SAS Institute).

Results

Based on the BMI criteria of the WHO, 3.3% of our study subjects were underweight, 28.1% were in the healthy weight range, 34.3% were overweight, and 34.3% were obese (18). The ethnic composition of the study population was 49.6% non-Hispanic White, 32.2% Hispanic, 13.2% African-American, 2.1% Asian, 2.5% unspecified ethnicity, and 1 American Indian. Hip and spine BMD (mean \pm SD) were 0.984 ± 0.115 g/cm² and 1.058 ± 0.110 g/cm², respectively (Table 1). Other relevant characteristics and nutrient intakes of the study subjects are presented (Table 1).

Determinants of BMD. Body weight, BMI, waist circumference, hip circumference, fat body mass, and lean body mass were significantly and positively correlated with BMD of both the lumbar spine and hip (Table 2). Height correlated only with spine BMD ($P < 0.0001$). Body fat mass as percent of the body weight correlated with hip ($P < 0.005$) but not with spine BMD. Age (Table 2), age of menarche, parity, and the number of pregnancies (results not shown) were not associated with BMD at the hip or spine.

Serum albumin and total protein correlated negatively only with hip BMD, and serum total protein and triglycerides correlated inversely only with spine BMD. Serum HDL-C and SHBG correlated negatively ($P < 0.0001$), whereas serum insulin and CRP ($P = 0.001$ for both) as well as testosterone ($P = 0.06$) correlated positively with hip BMD, but not with spine BMD. Plasma concentrations of estradiol and progesterone in these menstruating women were not associated with either hip or spine BMD by univariate analysis ($P > 0.16$ – 0.99).

Correlations between nutrients and BMD are also summarized (Table 2). Hip BMD correlated positively with total energy intake, sugars, proteins, and fats (all $P \leq 0.05$). Spine BMD was not associated with intakes of major nutrients other than fats.

Independent determinants of hip BMD. Intakes of all types of fatty acids, trans-fatty acids, SFA, monounsaturated fatty acids (MUFA), and (n-3) and (n-6) PUFA, were entered in the first level (model 1, nutrients) of the multilevel multiple regression analyses, with hip BMD as the dependent variable. Energy intake was the only independent predictor in the nutrient model and explained 3% of the variance in hip BMD (Table 3). SFA intake was an independent predictor of hip BMD in the nutrient model not including energy intake. Note that variable energy intake was correlated with SFA ($r = 0.86$; $P < 0.0001$).

Energy intake along with the blood chemistry variables, including total protein, albumin, alkaline phosphatase, and

TABLE 1 Selected characteristics of the study subjects¹

Variables	
BMD	
Total hip BMD, g/cm ²	0.984 (0.969, 0.998)
Lumbar spine BMD, g/cm ²	1.058 (1.044, 1.072)
Demographic and anthropometric	
Age, y	36.2 (35.9, 36.6)
Height, cm	161.4 (160.6, 162.3)
Weight, kg	74 (72.2, 75.8)
BMI, kg/m ²	28.4 (27.7, 29.1)
Waist circumference, cm	87.6 (86.1, 89.0)
Hip circumference, cm	109 (107.5, 110.5)
Waist:hip ratio	0.8 (0.79, 0.81)
Fat body mass, kg	28.1 (26.8, 29.3)
Lean body mass, kg	46.2 (45.4, 46.9)
Body fat, % body mass	36.9 (36.0, 37.7)
Reproductive	
Parity column percentage	
Yes, n (%)	214 (88.4)
No, n (%)	28 (11.6)
Age of menarche, y	12.7 (12.5, 12.8)
Complete pregnancies, n	2.2 (2.1, 2.4)
Blood chemistry/hormone	
Serum cholesterol, mmol/L	4.68 (4.58, 4.79)
Serum HDL-C, mmol/L	1.39 (1.35, 1.42)
Serum triglycerides, mmol/L	1.18 (1.09, 1.27)
Serum calcium, mmol/L	4.5 (4.45, 4.5)
Serum potassium, mmol/L	2.15 (2.14, 2.16)
Serum albumin, g/L	40.9 (40.4, 41.0)
Serum alkaline phosphatase, U/L	71.7 (69.5, 73.8)
Serum total protein, g/L	73.5 (73, 74)
Serum SHBG, nmol/L	100.5 (95.5, 105.4)
Serum CRP mg/L	6.96 (5.99, 7.93)
Plasma testosterone, nmol/L	0.03 (0.02, 0.03)
Plasma progesterone, nmol/L	33.7 (31.5, 35.6)
Plasma 17 β -estradiol, ² pmol/L	289.6 (272.4, 307.3)
Serum insulin, pmol/L	85.4 (77.1, 93.8)
Daily nutrient intake	
Energy intake, kJ	7331 (7038, 7624)
Carbohydrate, g	200 (191, 209)
Protein, g	67.6 (64.7, 70.5)
Fat, g	75.7 (72, 79.5)
Carbohydrate, % kJ	46 (45, 47)
Protein, % kJ	16 (15.5, 16.5)
Fat, % kJ	38.1 (37.3, 38.9)
Trans-fatty acids, g	5.5 (5.1, 5.9)
SFA, g	25.1 (23.8, 26.4)
MUFA, g	29.7 (28.1, 31.2)
PUFA, g	14.8 (13.9, 15.7)
(n-3) PUFA, g	1.4 (1.3, 1.6)
(n-6) PUFA, g	13.3 (12.4, 14.1)

