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Vitamin D Status and its Relationship to Body Fat, Final Height and Peak Bone Mass in Young Women

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ABSTRACT

Context: Vitamin D insufficiency has now reached epidemic proportions and has been linked to low bone mineral density (BMD), increased risk of fracture and obesity in adults. However, this relationship has not been well characterized in young adults.

Objective: To examine the relationship between serum 25-hydroxyvitamin D (25OHD), anthropometric measures, body fat (BF) and bone structure at the time of peak bone mass.

Design: Cross-sectional study.

Outcome Measures and Subjects: Anthropometric measures, serum 25OHD radioimmunoassay values, and CT and DXA values of BF and bone structure in 90 post-pubertal females, ages 16-22, residing in California.

Results: Approximately 59% of subjects were 250HD insufficient (≤29 ng/ml), and 41% were sufficient (≥30 ng/ml). Strong negative relationships were present between serum 25OHD and CT measures of visceral and subcutaneous fat and DXA values of BF. In addition, weight, body mass, and imaging measures of adiposity at all sites were significantly lower in women with normal serum 250HD concentrations than women with insufficient levels. In contrast, no relationship was observed between circulating 25OHD concentrations and measures of BMD at any site. Unexpectedly, there was a positive correlation between 25OHD levels and height.

Conclusions: We found that vitamin D insufficiency is associated with increased BF and with decreased height but not with changes in peak bone mass.

INTRODUCTION

Vitamin D, a key regulator of bone metabolism, is thought to play an important role in adipogenesis and the prevention of a variety of diseases, including osteoporosis, cancer, diabetes and immune disorders (1-3). It is derived from skin exposure to sunlight (vitamin D_3) or from food supplements (vitamin D_2 or D_3) and undergoes successive hydroxylations in the liver and kidneys to give rise to its active metabolite 1α,25 dihydroxyvitamin D ($1,25(OH)_2D$) (4). The vitamin D receptor (VDR) is widely distributed in various tissues including bone and fat and triggers most of the action of vitamin D (5). There are, however, significant discrepancies in the results of previous studies assessing the relation between vitamin D, bone health and adiposity.

While several studies in adults have shown that vitamin D increases bone mineral density (BMD) (6), prevents osteoporotic fractures (7, 8) and reduces the risk of falling in the elderly (9), other studies in adolescents and young adults have yielded conflicting results; some, but not all, found an association between vitamin D and bone mass (10-15). Moreover, a recent study in adults aged 30-79 suggests the relation between 25OHD levels and BMD is present in the white population, but not in African Americans or subjects of Hispanic ethnicity (16).

Obesity has now reached epidemic proportions and the combined percentage of overweight and obese individuals in the United States is staggering, approaching 32% in children and adolescents and 66% in young adults (17). Although vitamin D insufficiency is prevalent in this population, especially in low socio-economic groups (18, 19), limited information regarding the relationship between weight and vitamin D levels is available. Several studies have shown adult obesity to be inversely correlated with 25OHD levels (20-26) and it has been suggested that adipogenesis may be inhibited by $1,25(OH)_2D$ (27). Even obese adults who take supplemental vitamin D_2 and are exposed to UV light have 25OHD levels substantially lower than non-obese matched controls (24).

Discrepancies in the results from previous studies may, in part, be related to the use of dual-energy x-ray absorptiometry (DXA) to obtain bone and/or fat measures since this projection technique cannot correct for the influence of other soft tissues in the region of interest (28). In this investigation, to account for the influence of soft tissues on DXA bone measurements, we examined the relations between vitamin D, bone health and adiposity by using both DXA and computed tomography (CT). Additionally, the confounding effects of growth and development, aging, and gender on the relations between fat mass, bone mass and vitamin D, were controlled by including only sexually and skeletally mature young females ages 16-22 years.

MATERIALS AND METHODS

Study Subjects

This study was approved by the institutional review board at our institution, and informed consent was obtained from all parents and/or subjects. An initial interview was conducted to describe the purpose and aims of the study and the tests to be performed. Candidates for this study were excluded if they had a diagnosis of any underlying disease or chronic illness, if they had been ill for longer than two weeks during the previous six months, if they had been admitted to the hospital at any time during the previous three years, or if they were taking any medications including oral contraceptives. Candidates who were pregnant, had ever been pregnant, or with absence of menses for more than four consecutive months were also excluded from the study. To decrease the seasonal variability in biochemical determinations, all appointments were scheduled between May and October. In addition, all subjects had normal kidney function and normal liver function tests, and there was no evidence of liver abnormalities detected by CT.

