Vitamin D Status of Seminomadic Fulani Men and Women

Robert H. Glew, PhD; Michael J. Crossey MD; Jup Polanams, PhD; Henry I. Okolie, MD; Dorothy J. VanderJagt, PhD

The purpose of this study was to assess the vitamin D status of Fulani men and women in northern Nigeria. The Fulani are seminomadic pastoralists whose culture, economy, and diet are centered on cattle. Most of the foods consumed by the Fulani are not good sources of vitamin D. Also being Muslim, the women do not derive much benefit from the vitamin D–generating effects of sunlight due to their dress habits. Furthermore, childhood rickets is common in the region. Serum was collected from 22 Fulani men (age, 47.6 ± 8.3 years; body mass index [BMI], 21.1 ± 3.2 kg/m^2) and 29 women (age, 55.5 ± 13.5 years; BMI, 21.6 ± 3.1 kg/m^2) in rural northern Nigeria and analyzed for 25-hydroxyvitamin D_2 and D_3 using ultraperformance liquid chromatography coupled with mass spectrometry. Eighty-three percent of the women and 45% of the men had serum 25-hydroxyvitamin D levels in the hypovitaminosis D range (10-30 ng/mL). In the males, there was a strong negative correlation between serum vitamin D and BMI (r = –0.49, p = .022) and percent body fat (r = –0.51, p = .015). No such correlations were observed in the Fulani women. Our main conclusion is that about half the men and most of the women in the Fulani community where this study was conducted are inadequately nourished with respect to vitamin D. A high prevalence of hypovitaminosis D indicates an elevated risk for rickets in children and bone fractures in adults.

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Author Affiliations: Department of Biochemistry and Molecular Biology, School of Medicine, University of New Mexico, Albuquerque, New Mexico (Drs Glew and VanderJagt); TriCore Reference Laboratories, Albuquerque, New Mexico (Drs Crossey and Polanams); Department of Internal Medicine, Federal Medical Centre, Gombe, Gombe, Nigeria (Dr Okolie).
Correspondence: Dorothy J. VanderJagt, PhD, Department of Biochemistry and Molecular Biology, School of Medicine, MSC08-4670, 1 University of New Mexico, Albuquerque, NM 87131-0001 (dvanderjagt@salud.unm.edu).

INTRODUCTION

Vitamin D in its active form, 1,25-dihydroxyvitamin D, is required for calcium and phosphorus homeostasis and the maintenance of a healthy skeleton. In addition, a poor vitamin D status is associated with increased risk for cancer of the colon, breast, and prostate;[1-5] cardiovascular disease;[6] and diabetes.[7] For women of childbearing age, maternal vitamin D status is critical to the nutrition of the breastfed infant because the vitamin D content of breastmilk is correlated strongly with the mother's plasma vitamin D concentration.[8-11] For postmenopausal women and elderly males, vitamin D is essential for bone maintenance and decreased risk for osteoporosis, particularly in populations with a low intake of dietary calcium.[12,13]

Vitamin D is acquired from 2 sources: synthesis from 7-dehydrocholesterol in the skin and from the diet. 7-Dehydrocholesterol, an intermediate in the pathway of cholesterol synthesis, is converted to provitamin D_3 in the skin by UV-b irradiation (290-315 nm). Provitamin D_3, in turn, is converted thermally to vitamin D_3 (cholecalciferol). Except for fatty fish and certain fish oils, liver, and the yolk of eggs, the amount of vitamin D in most natural food sources is low; the majority of dietary vitamin D is obtained from vitamin D–supplemented foods. Foods can be supplemented with either vitamin D_3 or vitamin D_2 (ergocalciferol) that is produced by irradiating plant-derived ergosterol.

