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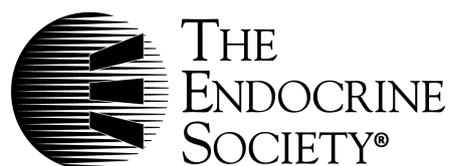
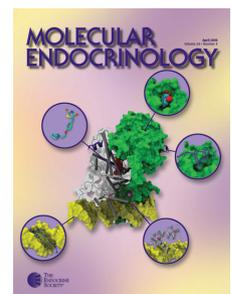
THE JOURNAL
OF CLINICAL
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& METABOLISM

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J. Clin. Endocrinol. Metab. 2005 90:635-640 originally published online Nov 16, 2004; , doi: 10.1210/jc.2004-1765

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Body Fat and Vitamin D Status in Black Versus White Women

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Obesity has been linked to lower serum 25-hydroxyvitamin D [25(OH)D] values, but whether this relationship plays a role in the poorer vitamin D status observed in blacks vs. whites is not clear. This study examines the relationship between serum 25(OH)D and percent body fat (%BF) by race in 6042 women (3567 non-Hispanic whites and 2475 non-Hispanic blacks), aged 12+ yr, from the third National Health and Nutrition Examination Survey (NHANES III, 1988–1994). Serum 25(OH)D values were measured with an RIA kit (DiaSorin), and %BF was calculated from bioelectrical impedance analysis. Adjusting for %BF only slightly reduced differences in mean serum 25(OH)D by race. The negative relationship be-

tween serum 25(OH)D and %BF was noticeably stronger in whites than in blacks of the same age. Within race, the relationship was stronger in younger than older individuals. Adjusting for confounders reduced, but did not remove, these differences in relationship strength. In conclusion, the serum 25(OH)D-%BF relationship in women varies both by race (stronger in whites than blacks) and age (stronger in younger than older persons). This complex relationship may explain why differences in obesity do not appear to play a major role in explaining variation in serum 25(OH)D by race. (J Clin Endocrinol Metab 90: 635–640, 2005)

SEVERAL STUDIES HAVE linked obesity with poorer vitamin D status, as reflected by lower serum 25-hydroxyvitamin D [25(OH)D] values (1–9). Both obesity and low serum 25(OH)D values are more common in African-American women than in Caucasian women (10–12), but few studies to date have examined the connection between these two variables by race. Results of these studies have conflicted. For example, Epstein *et al.* (13) and Nesby-O'Dell *et al.* (12) reported no relationship between serum 25(OH)D levels and obesity or body mass index (BMI) in blacks, whereas Parikh *et al.* (9) found a significant negative correlation between BMI and serum 25(OH)D in African-Americans.

The present study uses data from 6402 adolescent and adult females, aged 12 yr and older, from the third National Health and Nutrition Examination Survey (NHANES III, 1988–1994) to examine the extent to which body fat differences between the races contribute to serum 25(OH)D differences by race. In addition, it assesses whether the relationship between body fat and serum 25(OH)D varies by race in women, and if so, whether this difference is constant across age. Addressing these issues is pertinent in light of the heightened interest in the poorer vitamin D status of African-Americans coupled with their significantly higher prevalence of obesity (10–12).

Subjects and Methods

Subjects

NHANES III was conducted in 1988–1994 by the National Center for Health Statistics, Centers for Disease Control and Prevention

First Published Online November 16, 2004

Abbreviations: %BF, Percent body fat; BIA, bioelectrical impedance analysis; BMI, body mass index; FFM, fat-free mass; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)₂D, 1,25-dihydroxyvitamin D; QC, quality control; Res, resistance; TBF, total body fat.

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(Hyattsville, MD), to assess the health and nutrition status of a large representative sample of the noninstitutionalized, civilian United States population. Data were collected via household interviews, and direct standardized physical examinations were conducted in specially equipped mobile examination centers (14). NHANES III was designed to provide reliable estimates for three race/ethnic groups: non-Hispanic whites, non-Hispanic blacks, and Mexican-Americans. All procedures were approved by the National Center for Health Statistics institutional review board, and written informed consent was obtained from all subjects.

Serum 25(OH)D measurements were obtained from 18,875 individuals, aged 12 yr and older, in NHANES III. In the present study the sample was restricted to 3,567 non-Hispanic white and 2,475 non-Hispanic black females, aged 12+ yr, with nonmissing data for serum 25(OH)D and percent body fat (%BF). This sample represents 61% of non-Hispanic white or black females in this age range who were originally selected for the survey, 73% of those who were interviewed, and 83% of those who received physical examinations in the survey.