All potential participants underwent a general physical examination, including assessments of the degree of sexual development, and a radiographic examination of the left hand and

wrist. Only those who had reached sexual maturity, defined as Tanner V of breast development (29), and skeletal maturity, defined as epiphyseal closure in the phalanges and metacarpals using the radiographic atlas of Greulich and Pyle (30), were included in the study. Measurements of weight were obtained to the nearest 0.1 kg, using the Scale-Tronix (Scale-Tronix, Inc, Wheaton, Ill) and measurements of height were obtained to the nearest 0.1 cm, using the Harpenden stadiometer (Holtain Ltd, Crymmych, Wales). Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2); for the purpose of this study, subjects were divided into a lean group (BMI < 25) and an overweight group (BMI ≥ 25). Using this approach, 90 female subjects were enrolled in this study and underwent imaging determinations of bone and adipose tissue and biochemical measurements of calcium-regulating hormones.

Bone and Fat Measurements

DXA and CT determinations of bone and fat were performed on the same day by the same technologist. Using a Hologic QDR4500 (Hologic, Inc., Bedford, Massachusetts) DXA scanner, the bone mineral content (BMC; g) and the bone mineral density (BMD, g/cm^2) were measured for the total body and for the axial and appendicular skeleton independently. In addition, the total, subtotal (excluding the head), arms, trunk and legs fat mass (kg) was determined. The coefficients of variation (CV) for repeated DXA measurements of BMC, BMD, and fat mass at the various locations examined have been reported to range from 0.7 to 4.1%, and the radiation exposure is negligible (31).

For CT determinations, a General Electric Hilite Advantage scanner (General Electric Healthcare, Milwaukee, WI) with a standardized reference phantom for simultaneous calibration was used. In the axial skeleton, values for cancellous bone density (BD; mg/cm^3) and for the cross-sectional area (CSA; cm^2) were measured at the midportion of the first three lumbar vertebral bodies, and in the appendicular skeleton, the cross-sectional area (CSA; cm^2), cortical bone area (CBA; cm^2), and cortical bone density (CBD; mg/cm^3) at the midshafts of the femurs were obtained; CVs for these bone measurements in young adults were previously reported between 0.6 and 1.5% (32). Additionally, from the same cross-sectional abdominal images measurements of the visceral fat (VF; cm^2) and subcutaneous fat (SF; cm^2) were obtained. For the purpose of this study, SF was defined as the amount of adipose tissue located between the skin and the rectus muscles of the abdomen, the external oblique muscles, the broadest muscles of the back and the erector muscles of the spine at the level of the umbilicus. VF was defined as the intra-abdominal adipose tissue surrounded by the rectus muscles of the abdomen, the external oblique muscles, the lumbar quadrate muscle, the psoas muscles, and the lumbar spine at the same level. The CV for repeated measures of visceral and subcutaneous fat has been reported to range from 1.5% to 3.5% (33). The time to complete the CT scans was approximately 10 minutes and the effective radiation dose was approximately 0.1 mSv (34).

Biochemical Determinations

Serum levels of 25OHD were assayed using a radioimmunoassay as described by Hollis et al (35). The lower limit of detection was 5 ng/ml (12.5 nmol/L). Goat anti-25OHD was a gift from Dr. Bruce Hollis. ^{125}I-25-(OH)D_3 and Donkey anti-goat secondary antibody were purchased from Diasorin (Stillwater, Mn.). This assay recognizes equally 250HD2 and 250HD3 and shows no bias when compared to HPLC (36). Calculated assay precision for within assay variation averages 6% and for interassay 16%. For the purpose of this study and according to the current consensus, subjects were divided into a 25OHD sufficient, or normal, group (≥ 30 ng/ml) and an insufficient group (≤ 29 ng/mL). Intact PTH (1-84) was measured with an electrochemiluminescent assay (37). The sensitivity of the assay is 1.2pg/ml (0.127 pmol/L) and intra- and inter-assay variations are 1.9-4% and 2.6-6.5%, respectively. To minimize interassay variability all samples were analyzed simultaneously.

