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Influence of obesity on vitamin D–binding protein and 25-hydroxy vitamin D levels in African American and white women

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Abstract

25-Hydroxy vitamin D (25OHD) is lipophilic and highly bound to vitamin D–binding protein (VDBP) in plasma. In the present study, we examined VDBP and 25OHD levels by race and body mass index (BMI) in young adult women to determine whether circulating VDBP plays a role in the low levels of 25OHD with obesity and among African Americans. In agreement with previous studies, mean 25OHD levels were lower in African American women than in whites ($P < .01$). In a hierarchical multiple regression model, BMI was associated with 25OHD after adjustment for age in white women ($P = .02$, $R^2 = .10$) but not in African American women. The VDBP levels, by contrast, were similar in African Americans and whites, and were unrelated to BMI in either racial group. Furthermore, VDBP was unrelated to the plasma level of 25OHD. These data confirm an interaction between race and obesity in vitamin D metabolism, and imply that the carrier protein is not an important determinant of circulating 25OHD in women, nor is it affected by race or adiposity.

1. Introduction

Vitamin D is a unique nutrient because it is acquired primarily by exposure of the skin to sunlight, although dietary vitamin D in adequate doses is also biologically effective [1]. The nutritional status of vitamin D is defined by the serum level of its hepatic metabolite 25-hydroxy vitamin D (25OHD) whose production is not tightly regulated and reflects the amount of vitamin D in the circulation.

Serum levels of 25OHD are reduced in obese adults [2,3] and children [4]. Although the mechanism for low 25OHD in obesity is not well understood, the rapid clearance of radiolabeled cholecalciferol injected intravenously into adipose tissue [5] lead to the hypothesis that vitamin D is sequestered in fat, resulting in low plasma 25OHD levels in obesity. Wortsman et al [6] reported that the content of the vitamin D precursor 7-dehydrocholesterol and the conversion of 7-dehydrocholesterol to vitamin D by frozen skin explants exposed to simulated sunlight were similar in control and obese subjects. Moreover, they found that both the cutaneous and oral vitamin D pathways were influenced by obesity in that serum vitamin D2 levels increased less after orally administered vitamin D2 and circulating vitamin D3 levels rose less immediately after whole-body phototherapy in obese than in normal-weight adults [6]. One survey found that vitamin D intake was less in obesity especially among those who were obese and elderly [7], whereas no difference was found in sunlight exposure in relation to percentage of body fat in elderly men and women living in the Boston area [8]. Interestingly, the relationship between body fat and 25OHD may be stronger in whites than in African Americans [9]. Vitamin D is necessary to regulate the active transport of calcium in the intestine and thereby skeletal mineral content, and may also affect the skeleton directly. Recently, vitamin D deficiency has been linked to muscle strength and balance; metabolic syndrome; and the development of diabetes and cardiovascular disease, depression, multiple sclerosis, and several cancers [10].

Vitamin D–binding protein (VDBP; Gc-globulin) is the major transporter of vitamin D sterols in plasma. It is a 52- to 59-kd monomeric glycoprotein that is synthesized in hepatocytes and is structurally similar to albumin and

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Table 1
Characteristics of the women in the study population

	Total population	Whites	African Americans
No. of subjects	88	52	36
Age (y)	30.2 ± 7.4	30.4 ± 7.6	30.0 ± 7.1
BMI (kg/m^2)	29.6 ± 8.7	29.8 ± 7.9	29.3 ± 9.2
25OHD (ng/mL)	13.0 ± 11.8	15.4 ± 13.5	9.5 ± 7.8*
VDBP (mg/dL)	51.7 ± 15.0	52.9 ± 20.2	49.1 ± 12.8

* $P < .01$, African Americans vs whites (t test performed on log-transformed data).

α-fetoprotein. It has a relatively high affinity ($K_a = 5 \times 10^{-8}$ mol/L) for 25OHD and is present in substantial molar excess (5×10^{-6} mol/L) compared with 25OHD (5×10^{-8} mol/L), so that only 5% of the VDBP binding sites are estimated to be occupied by this ligand [11]. The VDBP levels are similar in men and women, but are increased in pregnancy [12] and after trauma [13], and are low in hypoproteinemia [12] and in undernourished patients [14]. No abnormality was found in VDBP levels in adults reported to be vitamin D deficient or those who were receiving vitamin D therapy [12]. However, VDBP-null mice [15] and mice deficient in the endocytic receptor megalin that resorbs VDBP in the proximal tubule cells of the kidney [16] have low levels of 25OHD.

Vitamin D–binding protein not only transports 25OHD, but acts as a scavenger for actin released into the plasma by lysed cells, enhances chemotaxis, regulates macrophage activity, and stimulates osteoclasts [17]. Because the effects of VDBP have important implications, we explored the role of VDBP in the low levels of 25OHD with obesity in women and looked for differences between whites and African Americans.