Variables

Serum 25(OH)D levels were assayed with an RIA kit (DiaSorin, Stillwater, MN). Assays were performed at the primary environmental and nutritional health laboratory for NHANES at the National Center for Environmental Health, Centers for Disease Control and Prevention (Atlanta, GA). The assay was designed to detect serum 25(OH)D values from 0–100 ng/ml (0–250 nmol/liter). Values below 5 ng/ml (12.5 nmol/liter) or above 70 ng/ml (175 nmol/liter) were verified by reassay. Sera from five measurements of bench quality control (QC) materials (*e.g.* identifiable to the analyst as a QC) and two levels of blind QC materials (*e.g.* not identifiable to the analyst) were tested on a regular basis throughout the survey period. The bench control materials were designed to cover a wide range of serum 25(OH)D values: 5 ng/ml (12.5 nmol/liter), 12 ng/ml (30 nmol/liter), 20 ng/ml (50 nmol/liter), 40 ng/ml (100 nmol/liter), and 100 ng/ml (250 nmol/liter). Two levels of blind QC pools were used to address low (~15 ng/ml or 37.5 nmol/liter) or elevated (~50 ng/ml or 125 nmol/liter) levels. A total of 13 blind QC pools aimed at low values and 11 aimed at high values were used during the survey. Assay coefficients of variation for these blind QC pools were 10–25% at the lower values of serum 25(OH)D (8–25 ng/ml or 20–62.5 nmol/liter) and 12–18% for high values (34–59 ng/ml or 85–147.5 nmol/liter). Long-term performance of this laboratory in the United Kingdom DEQAS international vitamin D proficiency testing program has been

excellent. Details of the assay method for this measurement have been previously reported (15).

The %BF was calculated using bioelectrical impedance analysis (BIA) data. A single, tetrapolar BIA measurement of resistance (Res) and reactance at 50 kHz was taken between the right wrist and ankle while the respondent was supine using a 1990B Bio-Resistance Body Composition Analyzer (Valhalla Scientific, San Diego, CA). Total body water and fat-free mass (FFM) were calculated from Res measurements using prediction equations that have been validated and published for NHANES III previously (16). Because these equations were based on Res data from RJL bioelectrical impedance analyzers (RJL, Clinton Township, MI), the NHANES III Res data had to be converted to RJL values before applying the equations, as described previously (16). Estimates of total body fat (TBF) and %BF were calculated as follows: TBF = FFM – body weight, and %BF = TBF/body weight. Body weight was measured to the nearest 0.01 kg using an electronic load cell scale (14).

Race and ethnicity were self-reported by the participants. Several additional variables were included in the study because previous studies suggested that they were related to serum 25(OH)D (11, 12), and preliminary analyses indicated they were also related to body fat and race and thus might confound the results of the present analysis. These included the following variables. 1) The month of blood collection was based on the date of the respondent's physical examination. 2) Physical activity was based on self-reported times per month that the respondent performed exercise that made him/her sweat for subjects aged 12–16 yr; for subjects aged 17 yr and older, it was based on self-reported times per month of performing various activities (walking a mile or more without stopping, jogging or running, bicycling, swimming, aerobics or aerobic dancing, other dancing, calisthenics or exercise, gardening or yard work, weight lifting, or other exercises, sports, or physically active hobbies). All activities for adults were included in the main analyses to be more comparable with the physical activity item for adolescents, which could not be separated into outdoor *vs.* indoor activities. Secondary analyses were performed for adults using only those activities likely to be performed outdoors (walking, jogging, or running; bicycling; swimming; and gardening and yard work) to compare with results based on all activities. 3) Dietary vitamin D intake from food was based on a single 24-h recall. The University of Minnesota Nutrition Coordinating Center food composition database was used for the vitamin D composition data (17, 18). 4) The frequency of milk consumption was based on self-reported times per month that the respondents drank milk or added it to cereal; cereal consumption was based on times per month that cold cereal was consumed. 5) Smoking was based on responses to questionnaire items asking whether the respondent had ever smoked at least 100 cigarettes and, if so, whether he/she currently smoked. Responses to these items were combined to identify current smoker or former smokers *vs.* never smokers. 6) Vitamin-mineral supplement use was based on a questionnaire item that asked whether the respondents had taken any vitamin or mineral supplements in the past month. 7) Current oral contraceptive or hormone therapy use were based on questionnaire items that asked about the use of birth control pills or estrogen (pills, cream, suppository, injection, or patch).