Statistical Analysis
A sample size of 90 subjects allows the determination of correlations greater than r = *.28* with 80% power. Statistical analysis was carried out using Statview (version 5.0.1; SAS Institute Inc., Cary, NC). Data were analyzed using simple linear regression analysis, multiple regression analysis and unpaired t-tests. All values are expressed as mean ± SD.

RESULTS

Relation between 250HD and subject characteristics
The age, anthropometric characteristics, and ethnic background of the women studied are described in Table 1. Weight and BMI were significantly higher, while height was significantly lower, in Hispanics than Caucasians. When all subjects were taken together, a significant positive correlation was found between height and 25OHD (Figure 1). In contrast, significant negative correlations were observed between 25OHD, weight and BMI (Figure 1). Multiple regression analysis showed that the negative relation between 250HD and weight and the positive relation between 250HD and height persisted even after accounting for differences in ethnic background.

Table 2 shows the mean values for 25OHD concentrations in lean (BMI < 25) and overweight (BMI ≥ 25) subjects. While mean serum values were significantly lower in Hispanics than in Caucasians, ethnic differences in 25OHD concentrations did not persist after adjusting for BMI (Table 2).

Thirty-seven (41%) women had normal 25OHD concentrations (≥ 30 ng/ml), while 53 (59%) women had insufficient 25OHD concentrations (≤ 29 ng/ml); of the insufficient group, 24 (45%) had values ≤20 ng/ml. Compared to women with normal 250HD values, vitamin D-insufficient subjects were of identical age, but were significantly shorter, heavier, and had greater BMI (Table 3). When the sufficient group was analyzed independently, no associations were present between vitamin D and any anthropometric measures. In contrast, there were significant negative correlations between 250HD and both weight and BMI (r's = -0.28 and -0.33; P's = 0.045 and 0.015, respectively), in the insufficient group.

A significant inverse correlation was found between 25OHD and PTH (r = -0.27; P = 0.01) and PTH values were higher in the insufficient than in the sufficient group (2.28 ± 0.88 and 1.92 ± 0.90, respectively; P = 0.025) (Figure 2).

Relation between 250HD and imaging measures of body fat and bone
CT measures for subcutaneous and visceral fat and DXA measurements for whole body fat, truncal fat and upper and lower extremity fat were significantly lower in women with normal 250HD concentrations than women with insufficient 250HD (Table 4). In contrast, there were no differences in CT or DXA values for bone in the axial and appendicular skeleton between women with sufficient and those with insufficient 250HD concentrations (Table 4).

Regardless of imaging technique, strong negative correlations were observed between all measures of body adiposity and 25OHD at all sites (Table 5). These associations were present when all women were considered together and when 250HD insufficient subjects were analyzed independently (Table 5); this relation was not present in women with sufficient 250HD. In contrast, regardless of whether all subjects were taken together or were separated by 250HD concentration group, no significant association was found between 25OHD levels and any DXA or CT bone phenotypes (data not shown).

DISCUSSION

We found a strong inverse correlation between weight and body mass and circulating vitamin D, and that young women with vitamin D-insufficiency were significantly heavier and had greater body mass than women with normal levels. Additionally, the results of this study showed significant reciprocal relations between 250HD and CT measures for subcutaneous and visceral fat and DXA measures of adiposity for the whole body, trunk and extremities. The high prevalence of vitamin D insufficiency in this young population living in a sun-rich area is

surprising and likely multifactorial. A recent report indicates that vitamin D insufficiency is common in children ages 6-21 living in the Northeastern United States and is associated with season, ethnicity/black race, age and vitamin D intake (18) but similar observations have not yet been reported in California. While vitamin D insufficiency was more common in Hispanics than in Caucasians in our study cohort, this difference did not persist after adjusting for BMI, indicating that the predominant risk factor was body fat rather than any variability in skin color attributed to ethnicity.

