

The Relationship between Obesity and Serum 1,25-Dihydroxy Vitamin D Concentrations in Healthy Adults

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Several previous reports of small cohorts have found significantly higher serum 1,25-dihydroxy vitamin D (1,25-vit D) in obese compared with nonobese whites. Based on these reports and on recent *in vitro* studies of adipocytes which suggest that administration of 1,25-vit D can stimulate lipogenesis and inhibit lipolysis, some investigators have proposed that high 1,25-vit D may play a role in promoting or maintaining adipocyte triglyceride stores in obese adults. To test the hypothesis that obesity is commonly associated with increased 1,25-vit D, we examined the relationships between calciotropic hormones and body adiposity in a large cohort of healthy adults. Serum intact PTH, 25-hydroxy vitamin D, and 1,25-vit D were measured in the postabsorptive state in 302 healthy adults who were Caucasian (n = 190; 71% female), African-American (n = 84; 89% female), and of other race/ethnicity (n = 28; 61% female). Results from the 154 obese subjects [body mass index (BMI) $37.3 \pm 5.8 \text{ kg/m}^2$; range, $30.1\text{--}58.2 \text{ kg/m}^2$] were

compared with those from 148 nonobese (BMI $25.6 \pm 2.9 \text{ kg/m}^2$; range, $18.0\text{--}29.9 \text{ kg/m}^2$) age-, race-, and sex-matched participants. Body composition was measured by dual energy x-ray absorptiometry. Serum intact PTH was positively correlated with both BMI ($r = 0.42$; $P < 0.0001$) and body fat mass ($r = 0.37$; $P < 0.0001$). Serum 25-hydroxy vitamin D was negatively correlated with BMI ($r = -0.4$; $P < 0.0001$) and body fat mass ($r = -0.41$; $P < 0.0001$). Serum 1,25-vit D was also negatively correlated with BMI ($r = -0.26$; $P < 0.0001$) and body fat mass ($r = -0.25$; $P = 0.0001$). Serum 1,25-vit D was significantly lower in obese than nonobese subjects (105.7 ± 41.1 vs. $124.8 \pm 36.7 \text{ pmol/liter}$; $P < 0.0001$) in both Caucasian and African-American adults. **We conclude that, because 1,25-vit D concentrations fall with increasing adiposity, it appears unlikely that elevation in 1,25-vit D is an important hormonal mechanism causing or maintaining obesity in adults.** (*J Clin Endocrinol Metab* 89: 1196–1199, 2004)

THE 1999–2000 NATIONAL Health and Nutrition Examination Survey (NHANES) found that the prevalence of obesity [body mass index (BMI) $\geq 30 \text{ kg/m}^2$] among adults in the United States has more than doubled since the NHANES II survey (1976–1980), so that now nearly one third of all U.S. adults are categorized as obese (1). Obese individuals are at risk for a number of metabolic and endocrine abnormalities (2). Among the endocrine derangements of obesity is hyperparathyroidism, believed to be secondary to hypovitaminosis D. Obese adults and overweight children have been found to have low 25-hydroxy vitamin D (25-OH-vit D) (3–6) and high intact PTH (iPTH) (3, 7).

Four previous studies have also reported that obese individuals have higher 1,25-dihydroxy vitamin D (1,25-vit D) serum concentrations than nonobese controls (3, 4, 7, 8). The finding of high serum concentrations of 1,25-vit D in obesity is of particular interest because recent reports have suggested that 1,25-vit D might play a role in predisposing overweight adults to further weight gain (as well as to hyperinsulinemia and insulin resistance) via the ability of 1,25-vit D to increase intracellular Ca^{2+} concentrations, particularly in adipocytes

(9). Investigators performing *in vitro* studies using human and murine adipocyte models have reported that, upon binding to the vitamin D receptor, 1,25-vit D can cause an increase in intracellular calcium that in turn can stimulate lipogenesis and inhibit lipolysis in adipocytes (10). 1,25-vit D has thus been identified as being a potential key player for stimulating triglyceride accumulation in obese subjects (9, 11). However, the four descriptions of high serum 1,25-vit D concentrations in obese humans (3, 4, 7, 8) were obtained from very small cohorts, with sample sizes of 12 (3), 13 (4), 14 (8), and 16 (7) obese subjects, all of whom appear to have been Caucasian. A review of the medical literature found no large studies that have examined serum 1,25-vit D as a function of body adiposity. We therefore examined hormones related to calcium homeostasis in a large adult cohort that was selected to be representative of the U.S. population, to test the hypothesis that obesity is associated with an increase in serum 1,25-vit D.

