Relation of body fat indexes to vitamin D status and deficiency among obese adolescents[1–4]

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ABSTRACT

Background: Data on the relation between vitamin D status and body fat indexes in adolescence are lacking.

Objective: The objective was to identify factors associated with vitamin D status and deficiency in obese adolescents to further evaluate the relation of body fat indexes to vitamin D status and deficiency.

Design: Data from 58 obese adolescents were obtained. Visceral adipose tissue (VAT) was measured by computed tomography. Dual-energy X-ray absorptiometry was used to measure total bone mineral content, bone mineral density, body fat mass (FM), and lean mass. Relative measures of body fat were calculated. Blood tests included measurements of 25-hydroxyvitamin D [25(OH)D], parathyroid hormone (PTH), osteocalcin, type I collagen C-telopeptide, hormones, and metabolic factors. Vitamin D deficiency was defined as 25(OH)D $<$ 20 ng/mL. PTH elevation was defined as PTH $>$ 65 ng/mL.

Results: The mean ($\pm$SD) age of the adolescents was 14.9 $\pm$ 1.4 y; 38 (66%) were female, and 8 (14%) were black. The mean ($\pm$SD) body mass index (in kg/m^2) was 36 $\pm$ 5, FM was 40.0 $\pm$ 5.5%, and VAT was 12.4 $\pm$ 4.3%. Seventeen of the adolescents were vitamin D deficient, but none had elevated PTH concentrations. Bone mineral content and bone mineral density were within 2 SDs of national standards. In a multivariate analysis, 25(OH)D decreased by 0.46 $\pm$ 0.22 ng/mL per 1% increment in FM ($\beta \pm$ SE, $P = 0.05$), whereas PTH decreased by 0.78 $\pm$ 0.29 pg/mL per 1% increment in VAT ($P = 0.01$).

Conclusions: To the best of our knowledge, our results show for the first time that obese adolescents with 25(OH)D deficiency, but without elevated PTH concentrations, have a bone mass within the range of national standards ($\pm$2 SD). The findings provide initial evidence that the distribution of fat may be associated with vitamin D status, but this relation may be dependent on metabolic factors. This study was registered at www.clinicaltrials.gov as NCT00209482, NCT00120146. *Am J Clin Nutr* 2009;90:459–67.

INTRODUCTION

Adequate vitamin D is essential for normal human growth and development, whereas vitamin D deficiency compromises long-term health and increases the risk of chronic disease (1). Health outcomes of severe vitamin D deficiency include rickets, osteoporosis, osteomalacia, increased risk of fracture, and tooth loss (1). Recent studies indicate that vitamin D insufficiency (less severe than deficiency) is associated with a wide range of illnesses and chronic conditions, including type 1 diabetes mellitus, hypertension, multiple sclerosis, and several types of cancers, such as breast, colon, and prostate cancer (1, 2). Thus, vitamin D status has far greater implications for health than has been previously acknowledged.

Obesity, defined as an elevated body mass index (BMI; in kg/m^2), is associated with a lower concentration of 25-hydroxyvitamin D [25(OH)D] and a higher concentration of parathyroid hormone (PTH) in adults (3). According to the National Health and Nutrition Examination Survey (NHANES; 1999–2000), 17.1% of children and adolescents were obese based on a BMI at or above the 95th percentile (4). Peak bone mass is attained during

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pubertal skeletal growth (5, 6), a period of time that has been associated with increased risk of vitamin D deficiency (7). In studies of obese adolescents in the United States, vitamin D deficiency has been correlated with greater weight and elevated BMI (7–10). However, specific data on the relation between vitamin D status and body fat indexes in this age group are lacking. Given the significance of bone mass accretion during adolescence and the prevalence of obesity, identification of factors associated with vitamin D status among obese adolescents is critical.

Body composition and skeletal growth are especially dependent on the interplay between the growth hormone (GH) axis and sex-steroid hormones during puberty (11). GH affects nitrogen and acid-base balance via an increase in hepatic glutamate production (12) and is implicated in the insulin resistance of puberty (13). However, evidence indicates that obesity may be characterized by a disruption in hormonal and nutrient metabolism: visceral fat has been associated with increased insulin resistance and progressive insulin insufficiency (13–15) and with anomalies in the GH axis [lower GH, normal insulin-like growth factor I (IGF-I) and IGF binding protein-3 (IGFBP-3), but lower IGFBP-1] (16) and the thyroid hormone axis [higher thyrotropin (TSH) and higher conversion of thyroxine (T_4) to triiodothyronine (T_3)] (17), whereas total-body fat mass (FM) has been characterized by higher leptin concentrations (18) and alterations in leucine metabolism (19). Therefore, the association of body fat indexes with vitamin D status may be dependent on these hormonal and nutrient factors. In addition, season, sex, race, diet, physical activity, height measures, and Tanner stage have been proposed as possible covariates of the association of body fat and 25(OH)D (8, 10, 20–23). The objective of this study was to identify factors associated with vitamin D status and deficiency and to further evaluate the relation of body fat indexes to vitamin D status and deficiency among obese adolescents.