¹ Values are means (95% CI), n = 242 or n (%)² n = 210.

HDL-C, were entered into model 2 (blood chemistry). Of these variables, only HDL-C and energy intake were significant independent predictors of hip BMD and explained 6% of the variance in BMD at this site.

Model 3 (hormones) included independent predictors from the first 2 models plus testosterone, progesterone, insulin, SHBG, and CRP. SHBG, energy intake, and HDL-C were the significant independent predictors and together explained 9%

of the variance in hip BMD. In model 3 in the absence of SHBG, CRP (but not testosterone or insulin) was also a positive independent predictor of hip BMD. Serum SHBG concentrations correlated negatively with serum concentrations of CRP ($r = -0.40$; $P < 0.0001$), insulin ($r = -0.34$; $P < 0.0001$), and testosterone ($r = -0.20$; $P = 0.002$). CRP concentrations correlated strongly with serum insulin ($r = 0.48$; $P < 0.0001$).

Model 4 (anthropometrics) included predictor variables from the first 3 models plus anthropometric variables, body weight, BMI, fat body mass, and lean body mass. Lean body mass was the only independent predictor of hip BMD in Model 4 (Table 3). Energy intake, HDL-C, and SHBG became insignificant after lean body mass was entered into the model. This may be due to a strong inverse correlation between lean body mass with SHBG ($r = -0.28$; $P < 0.0001$) and a strong positive correlation between SHBG with HDL-C ($r = 0.32$; $P < 0.0001$). Lean body mass alone accounted for 20% of the variance in hip BMD. Predictors in model 4 were further adjusted for the influence of ethnicity on hip BMD. African-American ethnicity was another independent predictor of hip BMD in model 5 and explained an additional 1% of the variance in hip BMD. Estradiol data were available for 210 subjects but not for 32 subjects, because the manufacturer had discontinued making the kit used for estradiol analysis. Luteal phase serum estradiol was not associated with hip BMD by univariate (Table 2) or multivariate analyses (Table 3, estradiol model). Lean body mass and African-American ethnicity remained the only independent predictors of hip BMD in the estradiol model.

Independent determinants of spine BMD. A statistical modeling approach similar to the one used for predicting hip BMD was applied to determine the independent predictors of spine BMD. All dietary fats, trans-fatty acids, SFA, MUFA, and (n-3) and (n-6) PUFA were entered in model 1 (nutrients) of multiple regression analysis with lumbar spine as the dependent variable (Table 4). Energy-adjusted intake of SFA was a positive independent predictor of spine BMD in model 1, explaining 4% of the variance. This variable was then entered into model 2 (blood chemistry) along with serum triglycerides, calcium, and total proteins. Serum triglycerides were an independent, negative predictor of spine BMD in model 2 and along with dietary SFA accounted for 6% of the variance in spine BMD.

Model 3 (hormones) included predictor variables from model 2 plus testosterone and progesterone. Neither testosterone nor progesterone was an independent predictor of spine BMD. Model 4 (anthropometrics) included the predictor variables from model 3 plus the anthropometric variables body weight, BMI, lean body mass, and fat body mass. Lean body mass was the additional independent predictor of spine BMD in model 4 ($P < 0.0001$) and together with triglycerides (a negative predictor; $P = 0.001$) explained 26% of the variance in spine BMD. In model 3, in the absence of triglycerides, height was an independent predictor of spine BMD. Note that in these subjects, serum triglycerides had a negative correlation with height ($r = -0.24$; $P = 0.0001$) and intake of SFA had a positive correlation with height ($r = 0.22$; $P = 0.0007$).