Data analyses

Sample weights were used when calculating point estimates. The use of these weights provides estimates representative of the civilian, non-institutionalized U.S. population at the time of NHANES III; the weights also account for oversampling and nonresponse in the survey. The analyses were performed using SUDAAN (19), a family of statistical procedures for analysis of data from complex sample surveys. Potential confounding variables were identified using regression or χ^2 analyses. Because responses to the physical activity variable were skewed, these data were analyzed after performing a log transformation. Age-adjusted means of the various confounding variables were calculated and compared between the two races using regression.

Regression analysis was also used to assess the effect of adjusting for body fat differences on racial differences in serum 25(OH)D values by calculating mean serum 25(OH)D values by race before and after adjusting for %BF. Age-adjusted mean serum 25(OH)D levels were also compared by quartile of %BF in white *vs.* black women. Finally, regression analyses were used to examine the relationship between serum

25(OH)D and body fat separately by race and age before and after adjusting for the confounding variables.

Several secondary analyses were also performed to address potential limitations in the study. For example, because BIA only provides an indirect measure of %BF, the analyses described above were repeated using BMI. Analyses were also repeated after restricting the sample to those examined between November and March (when UV radiation levels are low) as an additional way to address the unequal distribution of month of blood collection between blacks and whites (11). Analyses in which confounders were controlled were repeated for adults after restricting the physical activity variable to only those activities that were more likely to be performed outdoors, as a means of better assessing possible differences in sun exposure. To explore the serum 25(OH)D-%BF relationship by age in more depth, analyses were performed in whites after dividing the 50+ yr group into 50–64 and 65+ yr groups. Finally, physical activity levels were compared in obese individuals by race and age to assess whether potential sun avoidance by obese individuals varied by these factors.

A secondary analysis was also conducted to assess the extent to which differences in the serum 25(OH)D-body fat relationship by race were affected by the smaller variation in serum 25(OH)D distribution observed in black women. In this analysis, the relationship was examined in white women after restricting the sample to those with serum 25(OH)D values that fell between 6.7 and 42.5 ng/ml (16.8–106.3 nmol/liter); these values correspond to the 1st and 99th percentiles of the serum 25(OH)D distribution of the black women.

Results

Sample sizes by age and age-adjusted means or prevalences of selected confounding variables by race are shown in Table 1 for the study sample. Non-Hispanic black women were younger and had more body fat than white women. They had lower intakes of vitamin D from food, as assessed

TABLE 1. Selected characteristics of the sample by race/ethnicity

Variable	Non-Hispanic white women	Non-Hispanic black women
	n	
Sample size by age (yr)		
12–29	788	925
30–49	970	909
50–69	886	458
70+	923	183
	Mean	
Age (yr)	43.9 ^a	38.9 ^a
% BF ^b	33.9 ^a	37.4 ^a
Physical activity (times/month) ^{b,c}	15.2 ^a	13.1 ^a
Dietary vitamin D ($\mu\text{g}/\text{d}$) ^b	4.42 ^a	3.31 ^a
Milk consumption (times/month) ^b	25.0 ^a	15.4 ^a
Cereal consumption (times/month) ^b	10.4 ^a	8.7 ^a
	%	
Smoking ^b		
Current/former	48.4 ^a	36.9 ^a
Never	51.6	63.1
Vitamin-mineral supplement use ^b		
Yes	47.7 ^a	35.1 ^a
No	52.3	64.9
Current oral contraceptive or hormone therapy use ^b		
Yes	21.3 ^a	14.7 ^a
No	78.7	85.3
Month of blood collection ^b		
November–March	28.1 ^a	43.5 ^a
April–October	71.9	56.5

^a $P < 0.05$ for non-Hispanic white *vs.* non-Hispanic black.

^b Adjusted for age.

^c Geometric mean.

by a single 24-h recall, as well as lower usual intakes of two food sources of vitamin D, milk and cereal. They were more likely to be nonsmokers than white women, but less likely to perform physical activity or use vitamin-mineral supplements or female hormones. Finally, they were more likely to have had their blood collected during November–March; this occurred because NHANES collects data in the south during the winter (because the mobile exam centers are not designed for cold weather operation), and the majority of African Americans live in the south [~62% in 1990 according to data from the U.S. Census Bureau (20)].