SUBJECTS AND METHODS

Study subjects and design

We analyzed data for 58 adolescents who were screened for a weight-loss trial. The study was conducted from October 2003 to August 2007 at the 5 clinical sites of the Glaser Pediatric Research Network (*see* Acknowledgment) with the Data Coordination Center located at Children's Hospital of Boston (www.gprn.org). The inclusion criteria included age 13.0–17.9 y, BMI $\geq$95th percentile, weight $<$136 kg [limit for the dual-energy X-ray absorptiometry (DXA) scan]. None of the adolescents had diabetes or other known underlying disorders associated with obesity, participated in a medical or surgical weight-loss program, or used medication or substances that might affect growth and development, dietary intake, or physical activity.

Cross-sectional data included the following: *1*) demographic data (age, sex, race, ethnicity, medication use, smoking, illicit drug use, alcohol consumption, and month of the year); *2*) findings on physical examination (Tanner stage and anthropometric measurements); *3*) laboratory assessments (blood tests, CT scan, and whole-body DXA scan); and *4*) dietary data (food-frequency questionnaires) and physical activity data (uni-axial Computer Science & Applications accelerometer, MTI actigraph, model 7164; MTI Health Services, Fort Walton Beach, FL).

Race and ethnicity were self-identified at screening. Weight and height were measured in duplicate, and mean values were used for the analyses. A third measurement was obtained and included in the calculation of the means when the difference between the duplicates was $>$0.5 cm for height or $>$0.3 kg for weight. Weight was measured while the subjects were wearing light clothing and no shoes with a digital scale to the nearest 0.1 kg, and height was measured with a calibrated wall-mounted stadiometer to the nearest 0.1 cm. BMI was calculated as the mean weight (kg) divided by the mean height squared (m). The anthropometric measurements (percentiles and z scores) were calculated based on data from the Centers for Disease Control and Prevention (24).

Fasting blood samples were obtained for measurement of hemoglobin A_{1C} and serum or plasma insulin, glucose, glucagon, IGF-I, IGFBP-1, estradiol, free testosterone, TSH, T_4, total leptin, and lipids (total cholesterol, HDL and LDL cholesterol, and triglycerides). After 3 d of a high-carbohydrate diet ($\geq$150 g) and a 10-h fast, the subjects underwent a 3-h oral-glucose-tolerance test (OGTT; 75 g glucose). All laboratory assays were performed at Esoterix Clinical Trials Services, Calabasas Hills, CA, which provided the reference range data used for the analyses.

Fasting plasma samples for amino acids were stored frozen at –70°C until analyzed by using ion-exchange liquid chromatography (Amino Analyzer L-8800; Hitachi, Tokyo, Japan) at the Children's Hospital of Boston Chemistry Laboratory, Boston, MA. The CV for all amino acids per this method was $\leq$6%. Vitamin D status and bone metabolism were evaluated by using nonfasting plasma samples collected on the same day at each of the General Clinical Research Centers and at the same time of the day (midmorning) and stored frozen at –70°C until analyzed at the Vitamin D, Skin, and Bone Research Laboratory, Boston, MA (by MFH and TCC). Plasma 25(OH)D was measured by using an in-house competitive protein binding assay with intra- and interassay CVs of 8–12% and 10–15%, respectively, that were comparable with liquid chromatography–tandem mass spectroscopy (25). Plasma PTH was measured by using an enzyme immunoassay (EIA) kit with intra- and interassay CVs of 3–5% and 5–8%, respectively (Immunotopic Inc, CA). Osteocalcin, an osteoblast biologic marker, was measured by using an EIA kit with intra- and interassay CVs of 4.9–5.0% and 3.4–4.6%, respectively (Quidel Corporation, San Diego, CA). Type I collagen C-telopeptide (CTX), an osteoclast biologic marker, was measured by using an EIA kit with intra- and interassay CVs of 5.0–5.4% and 5.0–8.1%, respectively (Quidel Corporation).

The CT scans used were from GE, Siemens, or Philips, depending on the center. The DXA scans used were the Hologic 4500 and the Delphi-A (Hologic, Bedford, MA). Standard phantoms were circulated between 4 of the 5 sites for cross-calibration of the Glaser Network studies (26). All DXA scans were analyzed at University of California, San Francisco, by using standard software (Delphi Manual software; Hologic) and all CT scans were analyzed at Harvard by using standard software (Photoshop CS2; Adobe Systems, San José, CA). A CT scan slice was obtained at the L4–L5 intervertebral disk based on a scout radiograph to standardize the position of the scan to the nearest millimeter (27). The abdominal adipose tissue (AAT) area was calculated, and areas of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were delineated and subtracted from the AAT, at the Department of Radiology, Children's

Hospital of Boston, Boston, MA (by GAT). The percentage VAT was calculated as 100 times the VAT divided by the sum of VAT and SAT. Whole-body DXA measurements and used for 3-compartment body composition analysis including the determination of: *1*) total body FM; *2*) total bone mineral content (BMC); and *3*) lean mass. Percentage FM was calculated as 100 times FM divided by the sum of FM, BMC, and lean mass. Bone mineral density (BMD) was calculated based on BMC divided by surface area (in cm^2). Percentiles and *z* scores for BMC and BMD were calculated based on national references (28).