In view of the prevalence of both vitamin D insufficiency and obesity in children and adolescents, it is possible that vitamin D status is an independent predictor of weight gain. Several studies in the adult population suggest that obesity is associated with vitamin D insufficiency (20-24, 26, 38), and one indicates that low vitamin D intake is an independent predictor of obesity (25). Another investigation in post-menopausal women receiving calcium plus vitamin D reported a small effect on weight gain prevention as compared to placebo (39). Indeed, vitamin D has been shown to lower leptin concentrations and may therefore contribute to the maintenance of body mass (40). On the other hand, body fat may also contribute to low circulating vitamin D levels by trapping vitamin D in fat tissues (24). Thus, obesity may, in part, be a direct consequence of vitamin D insufficiency and/or may result in vitamin D insufficiency. It is noteworthy that vitamin D insufficiency has been implicated in numerous health conditions including osteoporosis, cancer, diabetes, and rheumatoid arthritis (1, 2, 41), and that increased body fat is also strongly associated with greater risk of diabetes and cancer (42). Consequently, vitamin D insufficiency may play an important role in the development of these various clinical conditions either directly or indirectly.

In addition to weight and body mass, we specifically determined fat content and fat distribution using DXA and CT. Previously, using bioelectrical impedance analysis in a large group of women of all ages, indirect measures of the percentage of body fat were found to be inversely related to circulating 25OHD; an association that was particularly noticeable in white females aged 12-49 (38). Another study using DXA also found a negative correlation between 25OHD and percentage of body fat, but not BMI, in healthy adult women (22). The current study extends these findings to a young population of white females and indicates a strong inverse correlation between body fat and 25OHD using total body measurements by DXA and site specific measurements by CT. Our data indicate that 25OHD is not only inversely correlated with total body fat, but also with specific measures of visceral fat and subcutaneous fat, suggesting that this relationship is independent of the site of fat accumulation.

Unexpectedly, there was a positive correlation between circulating 25OHD and height in the population studied. While vitamin D is key to skeletal development and its deficiency may result in short stature associated with rickets (15) none of the subjects in this study had any clinical or radiological evidence of rickets. A significant decrease in height was previously reported in adolescent girls aged 13 to 17 years who had vitamin D deficiency without any clinical evidence of rickets (10). Further studies are needed to determine the possible role of vitamin D in longitudinal bone growth in the absence of clinical evidence of rickets.

An intriguing result of this study was the absence of a correlation between vitamin D status and bone determinations, regardless of site or whether assessed by DXA or CT. Previous investigations in adults have indicated that vitamin D supplementation improved bone mineral density and reduced the risk of osteoporosis and fractures (6-8, 14). However, studies in adolescent females have yielded discrepant results; some reported an association between low bone mass and vitamin D insufficiency and low vitamin D intake (12, 13), while others, like us, found no such relation (10, 11). Although our population was comprised of Hispanic and Caucasian, this study was not powered to analyze Caucasian and Hispanic separately, and the possibility of ethnic

variability in the response to vitamin D exposure cannot be excluded. Similarly, our findings in females do not exclude the notion that vitamin D influences bone mass in adolescent and young adult males, as previously reported (43, 44). Despite these limitations, the results of the current study support the hypothesis that the negative effect of vitamin D insufficiency on bone mass may not be present in healthy young adults around the time that bone mass reaches its peak.

The use of two techniques for the accurate and independent assessment of the relations of vitamin D to bone and fat tissue, the use of the same technologist to obtain all CT and DXA measures, and the rigorous assessment of the sexual and skeletal development are major strengths of this study. Previous studies on the effects of vitamin D insufficiency on bone were mostly conducted using DXA, a technique that is low in cost, has minimal radiation exposure, and is readily accessible and easy to use. Although DXA values are influenced by changes in body configuration (28, 45, 46), and inherently underestimate bone acquisition in short and or overweight individuals (47), it should be noted that in spite of these limitations, our findings were similar regardless of technique.

In conclusion, our study indicates that vitamin D insufficiency is extremely common in young women living in a sun-rich area of the United States. It also supports the hypotheses that either vitamin D insufficiency is a risk factor for increased body fat or increased body fat is a risk factor for vitamin D insufficiency. The positive association between height and vitamin D status is unexplained and intriguing, and warrants further investigation. Our data, however, do not support a role for vitamin D in regulating bone mass acquisition around the time it reaches its peak.