2. Methods

2.1. Subjects

Serum specimens were analyzed from women aged 18 to 44 years who were evaluated at the University of Louisville Hospital Emergency Department from January to March 2007. Blood samples were collected throughout the day and night, and with no specific relationship to meals. Because VDBP is produced by the placenta [18], the analysis was restricted to women with a negative serum human chorionic gonadotropin pregnancy test result. Samples from 52 white and 36 African American women were available for study. Samples from 8 women who listed their race as "other" were not analyzed.

2.2. Assays

Serum levels of 25OHD were determined using an assay kit from ALPCO Diagnostics (Salem, NH). This competitive protein binding assay is based on the ability of a plasma extract or standard to compete for binding to VDBP. The assay recognizes 25OHD2 and 25OHD3 equally. The minimal detectable dose was approximately 3 ng/mL.

Vitamin D–binding protein was measured using a proportional enzyme-linked immunosorbent assay from ALPCO Diagnostics. The standards range from 2.2 to 60 ng/mL, and samples were diluted 1:40 000 with sample

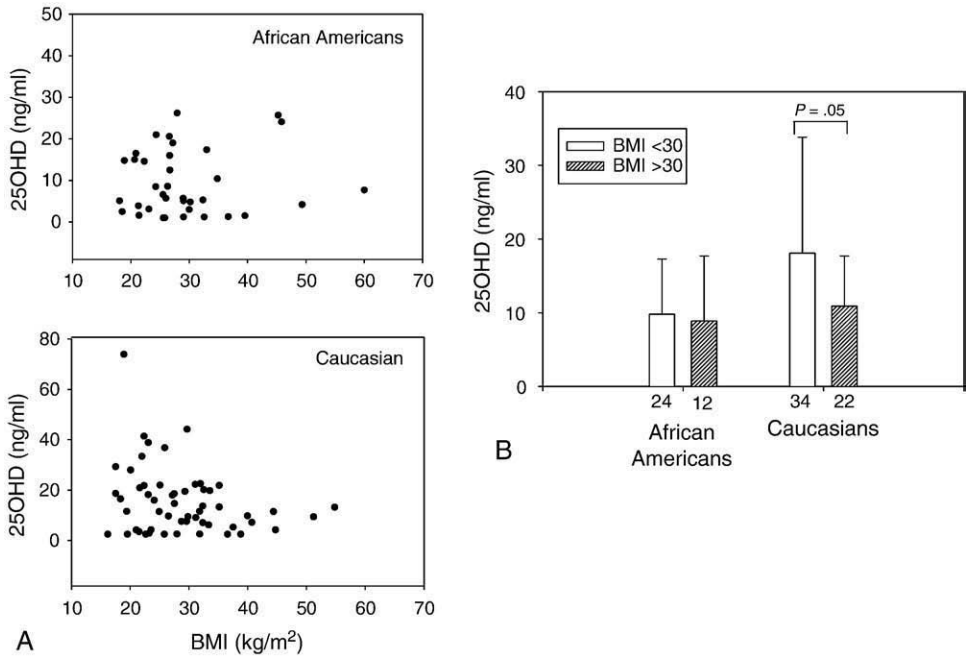

Fig. 1. A, Scattergrams relating circulating 25OHD levels in African American and white women to BMI. B, Bar graph comparing 25OHD levels (mean ± SD) in women who were obese (BMI >30 kg/m^2) and nonobese (BMI <30 kg/m^2).

buffer. The within-assay coefficient of variation of a representative sample diluted and assayed (n = 6) was 6.8%.

2.3. Data analysis

The levels of VDBP and 25OHD were natural log transformed to increase normality. To simplify the presentation and interpretation, however, we report descriptive statistics as mean ± SD of the nontransformed values. Student's *t* test was used to examine differences between whites and African Americans, and between normal weight and obesity. Linear regression was used to assess the relationship between VDBP and 25OHD. We also tested the association of body mass index (BMI) with 25OHD or VDBP by hierarchical multiple regression with adjustment for age within each race.

3. Results

The clinical characteristics of the study subjects are summarized in Table 1. Mean 25OHD levels were 13.0 ± 11.8 ng/mL (mean ± SD). Of the 88 women, 45 (51%) were severely deficient (<10 ng/mL), an additional 25 (28%) were deficient (10-20 ng/mL), 12 (14%) were relatively insufficient (20-30 ng/mL), and only 6 women (7%) had 25OHD levels that exceeded 30 ng/mL and would be considered to have adequate vitamin D [10]. The white and African American women studied did not differ with respect to age or BMI.