Dietary and supplement intakes in the past year were estimated by using the validated Youth Adolescent food-frequency questionnaire (YAQ; Channing Laboratory, Boston, MA). Physical activity was estimated by using the uni-axial Computer Science & Applications accelerometer (MTI actigraph, model 7164; MTI Health Services, Fort Walton Beach, FL) worn at the hip for $\geq$3 d (n = 24). Institutional Review Board approval for the data analyses was obtained from the 5 Glaser Pediatric Research Network sites (*see* Acknowledgment) and Boston University School of Medicine. Signed informed consent from a parent or other legal guardian of each subject, and age-appropriate assent was obtained before screening for the study.

Analyses

Concentrations of 25(OH)D and PTH were used as outcome variables for this secondary analysis. The concentration of 25(OH)D is currently considered the best indicator of vitamin D stores (29, 30). Vitamin D deficiency is best defined as a 25(OH)D concentration <20 ng/mL (29). However, much controversy exists over the best cutoff to define deficiency. Thus, the analysis included dichotomous and continuous outcome values for 25(OH)D. Because none of the adolescents had a PTH concentration >65 pg/mL (upper limit), we used PTH as a continuous variable in these analyses.

Several indexes were calculated based on OGTT or fasting blood samples to estimate insulin resistance and glucose tolerance in relation to vitamin D status for this sample (n = 58), whereas results for the overall study population are reported elsewhere. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as [glucose (mmol/L) × insulin (μU/mL)]/22.5 (31) to estimate fasting insulin resistance. The area under the curve (AUC) for insulin and glucose were calculated from the OGTT data by using the trapezoidal method. None of the adolescents were found to have type 2 diabetes mellitus at baseline based on their glucose fasting concentrations ($\geq$126 mg/dL, or 7 mmol/L) or during an OGTT (2-h glucose >200 mg/dL, or 11.1 mmol/L) (32).

Pearson correlation coefficients were calculated to assess simple associations between continuous variables, and point biserial coefficients (mathematically equivalent to Pearson correlation coefficients) and the association between continuous and dichotomous variables. Spearman correlation coefficients were also calculated to corroborate the Pearson correlations and a few minor discrepancies in inference were noted, all cases of borderline P value just above or below the conventional P = 0.05 cutoff. No variables were so severely skewed in distribution as to require transformation for these analyses. We based our judgment that transformation was not required on *1*) the close agreement of Pearson and Spearman correlations and *2*) the largely symmetric distribution of both dependent variables in regression analysis; the median lay within 2% of the mean for 25(OH)D and 9% for PTH. Student's t tests were used to compare means of continuous data between the 25(OH)D-deficient and not-deficient groups. Chi-square and Fisher exact tests were used to compare proportions for categorical data.

Multiple linear regression analysis was used to model continuous outcomes with body fat indexes after adjustment for potential confounding variables. In addition, we conducted multiple logistic regression analysis to examine the adjusted relation between body fat indexes and vitamin D deficiency. Independent variables included core variables that were found to significantly affect 25(OH)D concentrations in other studies, including age, sex, race, season, vitamin D intake, and height measures. Because relevant data for PTH is limited, age, sex, and black or African American race were used as core variables in the models of PTH. As suggested in the literature, we also examined the potential confounding effect of Tanner stage on the outcomes. Other potential confounding variables of the relation between fat indexes and outcome variables were identified based on significant correlations between sample characteristics and body fat indexes. Potential confounding variables were then added separately to the models that included the core variables. We also evaluated effect modification by sex and race by including interaction terms between variables. A small number of missing variables (DXA scan, CT scan, and HOMA-IR values) were imputed for use in regression analysis, by using the mean for the subject's race and sex (1–3 missing values) (33).

On the basis of a 2-sided test at 0.05, a sample of 58 subjects provided 80% power for detecting a correlation coefficient of 0.35 between 2 variables or for detecting R^2 values of 0.12, 0.20, and 0.26 by using multiple linear regression analyses with 1, 6, and 11 variables, respectively. All analyses were performed by using SAS software (version 9.1; SAS Institute Inc, Cary, NC).

RESULTS

Adolescents were 14.9 ± 1.4 y (mean ± SD) of age, 38 (66%) were female, and 8 (14%) were black or African American. The BMI was 36 ± 5, 29 subjects (50%) had a BMI between the 99th and 99.9th percentile, FM was 40.0 ± 5.5%, and VAT was 12.4 ± 4.3%. The mean 25(OH)D concentration was 24.7 ± 9.9 ng/mL. A total of 17 obese adolescents (29%) had a 25(OH)D concentration <20 ng/mL (vitamin D deficiency). None of the subjects had an abnormal PTH concentration; the highest value for PTH was 48 pg/mL. Characteristics of the sample are described in **Table 1** and **Table 2**.