Subjects and Methods

Subjects

Between the months of April 2002 and April 2003, we studied 302 healthy adults from the metropolitan Washington, D.C. area who were recruited through newspaper and radio advertisements. Recruitment did not vary by season. Race was self-assigned. Subjects were required to be free of significant medical illnesses and to be taking no medications on a regular basis known to impact body weight or calcium homeostasis. The clinical protocol was approved by the National Institutes of Child

Abbreviations: BMI, Body mass index; CV, coefficients of variation; DXA, dual energy x-ray absorptiometry; iPTH, intact PTH; 1,25-vit D, 1,25-dihydroxy vitamin D; 25-OH-vit D, 25-hydroxy vitamin D.

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Health and Human Development Institutional Review Board. Informed consent was obtained from all subjects. Financial compensation was provided for subjects' time and inconvenience.

Clinical protocol

Subjects were studied at the Warren Grant Magnuson Clinical Center of the National Institutes of Health (NIH). All visits were conducted in the morning, and subjects were asked to report after a 10-h overnight fast. Each subject underwent a full history and physical examination, anthropometric measurements, and whole-body dual energy x-ray absorptiometry (DXA) for body composition analysis. DXA was performed using the Delphi (Hologic Inc., Bedford, MA) array beam instrument (software version 11.2). Weight was measured to the nearest 0.1 kg using a digital scale (Life Measurement Instruments, Concord, CA) that was calibrated with a known weight before each measurement. Height was measured in triplicate to the nearest 1 mm using a stadiometer also calibrated before each set of measurements (Holtain Ltd., Crymch, UK). 25-OH-vit D measurements were performed using a competitive binding assay (Nichols Advantage, Nichols Diagnostics, San Clemente, CA) (12). Sensitivity of the assay was 7 ng/ml (17.5 nmol/liter), and the mean inter- and intraassay coefficients of variation (CV) were 8.9% and 5.7%, respectively. 1,25-vit D levels were measured using cartridge extraction and RIA (Mayo Medical Laboratories, Rochester, MN). Sensitivity of this assay was 10 pg/ml (26 pmol/liter), and its mean inter- and intraassay CV were 11.4% and 12%, respectively (12). iPTH measurements were made using a two-site immunoenzymometric assay (Nichols Advantage, Nichols Diagnostics) (13). Sensitivity was 1 pg/ml, and the mean inter- and intraassay CV were 7.9% and 4.7%, respectively. Other blood chemistries, such as hepatic, renal, and thyroid functions, were obtained through the hospital clinical chemistry laboratory by standard methods. All blood samples were collected between 0900 and 1100 h.

Statistical analysis

Data were analyzed using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL) and StatView version 5.01 (SAS Institute, Inc., Cary, NC). Unless otherwise indicated, data are reported as mean \pm SD. Correlations between parameters were evaluated using Spearman correlation coefficients. Comparisons between groups (*e.g.* between African-American and Caucasian subjects) were made using unpaired Student's *t* tests, ANOVA, or analysis of covariance as indicated. *P* value less than 0.05 was considered significant.

Results

Of the 302 enrolled subjects, the 152 obese subjects were similar in age, gender, and race to the 148 nonobese subjects (Table 1). Mean serum 25-OH-vit D in the obese group was

TABLE 1. Subject demographics and calcitropic hormone levels in obese and nonobese subjects