Table 2 compares mean serum 25(OH)D values by race before and after adjusting for %BF. Mean serum 25(OH)D values were 1.4–1.9 times higher in white women than in black women before adjusting for %BF. Adjusting for %BF reduced, but did not remove, the white/black difference; serum 25(OH)D values remained 1.3–1.9 times higher in whites. The lack of impact of %BF on racial differences in serum 25(OH)D is also evident by the consistent difference in age-adjusted mean serum 25(OH)D levels in white *vs.* black women in the different %BF quartiles (Fig. 1). The ratio of white/black means was 1.7 in the first three quartiles and 1.5 in the fourth, or highest, quartile of %BF.

To explore the basis for the lack of impact of body fat on racial differences in serum 25(OH)D, a regression of serum 25(OH)D on %BF was performed separately by race after adjusting for age or for all confounders. Results are shown in Table 3. %BF was significantly related to serum 25(OH)D in white women regardless of age, but among black women, the relationship was significant only among women less than 50 yr of age. Furthermore, the β coefficients for the relationship in white women were approximately 3–7 times larger than those in black women, indicating that the strength of the relationship is greater in whites than in blacks.

The strength of the relationship also varied by age within race, as suggested by the smaller β coefficients for women ages 50+ yr relative to those for the younger women in both races (Table 3). The lack of statistically reliable estimates for older black women precluded firm conclusions about the age relationship among blacks, but among white women, β coefficients were approximately 2–3 times higher in those ages 12–49 yr than in those over age 50 yr either before or after adjusting for other confounding variables. Additional explo-

TABLE 2. Mean serum 25(OH)D by race/ethnicity in women before and after adjusting for %BF

Age and adjustment factors	Non-Hispanic white	Non-Hispanic black	White/black ratio
12–29 yr			
Unadjusted	35.43	18.25	1.9
Adjusted for %BF	35.26	18.87	1.9
30–49 yr			
Unadjusted	30.99	17.12	1.8
Adjusted for %BF	30.72	18.88	1.6
50–69 yr			
Unadjusted	27.30	19.99	1.4
Adjusted for %BF	27.30	20.40	1.3
70+ yr			
Unadjusted	26.38	19.28	1.4
Adjusted for %BF	26.36	19.50	1.4

Values are expressed as nanograms per milliliter. To convert serum 25(OH)D to nanomoles per liter, multiply by 2.5.

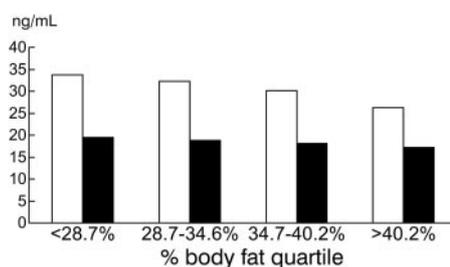


FIG. 1. Age-adjusted mean serum 25(OH)D (nanograms per milliliter) by %BF quartile in non-Hispanic white or black females, aged 12+ yr. To convert serum 25(OH)D values to nanomoles per liter, multiply by 2.5. □, Non-Hispanic white; ■, non-Hispanic black.

ration of the relationship in older white women revealed a tendency for the relationship to continue to weaken with age, as suggested by the larger β coefficient for women ages 50–64 yr ($\beta = -0.18$; $P = 0.09$) than for women ages 65+ yr ($\beta = -0.07$; $P = 0.64$).

Results of the secondary analyses of body fat and serum 25(OH)D performed to address various potential study limitations (*e.g.* use BMI instead of %BF, restrict sample to winter only, or restrict physical activity to likely outdoor activities) were similar to those obtained in the main analyses (data not shown). In addition, the frequency of physical activity did not differ in obese individuals by race or age (data not shown); this analysis was performed to assess whether sun avoidance in obese subjects might vary by these factors. Finally, results of the analysis performed to assess whether the smaller variation in serum 25(OH)D values among blacks accounted for its weaker relationship with body fat in this group suggested that this was not the case. Specifically, the β coefficient for %BF remained noticeably higher for white women ($\beta = -0.22$) than for black women ($\beta = -0.10$) even after restricting the sample to white women with serum 25(OH)D values that fell between the 1st and 99th percentiles of the serum 25(OH)D distribution of the black women.