Adolescents with a 25(OH)D concentration <20 ng/mL were more likely to be of black or African American race ($P < 0.001$) (Table 1). Concentrations of 25(OH)D were directly correlated with summer season ($P < 0.01$) and physical activity level ($P < 0.05$). FM and FM% were significantly higher in the vitamin D–deficient group than in the vitamin D–nondeficient group (Table 1). Likewise, FM and FM% were significantly and inversely related to increasing 25(OH)D concentrations ($P < 0.05$; Table 1). PTH concentrations were significantly and inversely associated with VAT% ($P < 0.05$) and were higher among those with younger Tanner stage ($P < 0.05$), which typically coincides with the peak of skeletal growth.

TABLE 1
Sample characteristics and univariate analysis[1]

Characteristic	Mean ± SD or *n* (%)	Mean ± SE or *n* (%)[2]		Correlation coefficient[3]	
	All (*n* = 58)	25(OH)D-deficient (*n* = 17)	Not 25(OH)D-deficient (*n* = 41)	25(OH)D	PTH
Demographic					
Age (y)	14.9 ± 1.4	15.1 ± 0.3	14.8 ± 0.2	−0.26[4]	−0.15
Female	38 (66)	13 (76)	25 (61)	−0.04	−0.20
Black or African American	8 (14)	7 (41)[5]	1 (2)	–0.52[5]	0.07
Hispanic	16 (28)	5 (29)	11 (27)	0.03	0.06
Summer	12 (21)	2 (12)	10 (24)	0.34[6]	−0.11
North of Atlanta	36 (62)	8 (47)	28 (68)	0.20	−0.19
Dietary nutrients and physical activity					
Calcium (mg/d)	928 ± 475	849 ± 99	961 ± 78	0.11	0.03
Magnesium (mg/d)	246 ± 107	235 ± 24	251 ± 17	0.02	−0.01
Vitamin D (IU/d)	229 ± 194	154 ± 33	259 ± 32	0.17	−0.01
Glycemic index	53 ± 3	53 ± 1	52 ± 1	−0.26	0.19
MVPA (min/d)[7]	41 ± 33	23 ± 8	45 ± 8	0.51[8]	0.01
Body composition, height, and development factors					
FFM (kg)	56.5 ± 9.6	57.1 ± 11.2	58.2 ± 1.4	−0.16	0.07
FM (kg)	39.8 ± 9.9	45.2 ± 2.0[6]	37.5 ± 1.5	−0.30[8]	−0.06
FM (%)[9]	40.0 ± 5.5	43.3 ± 0.9[6]	38.6 ± 0.9	−0.26[8]	−0.10
VAT (cm^2)	74 ± 36	70 ± 9	76 ± 6	0.00	−0.24
VAT (%)[10]	12.4 ± 4.3	11.2 ± 1.0	12.9 ± 0.7	0.09	–0.29[8]
Height (*z* score)	0.34 ± 0.98	0.15 ± 0.24	0.41 ± 0.15	0.20	0.08
BMI (kg/m^2)	36.0 ± 5.4	39.2 ± 1.4[6]	34.7 ± 0.8	−0.42[6]	−0.08
Tanner stage II-III, pubic hair	12 (21)	2 (12)	10 (24)	0.15	0.24[4]

[1] 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; FFM, fat-free mass; FM, fat mass; VAT, visceral adipose tissue; MVPA, moderate-to-vigorous physical activity (measured by the actigraph method).
[2] Student's *t* test was used to compare continuous variables by 25(OH)D-deficient group.
[3] Pearson correlation coefficient (continuous variables) or point biserial coefficient (categorical variable).
[4] $P > 0.05$ for Pearson correlation; Spearman correlation similar in magnitude, $P < 0.05$.
[5,6,8] Comparison of 25(OH)D-deficient with not-deficient or after testing for nonzero correlation: [5]$P < 0.001$, [6]$P < 0.01$, [8]$P < 0.05$.
[7] $n = 24$.
[9] Fat mass/(fat mass + lean mass + bone mineral content).
[10] VAT/VAT + subcutaneous adipose tissue.

Although TSH and T4 concentrations were within normal limits for all adolescents, TSH was positively associated with PTH concentrations, whereas T4 was inversely related to 25(OH)D concentrations ($P < 0.05$). In addition, leptin was significantly higher in the 25(OH)D-deficient group than in the 25(OH)D-nondeficient group, whereas an inverse association between leptin and PTH concentrations was observed ($P < 0.05$). Glutamate and free testosterone ($n = 41$) were lower in the 25(OH)D-deficient group than in the 25(OH)D-nondeficient group ($P < 0.05$).