	Obese (n = 154)	Nonobese (n = 148)	<i>P</i>
Females (%)	78	72	0.27
Race % (C/AA/O)	58/32/10	68/23/9	0.33
Age (yr)	37.6 \pm 9.4	36.6 \pm 11.4	0.38
Range (yr)	18.8–60.7	19.3–71.2	
BMI (kg/m ²)	37.44 \pm 6	25.66 \pm 3	<0.0001
Range (kg/m ²)	30.07–58.22	18.03–29.9	
25-OH-vit D (ng/ml)	23.5 \pm 12.2	31 \pm 14.4	<0.0001
Range (ng/ml)	7–87	7–77	
iPTH level (pg/ml)	59 \pm 21	48 \pm 15.7	<0.0001
Range (pg/ml)	18.8–122	17.3–87.6	
1,25 vit D (pg/ml)	44 \pm 15.3	52 \pm 15.3	<0.0001
Range (pg/ml)	10–100	18–110	

Values are mean \pm SD. C, Caucasian; AA, African-American; O, other. To convert to SI units, multiply by 2.496 for 25-OH-vit D (nmol/liter), by 2.6 for 1,25-vit D (pmol/liter), and by 0.1 for iPTH (pmol/liter).

23.5 \pm 12.2 ng/ml (58.7 \pm 30.5 nmol/liter), significantly lower (*P* < 0.0001) than that of the nonobese group (31 \pm 14.4 ng/ml; 77.4 \pm 35.9 pmol/liter). Serum iPTH concentrations were significantly higher (*P* < 0.0001) in obese subjects (53.8 \pm 19 *vs.* 43.4 \pm 14.3 pg/ml). Serum 1,25-vit D was significantly lower (*P* < 0.0001) in obese (44 \pm 15.3 pg/ml; 114.4 \pm 39.8 pmol/liter) compared with nonobese subjects (52 \pm 15.3 pg/ml; 135.2 \pm 39.8 pmol/liter). Serum calcium, ionized calcium, magnesium, and serum phosphorus levels were not significantly different in the two groups.

There was a positive association (*r* = 0.42; *P* < 0.0001) between iPTH and BMI (Fig. 1A) and DXA fat mass (*r* = 0.37; *P* < 0.0001; Fig. 1B), and a negative association (*r* = -0.4; *P* < 0.0001) between 25-OH-vit D and BMI (Fig. 1C) and DXA fat mass (*r* = -0.4; *P* < 0.0001; Fig. 1D). 1,25-vit D levels were also negatively correlated (*r* = -0.26; *P* < 0.0001) with BMI (Fig. 1E) and with body adiposity (*r* = -0.24; *P* = 0.0001; Fig. 1F). The prevalence of a high serum 1,25-vit D concentration (>67 pg/ml, greater than the upper limit of normal range for the assay) in the nonobese group (13.5%) was almost twice that of the obese group (7.2%; *P* = 0.11). When the data were stratified by race, we found that the inverse relationship between 1,25-vit D and BMI was present in both African-American (*n* = 84; *r* = -0.46; *P* < 0.0001) and Caucasian (*n* = 190; *r* = -0.18; *P* = 0.01) subjects. Similar results were found for the relationship between 25-OH-vit D and BMI (African-Americans, *r* = -0.37; *P* = 0.0006; *vs.* Caucasians, *r* = -0.428; *P* < 0.0001). In a multiple-regression model accounting for age, race, and gender, 1,25-vit D was independently and negatively associated with BMI (*r* = -0.26; *P* < 0.0001) as well as with DXA fat mass (*r* = -0.25; *P* < 0.0001).

Discussion

We found lower 25-OH-vit D and 1,25-vit D and higher iPTH concentrations in obese adults, independent of age, sex, or race. Previous studies have found negative associations between serum 25-OH-vit D and BMI (3–5, 14) and body fat mass (6). BMI has also been inversely correlated with peak serum vitamin D₃ levels after exposure of the skin surface to irradiation and with peak serum vitamin D₂ levels after oral ingestion of vitamin D₂ (5, 15). This apparent decrease in vitamin D bioavailability with increased adiposity has been hypothesized to be due to the increased sequestration of vitamin D in fat (4). Interestingly, the previously reported higher 1,25-vit D levels in obese subjects have been proposed to exert negative feedback control over the synthesis of 25-OH-vit D in the liver, thus accounting for the lower levels of 25-OH-vit D in obese subjects (14). Because on average the 1,25-vit D levels were not raised in the obese subjects in our study, it seems unlikely that their lower 25-OH-vit D levels could possibly be due to any type of negative feedback at the hepatic level from the circulating 1,25-vit D.