When model 4 was adjusted for ethnicity, African-American ethnicity was the only positive independent predictor of spine BMD. Lean body mass, triglycerides, and being African-American explained 28% of the variance in spine BMD. Forcing estradiol into the regression model (estradiol model, Table 4) did not change the outcome of the regression analysis.

TABLE 2 Pearson correlation coefficients (*r*) and *P*-values for hip BMD and lumbar spine BMD and selected independent variables¹

Variables	Total hip BMD		Spine BMD	
	<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value
Demographic and anthropometric				
Total hip BMD			0.55	<0.0001
Age	-0.08	— ²	-0.05	—
Height	0.06	—	0.31	<0.0001
Weight	0.45	<0.0001	0.36	<0.0001
BMI	0.42	<0.0001	0.21	0.0006
Waist	0.36	<0.0001	0.22	0.0004
Hip	0.37	<0.0001	0.25	<0.0001
Fat body mass	0.34	<0.0001	0.24	0.0001
Lean body mass	0.53	<0.0001	0.46	<0.0001
Body fat, % <i>body mass</i>	0.18	0.005	0.08	—
Blood chemistry/hormones				
Serum albumin	-0.13	0.04	-0.04	—
Serum alkaline phosphatase	0.11	—	-0.03	—
Serum calcium	0.08	—	0.12	0.05
Serum HDL-C	-0.19	0.003	0.06	—
Serum triglycerides	0.01	—	-0.15	0.02
Serum total protein	-0.11	—	-0.12	0.05
Plasma testosterone	0.12	—	0.02	—
Plasma progesterone	-0.09	—	0.06	—
Plasma 17 β -estradiol ³	-0.001	—	-0.03	—
Serum SHBG	-0.22	0.0004	0.02	—
Serum CRP	0.22	0.001	0.09	—
Serum insulin	0.21	0.001	0.07	—
Macronutrients				
Energy intake	0.16	0.01	0.10	—
Total carbohydrates	0.12	—	0.10	—
Total sugars	0.13	0.04	0.03	—
Total protein	0.13	0.04	0.06	—
Animal protein	0.13	0.04	0.07	—
Calcium	0.05	—	-0.02	—
Fats and fatty acids				
Total fats	0.18	0.005	0.16	0.01
Trans-fatty acids	0.06	—	0.07	—
SFA	0.17	0.01	0.17	0.01
MUFA	0.15	0.02	0.13	0.04
(n-3) PUFA	0.09	—	0.04	—
(n-6) PUFA	0.17	0.008	0.15	0.02

¹ *n* = 242.

² —, *P* > 0.05.

³ *n* = 210.

Discussion

In this study, our aim was to assess the influence of dietary fatty acids and sex steroids on BMD at 2 clinically important sites, the hip and the lumbar spine in a well-defined group of premenopausal women. In this convenient, cross-sectional study sample, mean BMD at these sites was consistent with the values reported in the literature for other study populations (19–21) and in univariate analyses correlated strongly with all anthropometric variables except height, which correlated only with spine BMD. In multiple regression models, lean body mass was the strongest independent predictor of BMD at both hip and spine. Height was not an independent predictor of spine BMD but correlated strongly with serum triglycerides, which was an independent predictor of spine BMD, as further discussed below.

Of all dietary nutrients examined, only fatty acids showed a consistent, significant positive relationship with both hip and spine BMD in univariate analyses (Table 2). Dietary (n-3) fatty acids have been implicated in skeletal health (22) and the lack of an association between (n-3) PUFA and BMD in our study could be attributed to the low mean intake of (n-3) fatty acids. The ratio of (n-6):(n-3) fatty acids (10.3:1) in these subjects was far below the optimal recommended dietary intake of (n-3) fatty acids to protect against chronic disease risk (23). Dietary fatty acids were independent predictors for BMD, but not after controlling for lean body mass. This attenuated independent association of SFA with BMD may be explained by the observation that all types of dietary fat, especially SFA (*r* = 0.29; *P* < 0.0001), had significant positive correlations with lean body mass.

Our findings that serum HDL-C and triglyceride concentrations were significantly correlated with hip and spine BMD, respectively, are in agreement with several previous reports (24–26). However, we showed further that HDL-C was not an independent predictor of BMD after adjusting for SHBG and lean body mass, because HDL-C was significantly correlated with SHBG and lean body mass. Our observation of a negative and independent association of serum triglycerides with spine BMD is consistent with the findings of Cui et al. (27). The exact mechanism underlying the relationship between serum lipids and spine BMD remains to be elucidated.