Discussion

Recent concerns have been raised about the high prevalence of low serum 25(OH)D values observed in African-Americans (12). The lower values observed in African-Americans have been attributed primarily to reduced synthesis of vitamin D in the skin due to greater skin pigmentation (21). Although differences in skin synthesis due to melanin undoubtedly play a major role in racial differences in serum 25(OH)D, other factors may also contribute. Obesity is of particular interest, given its link to lower serum 25(OH)D values and the fact that the prevalence of obesity is significantly higher in African-American women than in white women (10). However, to date, few studies have examined whether obesity is linked to the lower serum 25(OH)D levels in African-Americans, and these have produced conflicting results (9, 12, 13).

The present study found that adjusting for body fat differences did not noticeably reduce the differences in serum 25(OH)D levels between white and black women in any of the age groups examined. Subsequent analyses of the relationship between serum 25(OH)D and %BF within race/ethnic groups highlighted a likely explanation for this lack of

TABLE 3. Relationship between serum 25(OH)D and %BF by race/ethnicity and age

Age and race	Regression of serum 25(OH)D on %BF					
	Adjusted for age			Adjusted for all confounders ^a		
	β coefficient	<i>P</i>	White/black ratio	β coefficient	<i>P</i>	White/black ratio
12–29 yr						
Non-Hispanic white	–0.3838	0.0000		–0.3192	0.0000	
Non-Hispanic black	–0.1277	0.0001	3.0	–0.1117 ^b	0.0417	2.9 ^b
30–49 yr						
Non-Hispanic white	–0.4754	0.0000		–0.3721	0.0000	
Non-Hispanic black	–0.1302	0.0000	3.7	–0.1326	0.0007	2.8
50+ yr						
Non-Hispanic white	–0.1642	0.0002		–0.1854	0.0001	
Non-Hispanic black	0.0286 ^b	0.5997	5.7 ^b	0.0273 ^b	0.7721	6.8 ^b

^a Age, physical activity, month of blood collection, smoking, oral contraceptive or hormone use, micrograms of dietary vitamin D per day, frequency of milk or cereal consumption, and vitamin-mineral supplement use.

^b May be unreliable (SE/ β coefficient > 0.30).

effect, namely, that serum 25(OH)D is only weakly related to %BF in blacks.

More importantly, the present study suggests the relationship between serum 25(OH)D and body fat in women is complex overall, *e.g.* it depends on both race and age. In specific, the relationship is stronger in whites than in blacks at all ages. In addition, there is divergence by age within race; we found a significant, although weak, relationship among younger black women, but no relationship among older black women. The divergence in strength by age was also observed in white women, although the relationship remained statistically significant in older white women, aged 50+ yr.

The reason for the weaker relationship between body fat and serum 25(OH)D in blacks is not clear. Wortsman *et al.* (7) recently proposed that the lower serum 25(OH)D levels seen among obese whites was the result of increased sequestration of vitamin D in fat tissue. Whether this applies to blacks is uncertain. On the one hand, differences in fat metabolism observed by race [enhanced lipogenesis and reduced lipolysis in blacks (22–24)] seem more consistent with blacks having a greater ability to sequester vitamin D than whites, which should lead to a stronger body fat-vitamin D relationship in blacks. On the other hand, blacks have reduced synthesis of vitamin D in the skin due to the presence of more melanin, which absorbs the UV radiation needed for the skin synthesis to occur (21). Skin synthesis of vitamin D has been estimated to account for the majority of vitamin D in the human body (25). Thus, it is possible that body fat has a lower impact in blacks because there is less vitamin D formed in the skin to sequester.

Other mechanisms proposed to explain the link between obesity and serum 25(OH)D in whites include greater avoidance of the sun by obese persons (3), and negative feedback control on hepatic synthesis of serum 25(OH)D due to higher levels of serum 1,25-dihydroxyvitamin D [1,25(OH)₂D] in the obese (1). Sun avoidance could play a role in the differences in the body fat-serum 25(OH)D relationship by race if, for example, obese blacks were more likely to avoid the sun than obese whites. However, in the present study, self-reported physical activity levels did not differ significantly between obese whites and blacks, which is not consistent with that possibility. Serum 1,25(OH)₂D was not measured in NHANES III,

so its potential role in differences in the body fat-serum 25(OH)D relationship by race could not be evaluated in the present study. However, there are data to suggest racial differences occur in the relationship between serum 1,25(OH)₂D and obesity. Epstein *et al.* (13) reported that serum 1,25(OH)₂D values did not differ by obesity status in blacks, which suggests that feedback from serum 1,25(OH)₂D on hepatic synthesis of serum 25(OH)D might be affected less by obesity status in blacks than in whites. However, their study sample was small (12 nonobese and 10 obese blacks) and may have lacked the statistical power needed to detect a difference.