The Fulani of northern Nigeria are seminomadic pastoralists whose culture, economy, and diet are centered on cattle. Several factors place these Fulani at risk of hypovitaminosis D. First, their diet is rich in unfortified dairy products, in particular milk, cheese, butter oil, and yogurt,[14] which contain little vitamin D. Second, they do not take dietary supplements because of both economic considerations and lack of availability. Third, their heavily pigmented skin greatly reduces the rate of conversion of 7-dehydrocholesterol into vitamin D_3. Fourth, since essentially all of the pastoral Fulani of northern Nigeria are Muslims, cultural and religious customs dictate that Fulani women cover everything except the face and hands when they are outside the household.[15,16]

The literature contains several reports of the vitamin D status of pregnant and nonpregnant women in northern Nigeria. In their study of pregnant Nigerian women at term, Okonofua and coworkers[17] found that the plasma 25-hydroxyvitamin D concentrations were, on average, about 2-fold higher in the nonpurdah (not wearing veil) subjects; however, in general, the concentrations of 25-hydroxyvitamin D in all of the Nigerian women were greater than those of Caucasian women in the United Kingdom. In 1997, in a study of the vitamin D status of pregnant teenagers in northeastern Nigeria,[18] we found that the mean vitamin D concentration at all 3 trimesters was below the normal range of values for pregnant women in North America and Europe. In their 2006 comprehensive review of the world literature of the prevalence and causes of rickets, Thacher and colleagues[19] concluded that nutritional rickets exists along a spectrum that has isolated vitamin D deficiency at one end and to isolated calcium deficiency at the other end, and postulated that deficiencies of vitamin D and calcium interact with unknown genetic and environmental factors that lead to the development of rickets.

For the reasons described above, we therefore hypothesized that the content of vitamin D in the serum of Fulani adults in Nigeria is low. To this end, we assessed the vitamin D status of Fulani men and women residing in northcentral Nigeria who have dietary and cultural customs in common with Fulani in sub-Saharan Africa in general.

MATERIALS AND METHODS

Subjects

Healthy Fulani men and women between the ages of 18 and 72 years were recruited at various clinics at the Federal Medical Centre, Gombe, from among family members accompanying patients to the hospital. When out of doors, the women's clothing covered all but the hands and face. Exclusion criteria included: recent surgery or bone fracture, pregnancy or kidney disease, and tobacco use. Informed consent was obtained from each subject after the purpose and requirements of the study had been explained to them in English, or Hausa in those instances where the potential subject did not understand English sufficiently to consent to participate. Approval for the study was granted by the human research committee of the University of New Mexico Health Sciences Center and the ethics committee of the Federal Medical Centre-Gombe in Gombe, Nigeria.

Vitamin D Analysis

The concentration of 25-hydroxyvitamin D in serum is considered a reliable indicator of vitamin D status.[20] Serum concentrations of 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 were determined using an ultra-performance liquid chromatography system (Acquity UPLC) coupled to a Quattro Premier MSMS system (Waters, Milford, Massachusetts). Hexane (40 µL) was added to each serum sample (200 µL). After the mixture was vortexed for 30 sec, 300 µL of acetonitrile containing the internal standard (75 ng/µL) were added. The internal standard was 25-hydroxyvitamin D_3 (26, 26, 26, 27, 27, 27-d6) (Medical Isotopes, Pelham, New Hampshire). The samples were vortexed again for 30 sec, followed by centrifugation in an Eppendorf microfuge for 7 min at 14 000 RPM. The supernatant was then transferred to an autosampler vial and 25 µL injected into the UPLC system.

Samples were chromatographed using an Acquity UPLC BEH C18 column (1.7 µm, 2.1×50 mm) with the following gradient generated using mobile phase A: 2 mM ammonium acetate with 0.1% (v/v) formic acid in high-performance liquid chromatography (HPLC)–grade water and mobile phase B: 2 mM ammonium acetate with 0.1 % (v/v) formic acid in HPLC-grade methanol. The initial flow rate was 0.60 mL/min with 13.5% mobile phase A and 86.5% mobile phase B. At 0.95 min, the flow rate was increased to 0.85 mL/min with 0% mobile phase A and 100% mobile phase B for 2 min. The flow rate was then returned to 0.60 mL/min with 13.5% mobile phase A and 86.5% mobile phase B. The total run time was 2.5 min per sample. The column temperature was maintained at 45 ± 5ºC.