Studies in twins and families indicate that genetics explains 50–80% of the variance in BMD (4,5); therefore, other nongenetic factors such as the nutrients examined in this study are not expected to explain >20–50% of variance in BMD. Although small, the correlation coefficients of BMD (*r* = 0.15–0.18; Table 2) with dietary fats derived from means of 3 24-h food records may account for a physiologically relevant amount of the variance in BMD due to nongenetic factors. Also, the multivariate regression coefficient for energy intake plus HDL-C of 0.06 (model 2; Table 3) and SFA intake (*P* = 0.01) plus serum triglyceride concentration (*P* = 0.02) being an independent predictor of spine BMD (*R*² = 0.06; model 2; Table 4) suggest that dietary fats, while not independent predictors of BMD in the final regression models, may still influence BMD by modulating body mass. Such nongenetic factors are modifiable by prevention strategies and might have important public health implications.

We obtained blood samples during the luteal phase of a menstrual cycle and calculated meaningful average concentrations of all 3 sex steroids (including progesterone) for statistical analyses. Despite this, of the 3 sex steroids, only testosterone showed a weak correlation with hip BMD. Estrogen plays a critical role in maintaining bone mass, as evidenced by steep declines in BMD after menopause or oophorectomy (28,29). Estrogen inhibits bone resorption and estrogen replacement therapies can partially alleviate postmenopausal loss of BMD (30,31). We found no relationship between luteal phase plasma concentrations of estradiol, progesterone, or testosterone and BMD. Our findings and a prior report showing a lack of association of follicular phase estradiol with BMD (32) suggest that endogenous estrogen in ovulatory women may be sufficient to maintain bone mass. The luteal phase estradiol concentrations (mean \pm SD) of 79 \pm 36 pg/mL (290 \pm 132 pmol/L) in our premenopausal subjects were higher than the concentrations of 45–65 pg/mL (165–239 pmol/L) postulated by others as being the threshold concentration for preventing postmenopausal bone loss (31,33).

Body weight is an important determinant of BMD in women regardless of menopausal status (34,35). By univariate analyses,

TABLE 3 Independent predictors of total hip BMD by forward selection multilevel multivariate regression analyses ($n = 242$)

Variables	Models ¹					
	P-values (standardized estimates, β)					
	1	2	3	4	5	Estradiol
Energy intake, <i>kJ/d</i>	0.01 (0.16)	0.008 (0.17)	0.01 (0.16)	0.46 (0.04)	0.38 (0.05)	0.74 (0.02)
Serum HDL-C, <i>mmol/L</i>	NE ²	0.002 (-0.20)	0.03 (-0.14)	0.29 (-0.06)	0.16 (-0.08)	0.35 (-0.06)
Serum SHBG, <i>nmol/L</i>	NE	NE	0.01 (-0.17)	0.26 (-0.07)	0.21 (-0.07)	0.09 (-0.11)
Lean body mass, <i>kg</i>	NE	NE	NE	<0.0001 (0.48)	<0.0001 (0.44)	<0.0001 (0.46)
African-American ethnicity	NE	NE	NE	NE	0.04 (0.12)	0.03 (0.13)
Plasma estradiol, ³ <i>pmol/L</i>	NE	NE	NE	NE	NE	0.79 (0.02)
Model R^2	0.03	0.06	0.09	0.29	0.3	0.31

¹ Model 1 (fatty acids) included energy intake, trans-fatty acids, SFA, MUFA, (n-3) PUFA, linoleic acid [(n-6) PUFA]. Model 2 (blood chemistry) included independent predictor from model 1 plus blood chemistry variables, HDL-C, albumin, alkaline phosphatase, and total protein. Model 3 (hormones) included predictors from models 1 and 2 plus testosterone, progesterone, SHBG, CRP, and insulin. Model 4 (anthropometrics) included predictors from models 1-3 plus correlated anthropometric variables body weight, BMI, lean body mass, and fat body mass. Model 5 (race and ethnicity): African-American and Hispanic ethnicity were entered in the model with Caucasian as the reference ethnicity. Estradiol model: predictor variables in model 5 was adjusted for serum estradiol.

² NE, Not entered.

³ $n = 210$.

we showed that all anthropometric variables, except height, were strongly associated with hip BMD, but lean body mass was the strongest independent predictor of both the hip and lumbar spine BMD in these premenopausal women, an observation consistent with previous reports (36,37). After menopause, fat tissue is the major source of endogenous estrogen, which may explain the observations that fat mass is the major anthropometric determinant of BMD in postmenopausal women (38).