Osteocalcin was lower in the 25(OH)D-deficient group than in the 25(OH)D-nondeficient group ($P < 0.05$). All markers of bone metabolism (turnover), including CTX, osteocalcin, and total alkaline phosphatase concentrations, were significantly associated with PTH ($P < 0.05$). Mean BMC and BMD *z* scores were within normal range, as compared with the recently published US norms for age, race, and sex (28).

Potential confounding variables

As discussed above, potential confounding variables of the relation of body fat indexes (FM% and VAT%) to the outcomes (25(OH)D and PTH) were identified based on significant correlation coefficients between sample characteristics and the body fat indexes FM% and VAT% ($n = 58$). Sample characteristics significantly associated with FM% included sex, leptin, vitamin D intake, leucine, and free testosterone ($P < 0.05$). Sample characteristics associated with VAT% included moderate-to-vigorous physical activity (MVPA), HOMA-IR, ratio of triglycerides to HDL cholesterol, glutamate, TSH, and IGFBP-1 ($P < 0.05$). More detailed data on carbohydrate metabolism indexes and body fat indexes for the weight-loss trial are being published separately.

Multivariate analysis

After the core variables age, sex, black or African American race, summer, vitamin D intake, and height *z* score were adjusted for (**Table 3**), an increase of 1% in FM% was associated with a decrease in 25(OH)D of 0.46 ± 0.22 ng/mL ($P = 0.04$, $\beta \pm$ SE; Table 3). The association of FM% and 25(OH)D did not vary significantly by site ($P = 0.07$). Except for sex and height *z* scores, the other core variables age, black or African American race, summer season, and vitamin D intake were all significantly associated with 25(OH)D in this model. Each additional 100 IU

TABLE 2
Metabolic and bone health characteristics with univariate analysis[1]

Characteristic	Mean ± SD	Mean ± SE[2]		Correlation coefficients[3]	
	All (n = 58)	25(OH)D-deficient (n = 17)	Not 25(OH)D-deficient (n = 41)	25(OH)D	PTH
Metabolic factor					
HOMA-IR index	4.5 ± 3.2	5.3 ± 1.0	4.1 ± 0.4	−0.16	−0.08
Hemoglobin A_{1C} (%)	5.4 ± 0.3	5.3 ± 0.1	5.4 ± 0.1	−0.01	−0.14
TG:HDL-C	3.7 ± 4.9	2.8 ± 0.5	4.1 ± 0.9	0.03	−0.02
TSH (μU/mL)	1.59 ± 1.01	1.25 ± 0.13	1.73 ± 0.18	0.07	0.39[4]
T_4 (μU/dL)	7.6 ± 1.6	8.7 ± 0.4[5]	7.2 ± 0.2	−0.33[6]	−0.07
Leptin (ng/mL)	36 ± 19	43 ± 4[6]	33 ± 3	−0.17	−0.23[7]
Leucine (μmol/L)	125 ± 19	123 ± 5	126 ± 3	−0.05	0.23
IGF-I (ng/mL)	340 ± 94	343 ± 26	338 ± 14	0.05	0.24
IGFBP-1 (ng/mL)	2.4 ± 4.7	1.7 ± 0.4	2.7 ± 0.8	0.17	0.02
Glutamate (μmol/L)	57 ± 27	41 ± 4[4]	64 ± 4	0.23	−0.16
Glutamine (μmol/L)	520 ± 67	537 ± 16	513 ± 11	−0.18	0.13
Free testosterone (%)[8]	2.08 ± 0.77	1.60 ± 0.15[4]	2.30 ± 0.15	0.19	0.00
Estradiol (ng/dL)[8]	6.5 ± 6.1	6.0 ± 1.2	6.8 ± 1.3	0.06	−0.10
Bone health					
25(OH)D (ng/mL)	24.7 ± 9.9	12.7 ± 1.2	29.6 ± 1.0	1.00	0.07
PTH (pg/mL)	15.9 ± 9.8	14.4 ± 1.8	16.5 ± 1.7	0.07	1.00
CTX (ng/mL)	1.5 ± 0.9	1.2 ± 0.2	1.6 ± 0.1	0.19	0.27[6]
Osteocalcin (ng/mL)	45 ± 22	35 ± 3[6]	49 ± 4	0.35[4]	0.37[4]
Alkaline phosphatase (IU/L)	129 ± 21	118 ± 12[5]	134 ± 10	0.16	0.45[5]
DXA BMC (g)	2273 ± 401	2345 ± 93	2243 ± 65	−0.10	−0.09
DXA BMC (z score)	1.48 ± 1.39	1.43 ± 0.27	1.50 ± 0.25	0.07	−0.18
DXA BMD (g/cm^2)	1.09 ± 0.12	1.12 ± 0.03	1.08 ± 0.02	−0.13	−0.17
DXA BMD (z score)	0.86 ± 1.39	0.83 ± 0.25	0.87 ± 0.26	0.06	−0.19

[1] 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; HOMA-IR, homeostasis model assessment of insulin resistance; DXA, dual-energy X-ray absorptiometry; BMC, bone mineral content; BMD, bone mineral density; TG, triglycerides; HDL-C, HDL cholesterol; CTX, type I collagen C-telopeptide; IGF-I insulin-like growth factor I; IGFB-1, IGF binding protein-1; TSH, thyrotropin; T_4, thyroxine.