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Table 1.
Age and anthropometric characteristics of 90 women separated by ethnic background

	All (n = 90)	Hispanic (n = 53)	Caucasian (n = 37)	P
Age (yrs)	19.4 ± 1.5 (16.3 – 22.8)	19.6 ± 1.4 (17.0 – 22.8)	19.1 ± 1.6 (16.3 – 22.2)	0.100
Weight (kg)	68.3 ± 17.5 (45.5 – 126.0)	72.7 ± 20.5 (45.5 – 126.0)	61.9 ± 8.8 (45.6 – 90.3)	0.003
Height (cm)	162.9 ± 4.7 (153.9 – 171.8)	161.6 ± 4.7 (153.9 – 171.8)	164.8 ± 4.1 (156.3 – 170.8)	0.001
BMI	25.7 ± 6.3 (16.7 – 44.5)	27.7 ± 7.1 (17.6 – 44.5)	22.8 ± 3.5 (16.7 – 35.6)	<0.001

Values are expressed as mean ± SD and (range).
P-values indicate results of unpaired t-tests between ethnic backgrounds.

Table 2.
25OHD concentrations (ng/ml) of 90 women separated by ethnicity and body mass

	25OHD (ng/ml)		
	All (n = 90)	Hispanic (n = 53)	Caucasian (n = 37)
All BMI (n = 90)	30.1 ± 13.0 (6.7 – 69.6)	26.6 ± 12.3[†‡] (6.7 – 67.3)	35.1 ± 12.4 (14.2 – 69.6)
Lean (BMI < 25) (n = 51)	34.3 ± 13.8* (15.2 – 69.6)	31.2 ± 14.6 (15.2 – 67.3)	36.6 ± 12.9 (16.1 – 69.6)
Overweight (BMI ≥ 25) (n = 39)	24.6 ± 9.5 (6.7 – 46.0)	23.3 ± 9.3 (6.7 – 44.9)	29.7 ± 9.3 (14.2 – 46.0)

Values are expressed as mean ± SD and (range).
*Indicates a significant difference between lean and overweight subjects (P <0.001)
[†] Indicates a significant difference between Hispanics and Caucasians (P = 0.002)
[‡] ANOVA analysis indicates no statistical difference between Hispanics and Caucasians when adjusted for BMI (P = 0.09)

Table 3.
25OHD values, age, and anthropometric characteristics of 90 women separated by 25OHD concentration groups

	All (n = 90)	Sufficient (n = 37)	Insufficient (n = 53)	P-values
25OHD (ng/ml)	30.1 ± 13.0 (6.7 – 69.6)	42.4 ± 10.1 (30.0 – 69.6)	21.5 ± 5.9 (6.7 – 29.6)	<0.001
Age (yrs)	19.4 ± 1.5 (16.3 – 22.8)	19.2 ± 1.6 (16.3 – 22.8)	19.5 ± 1.4 (17.0 – 22.87)	0.408
Weight (kg)	68.3 ± 17.5 (45.5 – 126.0)	63.9 ± 11.9 (45.6 – 113.0)	71.3 ± 20.0 (45.5 – 126.0)	0.046
Height (cm)	162.9 ± 4.7 (153.9 – 171.8)	164.1 ± 3.9 (156.8 – 170.3)	162.1 ± 5.1 (153.9 – 171.8)	0.048
BMI	25.7 ± 6.3 (16.7 – 44.5)	23.7 ± 4.6 (16.7 – 43.9)	27.1 ± 7.1 (17.6 – 44.5)	0.014

Values are expressed as mean ± SD and (range).
P-values indicate results of unpaired t-test between 25OHD concentration groups.

Table 4.
CT and DXA fat and bone measurements in 90 women separated by 25OHD concentration groups