A negative association between serum SHBG and total hip BMD by univariate analysis was observed in this study of premenopausal women (Table 2) and in a prior study of postmenopausal women (31) and also noted by multivariate analyses in this study (Table 3) and that of Pluijm et al. (7) in postmenopausal women. However, by multilevel multivariate analyses, we further showed that the independent association of SHBG with hip BMD was attenuated upon the addition of lean body mass to the regression analysis (Table 3). Thus, SHBG is not an independent predictor after adjusting for anthropometric variables and this may be explained by a strong inverse correlation of SHBG with measures of body composition in pre- (F. Nayeem,

M. Nagamani, K. E. Anderson, Y. Huang, J. J. Grady, and L-J. W. Lu, unpublished data) and postmenopausal women (39).

African-American women have, on average, higher BMD and lower fracture risk than non-Hispanic White women (40-42). Although only 13% of the subjects in our study were African-American, this ethnicity had a positive influence on BMD at the hip and spine in models adjusted for all other covariates and using Caucasians as the reference ethnicity.

Strengths of our study are the inclusion of subjects of multiple ethnic backgrounds (32% being Hispanic), a wide range of BMI (including obese women) during the peak period of BMD, and the use of 3 24-h recall food records for assessing dietary intake, which is considered a more reliable method than FFQ (43). We obtained blood samples from women who were not using exogenous hormones on 3 different days during the luteal phase of the cycle to optimally estimate serum concentrations of estradiol, progesterone, and testosterone. The multilevel multivariate analysis approach allowed us to examine inter-relationships between many predictor variables and BMD. Weaknesses of the study include lack of data on physical activity and family history of

TABLE 4 Independent predictors of spine BMD by forward selection multilevel multivariate regression analyses ($n = 242$)

Variables	Models ¹					
	P-values (standardized estimates, β)					
	1	2	3	4	5	Estradiol
Energy intake, <i>kJ/d</i>	0.15 (-0.18)	0.15 (-0.21)	0.15 (-0.21)	0.11 (-0.18)	0.09 (-0.18)	0.1 (-0.21)
Saturated fatty acids, <i>g/d</i>	0.01 (0.33)	0.01 (0.32)	0.01 (0.32)	0.11 (0.18)	0.07 (0.20)	0.09 (0.23)
Serum triglycerides, <i>mmol/L</i>	NE ²	0.02 (-0.14)	0.02 (-0.14)	0.001 (-0.19)	0.01 (-0.15)	0.005 (-0.18)
Lean body mass, <i>kg</i>	NE	NE	NE	<0.0001 (0.47)	<0.0001 (0.42)	<0.0001 (0.42)
African-American race	NE	NE	NE	NE	0.01 (0.15)	0.03 (0.14)
Plasma estradiol, ³ <i>pmol/L</i>	NE	NE	NE	NE	NE	0.85 (0.01)
Model R^2	0.04	0.06	0.06	0.26	0.28	0.27

¹ Model 1 (fatty acids) included energy intake, trans-fatty acids, SFA, MUFA, (n-3) PUFA, (n-6) PUFA (linoleic acid). Model 2 (blood chemistry) included predictors from model 1 plus blood chemistry variables, calcium, total protein, and triglycerides. Model 3 (steroid hormones) included predictors from model 2 plus testosterone and progesterone. Model 4 (anthropometrics) included predictors from models 1 and 2 plus anthropometric variables, body weight, BMI, lean body mass, and fat body mass. Model 5 (ethnicity): African-American and Hispanic ethnicity were entered in the model with Caucasian as the reference ethnicity. Estradiol model: predictor variables in model 5 were adjusted for estradiol.

² NE, Not entered.

³ $n = 210$.

osteoporosis. The narrow age range of our study cohort precludes any application of our study results to the postmenopausal population.

In summary, we found in this cross-sectional study that the most important positive predictors of BMD at both hip and spine were lean body mass and African-American ethnicity. Serum triglyceride concentration was an independent negative predictor of spine BMD. Dietary fatty acids did not predict BMD but may indirectly influence BMD by affecting anthropometric measures. Circulating concentrations of estradiol, which are important in postmenopausal women, and progesterone and testosterone, were not associated with BMD at either skeletal site. These results suggest that future intervention studies to prevent loss of bone mass in menstruating premenopausal women should focus on strategies to increase or maintain lean body mass.

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