[2] Student's t test was used to compare continuous variables by 25(OH)D-deficient group.

[3] Pearson correlation coefficient (continuous variables) or point biserial coefficient (categorical variable).

[4–6] Comparsion of 25(OH)D-deficient with not-deficient or after testing for nonzero correlation: [4]$P < 0.01$, [5]$P < 0.001$, [6]$P < 0.05$.

[7] $P > 0.05$ for Pearson correlation; Spearman correlation similar in magnitude, $P < 0.05$.

[8] n = 41.

vitamin D/d intake over the past year was associated with an increase in 25(OH)D of 1.10 ± 0.54 ng/mL ($P < 0.05$). Thus, using this model, a supplement of 1000 IU vitamin D/d might theoretically be associated with an increase in 25(OH)D of 11 ng/mL.

Although height z score was not significantly associated with 25(OH)D, height z score was kept in the model because the effect of FM% and vitamin D intake on 25(OH)D were modified by height z score (data not shown). The addition of single variables such as leptin to the core model attenuated the magnitude and the strength of the association between FM% and 25(OH)D. The addition of free testosterone increased the magnitude and enhanced the association between FM% and 25(OH)D (Table 3). This increase was observed regardless of whether the core model included or excluded the 17 subjects with missing free testosterone measurements. Interaction terms with sex and race were not added to the models because they were not significant.

After the core variables were adjusted for, the risk of vitamin D deficiency increased by 1.78 with each percentage increase in FM% (adjusted odds ratio: 1.78; 95% CI: 1.27, 2.50; $P = 0.001$). Each additional intake of 100 IU vitamin D/d over the past year was associated with a decreased risk of vitamin D deficiency (adjusted odds ratio: 0.37; 95% CI: 0.17, 0.80; $P = 0.01$). The results for added covariates to the logistic core model were comparable with those in the linear model (all $P > 0.05$), but the effect for FM% was more statistically significant (data not shown).

The core model for PTH and VAT% is shown in **Table 4**. After age, race, and sex were adjusted for, an increase of 1% in VAT% was associated with a decrease in PTH of 0.78 ± 0.29 pg/mL ($P = 0.009$; Table 4). This association did not vary significantly by site ($P = 0.27$). Overall, and as shown in Table 4, the single addition of potential confounding variables (HOMA-IR, glutamate, or ratio of triglycerides to HDL cholesterol) to the core variables age, sex, and black or African American race did not alter the magnitude or strength of the relation of VAT% to PTH. The addition of MVPA (n = 24) increased the coefficient for VAT% and the fraction of variance explained; this increase was observed regardless of whether the core model included or excluded the 34 subjects without MVPA measurements. When TSH was added to the core model, the magnitude of the association of VAT% to PTH was attenuated (Table 4). In that model, each 0.1-μU/mL increase in TSH was associated with an increase in PTH of 0.26 ± 0.13 pg/mL ($P < 0.05$), but each percentage increase in VAT was no longer significantly associated with PTH (−0.56 ± 0.30 pg/mL; $P = 0.07$). Finally, the

TABLE 3
Multiple linear regression analysis of 25-hydroxyvitamin D [25(OH)D] (ng/mL) in 58 obese adolescents

Model and variable[1]	Adjusted coefficient ± SE[2]	Partial R^2 value[3]	P^4
Core			
Fat mass (%)[5]	−0.46 ± 0.22	0.08	0.04
Age (y)	−1.90 ± 0.72	0.12	0.01
Female vs male	1.53 ± 2.47	0.01	0.54
Black or African American vs others	−14.42 ± 2.74	0.36	<0.001
Summer vs other seasons	6.51 ± 2.36	0.13	0.008
Vitamin D intake (100 IU/d)	1.10 ± 0.54	0.08	<0.05
Height *z* score, 0.1 SD	0.13 ± 0.10	0.03	0.21
Core + free testosterone[6]			
Fat mass (%)	−0.71 ± 0.32	0.13	0.03
Free testosterone (%)	−2.33 ± 1.87	0.05	0.22
Core + leucine			
Fat mass (%)	−0.48 ± 0.22	0.09	0.04
Leucine (μmol/L)	−0.03 ± 0.06	0.01	0.56
Core + leptin			
Fat mass (%)	−0.41 ± 0.27	0.04	0.14
Leptin (ng/mL)	−0.03 ± 0.08	<0.01	0.73

[1] Core model includes percentage of fat mass and variables suggested in models of 25(OH)D in the literature. Other variables were added individually to the core model if they were identified as a potential confounding variable on the basis of their correlation with percentage of fat mass ($P < 0.05$).

[2] Increase in 25(OH)D (in ng/mL) per indicated increment in covariate, adjusted for all other variables in the model.