The lower bioavailable vitamin D observed in obese individuals in our study is the most likely cause of their higher iPTH levels, as has been suggested in previous studies that have simultaneously measured 25-OH-vit D and PTH levels in obese subjects (3, 5, 16). The higher PTH concentrations found in obesity would be expected to stimulate the 1- α -hydroxylase enzyme that converts 25-OH-vit D to 1,25-vit D

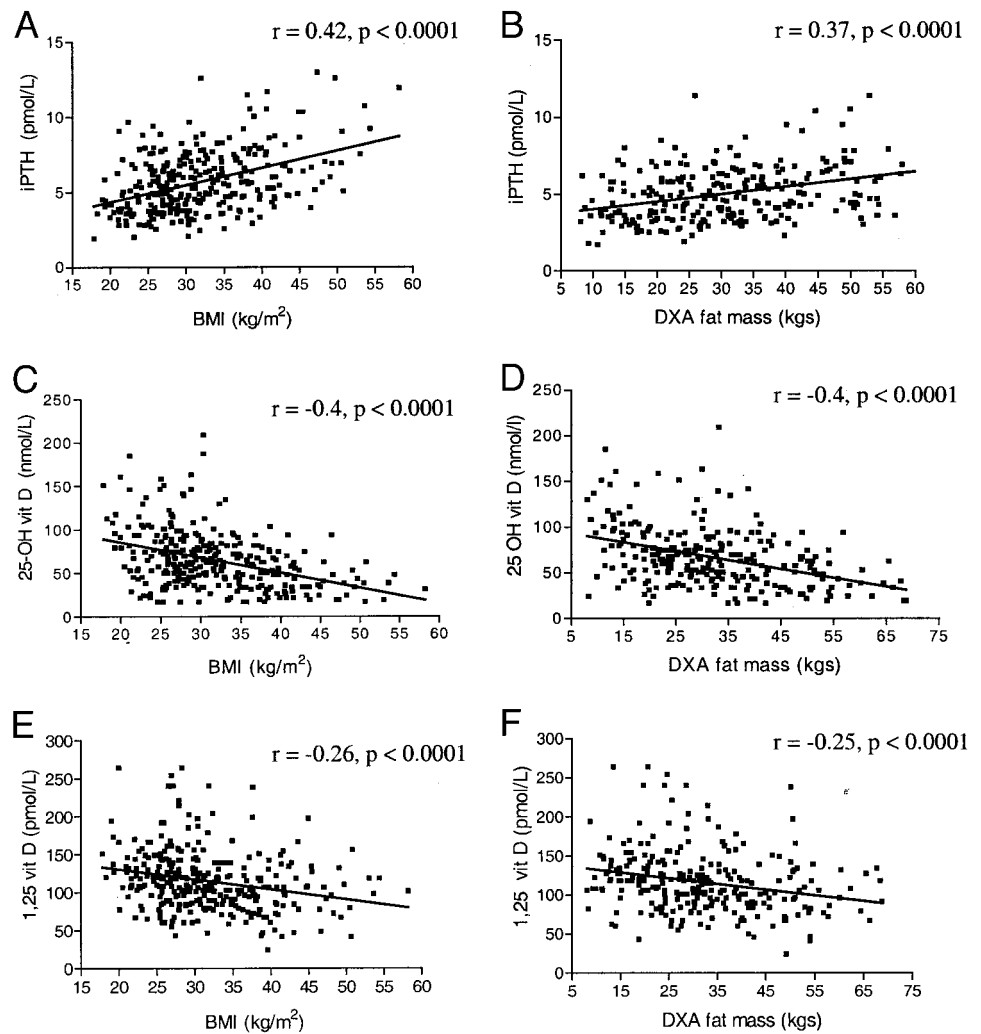


FIG. 1. Relationships between calcitropic hormones, BMI, and body fat mass as measured by DXA scans.