		All (n= 90)	Sufficient (n = 37)	Insufficient (n = 53)	P
Fat Phenotypes					
CT	Subcutaneous (cm^2)	252.8 ± 152.7	203.3 ± 98.9	288.1 ± 174.0	0.029
	Visceral (cm^2)	36.46 ± 42.89	24.74 ± 33.88	44.81 ± 46.83	0.009
DXA	Total (kg)	24.81 ± 11.79	21.59 ± 7.67	27.10 ± 13.62	0.029
	Trunk (kg)	11.29 ± 5.76	9.35 ± 3.82	12.69 ± 5.61	0.006
	Arms (kg)	1.64 ± 1.02	1.34 ± 0.61	1.85 ± 1.19	0.019
	Legs (kg)	4.75 ± 1.91	4.33 ± 1.38	5.05 ± 2.17	0.077
Bone Phenotypes					
CT	Vertebral BD (mg/cm^3)	299.1 ± 43.5	294.4 ± 37.3	302.5 ± 47.5	0.392
	Vertebral CSA (cm^2)	8.78 ± 1.32	8.73 ± 1.21	8.83 ± 1.40	0.723
	Femoral CBD (mg/cm^3)	1234 ± 36	1236 ± 37	1233 ± 37	0.763
	Femoral CBA (cm^2)	4.23 ± 0.53	4.24 ± 0.39	4.23 ± 0.61	0.955
	Femoral CSA (cm^2)	5.11 ± 0.72	5.07 ± 0.57	5.14 ± 0.81	0.649
DXA	Total BMC (g)	2105 ± 298	2110 ± 272	2101 ± 317	0.886
	Total BMD (g/cm^2)	1.11 ± 0.07	1.11 ± 0.08	1.11 ± 0.07	0.919
	Trunk BMC (g)	168.0 ± 26.9	161.8 ± 19.3	172.5 ± 30.6	0.771
	Trunk BMD (g/cm^2)	0.88 ± 0.07	0.88 ± 0.07	0.88 ± 0.08	0.884
	Hip BMC (g)	39.56 ± 6.16	40.19 ± 4.95	39.12 ± 6.90	0.421
	Hip BMD (g/cm^2)	1.05 ± 0.11	1.05 ± 0.08	1.04 ± 0.13	0.663
	Arms BMC (g)	138.8 ± 23.9	137.8 ± 18.0	139.5 ± 27.4	0.933
	Arms BMD (g/cm^2)	0.73 ± 0.06	0.74 ± 0.06	0.73 ± 0.06	0.446
	Legs BMC (g)	389.9 ± 62.7	392.8 ± 50.4	387.8 ± 70.5	0.715
	Legs BMD (g/cm^2)	1.17 ± 0.09	1.17 ± 0.08	1.16 ± 0.10	0.586

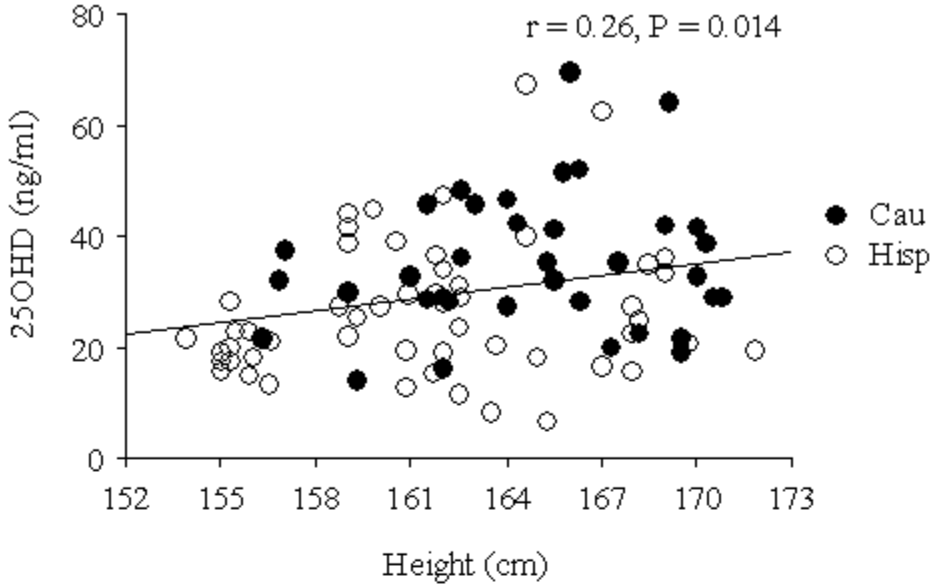
P-values indicate results of unpaired t-test between 250HD concentration groups.