[3] Fraction of variance explained in addition to that explained by other variables in the model. Total adjusted R^2 for the core model is 0.48.

[4] *P* tests for nonzero coefficient.

[5] Fat mass/(fat mass + lean mass + bone mineral content).

[6] $n = 41$.

addition of IGFBP-1 increased the magnitude of the effect of VAT% on PTH and the strength of the association (Table 4), but each 1-ng/mL increase in IGBP-1 was not significantly associated with PTH (−0.47 ± 0.29 pg/mL; $P = 0.12$). Interaction terms with race and sex were not added to the models because they were not significant.

DISCUSSION

Overall, the study findings indicate that body fat indexes are associated with vitamin D status. Results from these analyses confirm earlier findings of higher BMI with vitamin D deficiency in childhood. Despite the fact that 29% of the adolescents were 25(OH)D-deficient, PTH concentrations and bone mass were within normal limits. To our knowledge, this was the first study to show that obese adolescents with vitamin D deficiency and without elevated PTH have normal bone mass. In the multivariate analysis, higher FM% was associated with lower 25(OH)D and a higher risk of vitamin D deficiency, whereas higher VAT% was associated with lower PTH. To our knowledge, this was the first study to show an inverse relation of VAT% and PTH concentrations in obese adolescents.

Most of the evidence of an association between body fat and vitamin D deficiency or vitamin D status comes from adult studies (34). A recent cross-sectional study in Philadelphia (10) of obese and nonobese persons aged 6–21 y with DXA scans (n = 382, 4% with obesity) showed that concentrations of 25(OH)D were more likely to be lower (<30 ng/mL) in those with greater BMI *z* scores and FM, but not FFM (11). Using DXA to measure FM, we showed that obese adolescents with and without vitamin D deficiency (<20 compared with ≥20 ng/mL) differed according to FM and FM%, but not lean mass. As opposed to the Philadelphia study, we found that the relation of FM% to 25(OH)D and to vitamin D deficiency remained significant after potential confounding variables were adjusted for. The differences between study findings may be explained in part by differences in study design and underlying sample characteristics. Interestingly, the inverse association of circulating 25(OH)D with obesity has been attributed to storage or degradation of vitamin D in adipose tissue (35).

PTH concentrations are expected to be elevated in growing children and in subjects with vitamin D deficiency. About 21% of the adolescents in our study were of Tanner stage II-III, when velocity of growth is expected to be at its peak, and 29% were found to be deficient in vitamin D. Although PTH was higher among obese adolescents of Tanner stage II-III, PTH was within the normal range. Other investigators previously proposed possible compensation mechanisms to explain normal PTH concentrations in the presence of vitamin D deficiency. Current proposed mechanisms include nutrient receptors anomalies (36) or intracellular magnesium deficiency (37). This blunted response of PTH has been described in the adult literature as functional hypoparathyroidism (36–38). Similarly, the anticipated compensatory increase in PTH in response to vitamin D supplementation among preadolescent African American children with vitamin D deficiency was observed among the nonobese but not the obese preadolescents ($P < 0.05$) (20). As described in a study of obese preadolescents (20), we found in our study of obese adolescents that increased vitamin D intake over the past year and 25(OH)D were not associated with higher PTH concentrations.

TABLE 4
Multiple regression analysis of parathyroid hormone (PTH) in 58 obese adolescents[1]

Model and variable[2]	Adjusted coefficient ± SE[3]	Partial R^2 value[4]	P[5]
Core			
VAT (%)[6]	−0.78 ± 0.29	0.12	0.009
Age (y)	−1.17 ± 0.89	0.03	0.20
Female vs male	−6.22 ± 2.64	0.09	0.02
Black or African American vs others	1.23 ± 3.50	<0.01	0.73
Core + MVPA[7]			
VAT (%)	−1.06 ± 0.47	0.22	0.04
MVPA (min)[6]	0.09 ± 0.06	0.11	0.16
Core + IGFBP-1			
VAT (%)	−0.99 ± 0.32	0.16	0.003
IGFBP-1 (ng/mL)	−0.47 ± 0.29	0.05	0.12
Core + TG:HDL-C			
VAT (%)	−0.76 ± 0.31	0.11	0.02
TG:HDL-C unit	−0.05 ± 0.28	<0.01	0.85
Core + HOMA-IR			
VAT (%)	−0.74 ± 0.31	0.10	0.02
HOMA-IR unit	−0.21 ± 0.42	<0.01	0.61
Core + glutamate			
VAT (%)	−0.75 ± 0.31	0.10	0.02
Glutamate (μmol/L)	−0.01 ± 0.05	<0.01	0.81
Core + TSH			
VAT (%)	−0.56 ± 0.30	0.06	0.07
TSH (0.1 μU/mL)	0.26 ± 0.13	0.07	<0.05

[1] MVPA, moderate-to-vigorous physical activity; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triglycerides; HDL-C, HDL cholesterol; IGFB-1, insulin-like growth factor binding protein-1; TSH, thyrotropin; VAT, visceral adipose tissue.