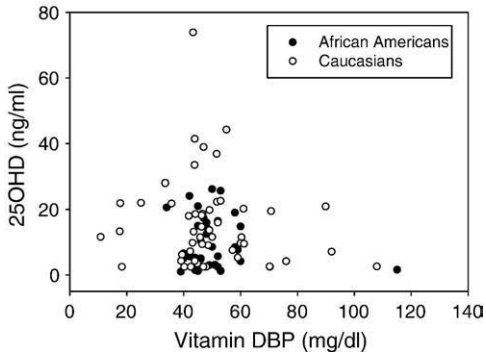

Fig. 3. Scattergram relating serum levels of VDBP to 25OHD in African American and white women.

In agreement with previous studies, mean 25OHD levels were lower in African American women than in whites (9.5 ± 7.8 vs 15.4 ± 13.5 ng/mL, $P < .01$). Among the African American women, 22 (61%) had levels less than 10 ng/mL, an additional 9 women (25%) had values of 10 to 20 ng/mL, 5 (14%) had values of 20 to 30 ng/mL, and none had adequate levels of vitamin D. The VDBP levels were similar in African American and white women.

Fig. 1A and B are scattergrams illustrating the relationship between BMI and 25OHD levels by race, and Fig. 1C summarizes the impact of obesity in women (BMI >30 kg/m^2) on 25OHD levels by race. Serum 25OHD levels were lower ($P = .05$) in obese than in normal-weight white women, whereas 25OHD levels in African American

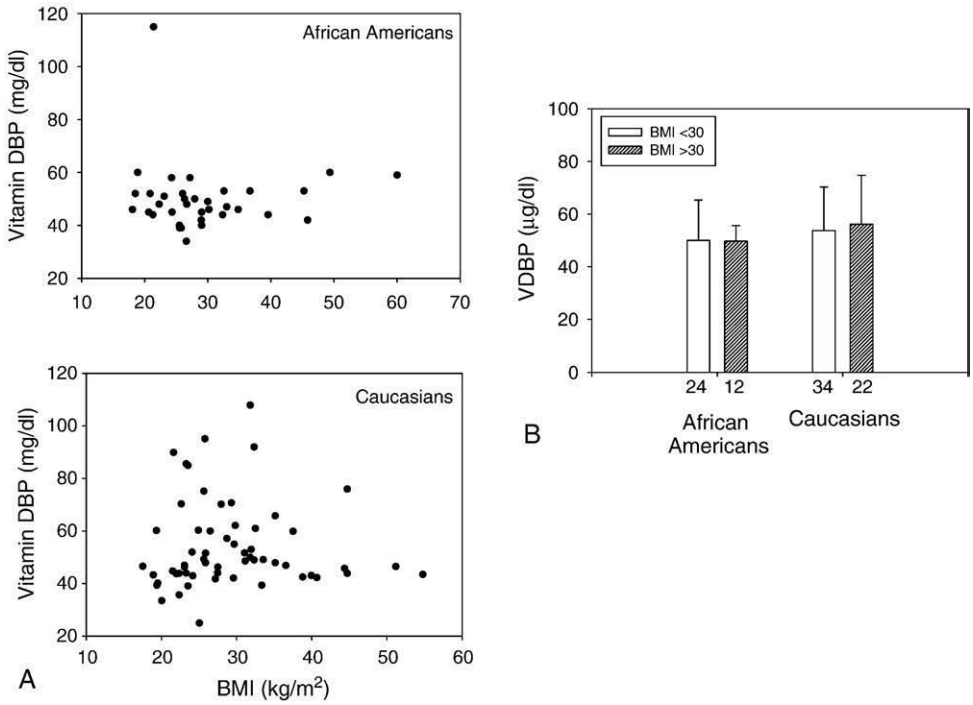

Fig. 2. A, Scattergrams relating circulating VDBP levels in African American and white women to BMI. B, Bar graph comparing VDBP levels (mean ± SD) in women who were obese (BMI >30 kg/m^2) and nonobese (BMI <30 kg/m^2).

women were unaffected by obesity. In a hierarchical multiple regression model, BMI was associated with 25OHD after adjustment for age in white women ($P = .02$, $R^2 = .10$) but not in African American women. As shown in Fig. 2, unlike 25OHD, VDBP levels were unrelated to BMI in either white or African American women.

Fig. 3 shows the relationship between serum levels of VDBP and 25OHD. It is interesting to note that the range of values for both 25OHD and VDBP was far greater in whites than in African Americans; however, no relationship between VDBP and 25OHD was found.

4. Discussion

Severe vitamin D deficiency was extremely common in young adult women visiting an inner city hospital emergency department in the winter. Using a definition of 10 ng/mL for severe vitamin D deficiency, 55% of the women studied were severely vitamin D deficient. Our findings confirm the lower mean levels and higher prevalence of vitamin D deficiency among African Americans, of whom 61% had levels that were less than 10 ng/mL. Lower levels of vitamin D in blacks are thought to reflect increased skin pigmentation and reduced vitamin D production as well as less vitamin D intake with less consumption of milk and milk products.