in humans. However, the present study demonstrates that despite their higher PTH levels, obese adults have lower 1,25-vit D levels than nonobese controls. The apparent negative relationship between 1,25-vit D and body weight was found over a wide range of BMI (from 18 to 56 kg/m²) in this study sample. A similar phenomenon of low 25-OH and 1,25-vit D levels with high PTH levels has also been reported in elderly institutionalized adults (17). The low 1,25-vit D in the face of significantly elevated PTH levels in institutionalized individuals was attributed to low 25-OH-vit D levels (low substrate) or a smaller renal mass (hydroxylase enzyme deficiency). The latter reasoning seems unlikely to be the cause of lower 1,25-vit D levels in the obese subjects of the present study, all of whom had normal renal function, but it is possible that the decreased availability of 25-OH-vit D levels in most obese subjects could at least in part account for their decreased 1,25-vit D concentrations. Irrespective of the actual mechanism, it is clear that, contrary to previous reports of studies that included relatively few obese subjects (3, 4, 7, 8), 1,25-vit D levels are not usually elevated in obese adults.

The present study is somewhat limited by the fact that calcitropic hormone levels were measured only one time in

each subject. Based on the observation that serum 1,25-vit D concentrations may be directly correlated with observed daily changes in body weight ($r = 0.68$; $P < 0.001$) and with caloric intake per kilogram per day ($r = 0.39$; $P = 0.01$), Lemann *et al.* (18) have concluded that an accurate assessment of serum 1,25-vit D in an individual requires several measurements over a time period during which body weight is stable. We believe that the data of the present study are reliable because none of our study subjects reported a change of more than 3% of their body weight for the 2 months preceding their clinic visit and because the large sample size ensures that individual variations could not account for the observed associations.

Differences in individual assays for measuring 1,25-vit D between the previous studies (3, 4, 7, 8), all of which were reported more than a decade ago, and the present study have to be considered as well while interpreting the results. The older studies used a competitive protein binding assay (sensitivity, 14 pmol/liter; inter- and intraassay variations, 11%) (19), either with (8) or without (3, 4) the use of HPLC technique (20) to separate the 1,25-vit D from other vitamin D metabolites, whereas we used the currently available RIA with the using the cartridge extraction method (12). The

hitherto unknown effects of obesity on various vitamin D binding proteins that in turn bind to different vitamin D metabolites may account for at least some of the differences between obese and nonobese subjects in the levels of 25-OH-vit D, but not for variations in 1,25-vit D, which largely circulates in an unbound fashion in the serum and mediates its target nuclear actions through a cytosolic 1,25-vit D receptor binding protein to which it binds with high specificity and low affinity (21, 22). Studies in large cohorts using the older competitive protein binding assays are needed to determine whether the results reported in the current investigation would be altered by the use of such assays.

Recent *in vitro* experiments suggest that 1,25-vit D induces a dose-responsive (1–50 nM) increase in intracellular calcium along with a 50–100% increase in adipocyte fatty acid synthase expression and activity, a significant increase in glycerol-3-phosphate dehydrogenase activity, and a significant inhibition of basal as well as isoproterenol-stimulated lipolysis (10). These data have led some researchers to suggest that 1,25-vit D might exert a coordinated control over lipogenesis and lipolysis via its modulation of adipocyte calcium signaling. The previous findings of high serum 1,25-vit D in obese individuals was then interpreted as a potentially important cause for elevated intraadipocyte calcium levels that would lead to increased lipogenesis and decreased lipolysis in the obese (9). 1,25-vit D-mediated signaling pathways in the regulation of adipocyte energy metabolism have therefore been suggested as suitable targets for the development of pharmacological as well as nutritional interventions for the prevention of increased adiposity (10). Our finding of a negative association between 1,25-vit D concentrations and adiposity is not consistent with the notion that 1,25-vit D plays an important role in mediating increased fat mass in humans. PTH has also been shown to raise intracellular calcium levels in human adipocytes (11). It thus remains possible that the elevated iPTH we observed in obese subjects (and attributed to low 25-OH-vit D concentrations) may play a causal role in the pathogenesis of increased adiposity. However, given the relatively low amount of body weight variance for which iPTH accounts, it would seem that calcitropic hormones play a relatively limited role in the pathogenesis of obesity.

In conclusion, contrary to previously published data from small sample studies, 1,25-vit D levels are not usually elevated in obese adults; rather, there is a negative relationship between serum 1,25-vit D and adiposity. These data do not support the hypothesis that 1,25-vit D is the one of the key factors that would predispose obese adults to gain additional body fat.

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