[2] Increase in PTH (pg/mL) per indicated increment in covariate, adjusted for all other variables in model.

[3] Core model includes VAT (%), age, female sex, and race. Single variables were added individually to the core model when identified as a potential confounding variable on the basis of their correlation with VAT (%) ($P < 0.05$).

[4] Fraction of variance explained in addition to that explained by other variables in the model. Total adjusted R^2 for the core model is 0.13.

[5] P tests for nonzero coefficient.

[6] VAT/(VAT + subcutaneous adipose tissue area).

[7] $n = 24$.

Studies of BMC in obese adolescents are rare and show conflicting results. BMC among obese and nonobese adolescents has been described as similar (39), lower (40), or higher (41, 42). The difference in BMC between obese and nonobese adolescents observed between studies was probably due to the underlying sample characteristics and lack of consistent adjustment for covariates. In our study, we showed that the mean BMC was higher than the national average, and all adolescents were found to be within the normal range (±2 SD) based on national standards. As opposed to other studies, we compared BMC (g) and their z score (28) by vitamin D–deficient group and vitamin D status. In our study, we found no significant difference in BMC or BMD according to vitamin D deficiency or status.

There is evidence of an association of insulin resistance and insufficiency with 25(OH)D concentrations in some pediatric studies (21, 23) but not other studies (43). Like Reinehr et al (43), we found no significant relation between insulin indexes and 25(OH)D in adjusted and unadjusted models, even after adjusting for Tanner stage ($P > 0.05$; data not shown). These results may have been due to differences in study design and underlying characteristics. There is also evidence that the thyroid hormone axis may play a role in skeletal growth and bone metabolism. Evidence comes from pediatric studies of hypothyroidism characterized by delayed bone age and short stature but also from supplementation studies of thyroid hormone in adults resulting in bone loss (44). TSH concentrations have been positively associated with higher BMI in adults and with higher bone mass. We found in our study that TSH was associated with PTH and VAT%. In fact, progressive central fat accumulation has been associated with elevated TSH in adults (45).

The limitations of this study included its cross-sectional design, which does not provide information on causality. However, we reproduced prior causal relations identified in other studies (animal and human studies), and we provided data on dietary intake and supplements in the past year (YAQ). Although the rate of vitamin D deficiency may not be generalizable to all populations, findings can be used in future studies as the sample had a racial mix similar to representative national samples. Geographic boundary rather than ultraviolet light B measurements was provided. Black or African American race in this sample should be interpreted as a composite of factors including racial-related factors, geographic boundary at blood drawn, and skin color. Despite incomplete data on several components for the endocrine axis and physical activity measures, we were able to provide initial evidence for some intriguing relations.

These study findings have several implications. First, investigators have proposed that higher body fat leads to increased sequestration of vitamin D in adipose tissue, which results in

reduced bioavailability and lower serum vitamin D concentrations (33). In that case, one would be concerned that 25(OH)D concentrations may not be a good indicator of vitamin D stores in obese adolescent. Our study showed that the relation of body fat to 25(OH)D is highly confounded, and its magnitude of effect is small. Second, results suggest that 1000 IU vitamin D/d would be necessary to increase the concentration of 25(OH)D by 11 ng/mL or decrease the odds of vitamin D deficiency by a factor of 0.00005 over a year among obese adolescents. Third, the assumption that bone mineralization is lower based on measurements of 25(OH)D <20 ng/mL without measurements of PTH concentrations may be irrelevant among obese adolescents, because subjects with vitamin D deficiency and nonelevated PTH concentrations in our study had normal bone mass (BMC and BMD) on DXA scan. Finally, the relation of TSH and PTH concentrations among obese adolescents is intriguing and should be examined further.

In conclusion, obese adolescents with low 25(OH)D concentrations or 25(OH)D deficiency but normal PTH concentrations have higher FM% and normal BMC and BMD values. The study findings provide initial evidence that VAT% is associated with PTH, but this relation may not be independent of TSH. More studies are needed to evaluate the connection between regulatory factors of energy metabolism and bone metabolism in obese adolescents.

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The authors' responsibilities were as follows—CML: chaired the writing committee for the vitamin D study; CML and MFH: conceived and designed the vitamin D study; HAF and CML: analyzed the data; CML, HAF, EVS, AM, DMW, PDKL, SHA, SEG, WJK, GAT, TCC, and MFH: interpreted the data; EVS: supervised the bone mass calibration for the network; GAT: interpreted the radiology tests for the network; TCC and MFH: supervised the laboratory testing for the vitamin D study; CML: drafted the manuscript; and HAF, EVS, AM, CS, DMW, PDKL, SHA, SEG, MSW, WJK, GAT, TCC, and MFH: critically reviewed the manuscript for intellectual content. None of the authors reported any financial conflicts of interest related to the present article; however, PDKL disclosed that he was employed at EMD Serono Inc, Rockland, MA, but that his participation in this study and in the preparation of this manuscript was unrelated to his current employment.

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