This study, like the data from the National Health and Nutrition Examination Survey III [9], reveals that obesity impacts vitamin D levels to a greater extent in white than in African American women, although other studies have found lower 25OHD levels with obesity in both racial groups [19]. The reason for lower levels of 25OHD in obese white but not African American women is not certain. One idea is that low mean 25OHD levels mask the additional effect of obesity in African Americans [20]. However, prepubertal black girls had a more pronounced seasonal variation in plasma 25OHD than whites despite lower mean levels [21]; and young adult African American women had lower levels than whites across all seasons, with a smaller difference between winter and summer values in the former [22]. A second hypothesis to explain the interaction between obesity and race relates to the racial difference in adipose distribution, with African Americans having more subcutaneous adipose tissue and less visceral adipose tissue than whites in most studies [23-25]. In older adults, truncal fat mass was a slightly stronger predictor of 25OHD than was total fat mass in 1 study [26]; but in another study, 25OHD was related to leg fat but not truncal fat [27]. Tumor necrosis factor α is expressed in adipocytes and is increased in obesity [28], and was found to increase the conversion of 7-dehydrocholesterol to calcitriol by cultured human keratinocytes [29]. Thus, a third hypothesis is that cytokine regulation of vitamin D metabolism influences the impact of obesity and race on 25OHD.

Unlike the results for 25OHD, VDBP levels were unaffected by race or BMI in either African American or white women. Several studies have examined potential regulators of circulating VDBP concentrations. Of direct relevance to our study are seemingly conflicting reports that VDBP concentrations were positively related to BMI and fat mass in elderly Belgian men [30] but were unrelated to BMI or total or truncal fat mass by dual-energy x-ray absorptiometry in middle-aged and elderly men and women in New Zealand [26]. No previous studies were identified that examined results in nonwhite racial groups. Male Sprague-Dawley rats fed a high-fat diet had elevated levels of VDBP [31]. There is 1 study describing a diurnal variation in VDBP, with stable levels throughout the day and evening but 15% lower levels in the early morning hours [32]. Levels of VDBP appear to be similar in children and adults but rise during pregnancy [33], are increased by oral contraceptives [34] and perhaps in acromegaly [34], and are low with poor nutritional status [12,14,35]. Finally, there are 3 common VDBP alleles (*Gc1s*, *Gc1f*, and *Gc2*) that differ in amino acid sequence and glycosylation (galactose and sialic acid in both *Gc1s* and *Gc1f*; galactose only in *Gc2*) that influence the level of VDBP in plasma [36].

Although nearly 90% of the 25OHD in the circulation is bound to VDBP, the level of VDBP in plasma does not appear to be an important determinant of the level of circulating 25OHD as indicated by the scattergram relating VDBP to 25OHD. This finding agrees with earlier studies showing similar VDBP values in controls, vitamin D–deficient adults, and adults treated with vitamin D [12]; in subjects with various illnesses that influence vitamin D metabolism [34]; and, in 1 recent cross-sectional study in elderly men and women [26]. Presumably, most of VDBP binding sites are unliganded because of high levels in relation to the circulating levels of 25OHD, allowing for VDBP and 25OHD levels to vary independently.

Megalin is an endocytic receptor that resorbs the VDBP-25OHD complex in the proximal tubule cells of the kidney, and megalin-deficient mice have low levels of 25OHD [16]. Normal serum levels of VDBP in obese women suggest that loss of vitamin D in urine is not the explanation for low 25OHD in obesity and, by implication, megalin expression and function in the kidney are not altered in obesity. Because of the many health risks shared by obesity and vitamin D deficiency, this issue deserves further study.

This study has several limitations. Dietary vitamin D intake and supplementation were not assessed. We also did not measure exposure to sunlight or time spent outdoors. We calculated BMI but did not examine percentage of body fat or adipose tissue distribution. Race was self-reported. A variety of clinical problems brought the women to the emergency department, which could have influenced the results. The time of the day of the blood sampling and the relationship to meals varied. The performance of 25OHD assays has been a subject of debate [37], and the competitive protein binding assay used in this research may have over- or underestimated 25OHD levels in a systematic fashion.

Finally, the analyses would be more robust if more subjects had been studied.

Vitamin D deficiency has been linked to muscle strength and balance; the metabolic syndrome; and the development of diabetes and cardiovascular disease, depression, multiple sclerosis, and several cancers [10]; and it is tempting to propose that that the link between obesity and these morbidities partly involves vitamin D. If so, it is clear from our results that young women of reproductive age need to be educated on the importance of vitamin D supplementation.

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