In the present study, the serum 25(OH)D-body fat relationship was also weaker in older individuals relative to younger subjects of the same race. The mechanisms outlined above for the racial difference in this relationship may also apply to the age-related difference. For example, older individuals have a reduced ability to synthesize vitamin D in the skin (26), which could result in less vitamin D available to sequester. Another possibility, *e.g.* differences in sun avoidance in older *vs.* younger obese persons, does not appear to explain the findings in the present study, because self-reported physical activity levels did not differ significantly between obese younger *vs.* older persons when analyzed in broad age groups among whites. Whether differences in serum 1,25(OH)₂D levels by age play a role is harder to assess, given the complex age patterns displayed by this variable; levels appear to increase up until age 65 yr, then decline (27). The present study could not directly examine the role of 1,25(OH)₂D, but the serum 25(OH)D-%BF relationship was noticeably weaker in white women aged 65+ yr [in whom serum 1,25(OH)₂D values are presumably lower] than in white women aged 50–64 yr, which is not consistent with higher serum 1,25(OH)₂D levels explaining age differences in this relationship. More work is clearly needed to better understand the mechanisms underlying the differences in the relationship between body fat and serum 25(OH)D by age as well as by race.

The present study has several limitations, including the use of %BF estimates based on BIA. BIA does not directly measure body fat; instead, it measures total body water, from which FFM and fat mass are subsequently derived using prediction equations. An NIH panel concluded that BIA was useful for body composition analysis in most individuals

who do not have conditions with major disturbances in body water (28), but they also noted that the validity of the FFM and fat mass estimates depends on the validity of the prediction equations. An extensive study was undertaken to develop and evaluate prediction equations for the BIA data collected in NHANES III; this multicenter study used a multicompartiment model to assess body composition in more than 1800 individuals (16). The evaluation indicated that the equations have excellent precision overall, but they did not perform as well in blacks as in whites (16). In specific, total body water and FFM were underpredicted in blacks; this systematic difference could affect comparisons between races (29). Two approaches were used to address the potential impact of this difference: 1) the relationship between %BF and serum 25(OH)D values was examined separately within each racial group as part of the main analyses in the present study, because, according to Chumlea *et al.* (29), the NHANES III body composition estimates from BIA are probably adequate for comparisons within racial groups; and 2) the relationship between body fat and serum 25(OH)D was assessed in secondary analyses using BMI rather than %BF. Results from both sets of analyses supported the finding of a reduced relationship between body fat and serum 25(OH)D in blacks.

Other limitations include differences in the season during which blood was collected by race, with more blacks having blood collected in the winter. However, we controlled for month of blood collection in all main analyses, and the results of the secondary analysis in which the sample was restricted to those with blood collected in winter were similar to the main analyses as well. NHANES III also lacks data on sun exposure and serum 1,25(OH)₂D. Physical activity level was used as a proxy for sun exposure in the present study, but it is only an approximation in light of the inability to define outdoor activities with certainty and the fact that sun exposure during nonleisure physical activity is not assessed. There is also potential for nonresponse bias in the sample, because not all of those who were selected to participate in the survey did so. Nonresponse bias in the sample that came to the mobile exam centers in NHANES III is reduced to some extent by a nonresponse adjustment factor included in the calculation of the sample weights (30). After applying these weighting adjustments, differences between examinees and nonexaminees were minor (30). However, about 17% of the eligible women who came to the mobile exam centers did not have usable serum 25(OH)D data, and this nonresponse is not addressed by the sample weight adjustments.

In conclusion, the relationship between body fat and serum 25(OH)D in women is complex; it is weaker in blacks than in whites, and within race, it is weaker in older than in younger subjects. Thus, it appears to depend on both race and age. The weaker relationship in African-Americans probably explains why the higher body fat levels in black women did not appear to play a major role in explaining their lower serum 25(OH)D values relative to those in white women. More work is needed to identify the mechanisms that underlie these variations in the relationship between body fat and vitamin D status.

Acknowledgments

Received September 3, 2004. Accepted November 4, 2004.

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A portion of this work was presented at the 26th Annual American Society for Bone and Mineral Research Meeting, Seattle, WA, October, 2004.

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