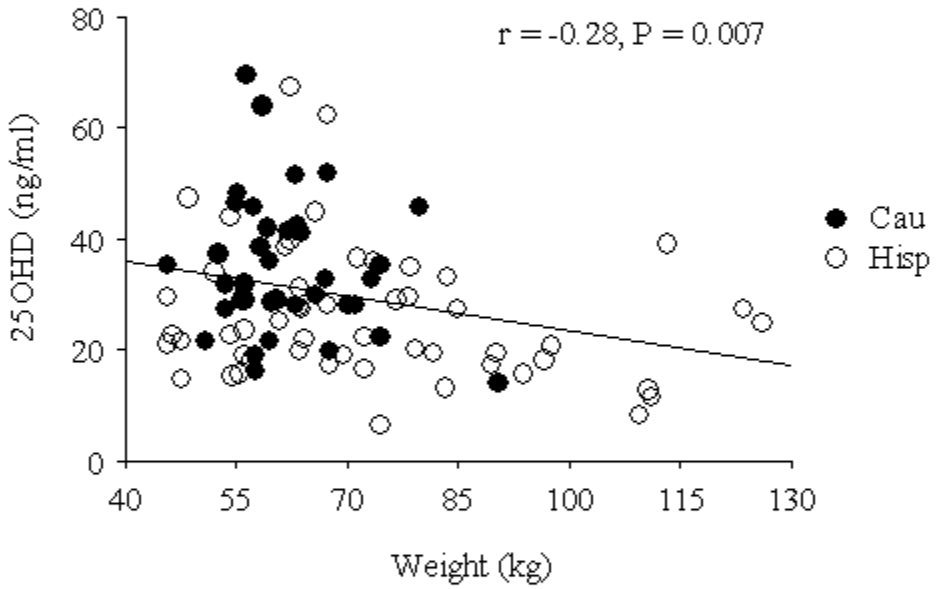
Table 5.
Relations between 25OHD concentrations and imaging measures of fat and bone in 90 women

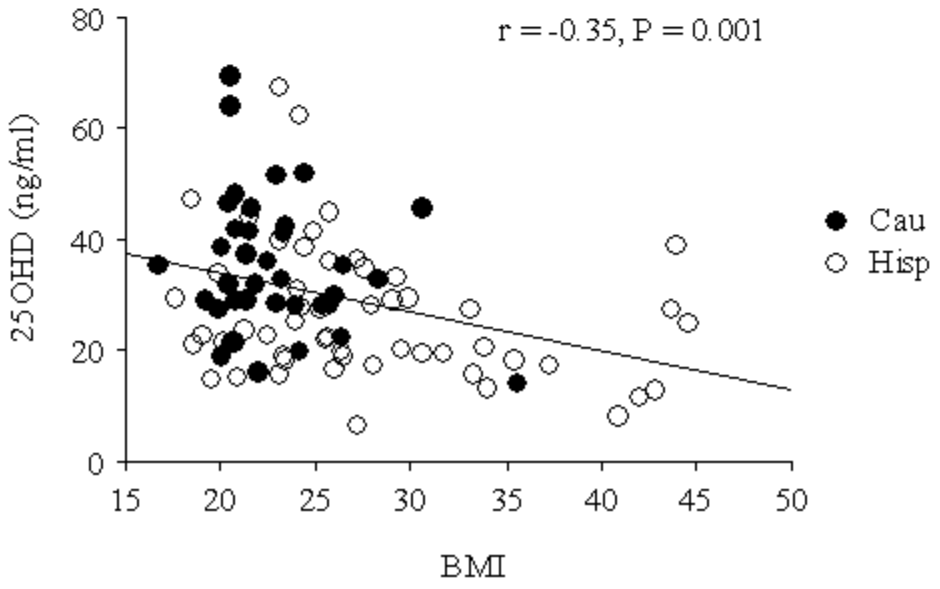
		All (n = 90)		Sufficient (n = 37)		Insufficient (n = 53)	
		r	P	r	P	r	P
Fat Phenotypes							
CT	Subcutaneous	-0.36	<0.001	-0.19	0.261	-0.32	0.019
	Visceral	-0.28	0.007	-0.05	0.769	-0.30	0.031
DXA	Total	-0.32	0.002	-0.18	0.303	-0.32	0.022
	Trunk	-0.37	<0.001	-0.16	0.333	-0.35	0.011
	Arms	-0.29	0.006	-0.16	0.204	-0.33	0.025
	Legs	-0.33	0.001	-0.22	0.342	-0.31	0.016

Figure Legend

Figure 1. Relation between vitamin D concentrations and height, weight and BMI.


r = 0.26, P = 0.014
25OHD (ng/ml)
0
20
40
60
80
152
155
158
161
164
167
170
173
Height (cm)
Cau
Hisp


r = -0.28, P = 0.007
25OHD (ng/ml)
0
20
40
60
80
40
55
70
85
100
115
130
Weight (kg)
Cau
Hisp


r = -0.35, P = 0.001
25OHD (ng/ml)
0
20
40
60
80
15
20
25
30
35
40
45
50
BMI
Cau
Hisp