A serum-based vitamin D standard containing 25-hydroxyvitamin D_3 at a target value of 95.9 ng/mL and 25-hydroxyvitamin D_2 at a target value of 56.2 ng/mL was used for calibration (Chromsystems, Munich, Germany). The actual concentration of the standard was

Table 1. Anthropometric Characteristics and Vitamin D Status of Fulani Males and Females

Parameter	Females (n = 29)	Males (n = 22)
Age, y	55.5 (13.5)[a]	47.6 (8.3)
Height, m	1.67 (0.6)	1.60 (0.5)[b]
Weight, kg	60.3 (8.1)	54.2 (9.9)[b]
Body mass index, kg/m^2	21.6 (3.1)	21.1 (3.2)
Fat, kg	8.7 (4.6)	15.0 (6.9)[b]
Fat, %	13.8 (5.6)	26.5 (7.4)[b]
Fat-free mass, kg	51.6 (4.5)	39.2 (3.9)[b]
Fat-free mass, %	85.9 (6.4)	73.5 (7.4)[b]
Total vitamin D, ng/mL	23.7 (1.4)	31.9 (1.6)[b]

[a] The number in parentheses indicates 1 SD.

[b] The difference between the male and female values was significant at $p < .001$.

lot dependent. Quality control was monitored using lyophilized serum controls at 2 levels (UTAK Laboratories Inc, Valencia, California) that were reconstituted according to the manufacturer's instructions. The target value of the low control was 30 ng/mL (range: 21-39 ng/mL) and the target value of the high control was 73 ng/mL (range, 51-95 ng/mL). A negative control consisting of the acetonitrile/internal standard solution was also included. All 3 quality-control levels were analyzed with the first sample run of the day. Over the next 24-hour period, only 1 control level was used with each batch of samples.

Anthropometric Assessments

A stadiometer accurate to 0.25 cm and a battery-operated scale accurate to 0.5 kg were used to determine, respectively, the height and weight of each subject. Blood pressure was measured twice using a nylon cuff and latex inflation system (Prestige Medical Inc, Northridge, California) and the 2 sets of values were averaged. Fat-free mass and body fat were estimated by bioelectrical impedance analysis conducted using a portable analyzer (Quantum, RJL Systems, Clinton Township, Michigan) as described elsewhere.[14] Resistance and reactance values, together with age, gender, weight, and height, were used to calculate fat-free mass and body fat using software provided by the manufacturer.

Statistical Analyses

Results are reported as means ± 1 SD. Descriptive statistics, group comparisons between men and women, and correlations between serum vitamin D concentrations and body composition parameters were performed using the Number Cruncher Statistical Software (Version 2001, NCSS, Kaysville, Utah). Spearman rank correlation coefficients were used to eliminate the effect of extreme values. The Fisher's exact test was used to test for differences in proportions. A p value of ≤.05 was considered significant.

RESULTS

Anthropometric characteristics of the subjects. The anthropometric characteristics of the subjects are summarized in the Table 1. There was no statistically significant difference in the ages of the male and female subjects. Although the men and women had comparable body mass index (BMI) values, the women had significantly greater fat-free mass and percent of fat-free mass, but lower body fat and percent of body fat than the men.

Vitamin D Analyses

The mean serum 25-hydroxyvitamin D_3 concentration of the 29 women was 23.7 (±1.4) ng/mL and was statistically different from the mean value of 31.9 (±1.6) ng/mL for the 22 male subjects (p < .001). Except for 1 female subject whose serum contained 29 ng/mL vitamin D_2, none of the other 50 male or female subjects in our study had detectable vitamin D_2. Therefore, in this report, the concentration of 25-hydroxyvitamin D_3 denotes total 25-hydroxyvitamin D.

It is widely agreed that a 25-hydroxyvitamin D concentration of less than 20 ng/mL indicates vitamin D deficiency, whereas a 25-hydroxyvitamin D concentration in the 21 ng/mL to 29 ng/mL range is regarded as indicating insufficiency.[21] Twenty-four of 29 (83%) Fulani women had a serum 25-hydroxyvitamin D concentration below 30 µg/mL, but only 10 of 22 (45%) of the men had a vitamin D concentration below the lower limit of normal (Table 2). Fourteen of the women (48%) had a 25-hydroxyvitamin D value between 21 ng/mL and 29 ng/mL, indicating insufficiency, and 10 (34%) had a value below 20 ng/mL, indicating vitamin D deficiency. In contrast, only 1 of the male subjects (5%) was vitamin D deficient and 9 of 22 (40%) had a serum 25-hydroxyvitamin D level between 20 ng/mL and 29 ng/mL.

When the serum 25-hydroxyvitamin D concentration was plotted vs BMI and various body composition parameters, including body fat, percent body fat, fat-free mass and percent fat-free mass, the only statistically significant correlations we found were between vitamin D and BMI (Figure 1, r = –0.48, p = .022), and vitamin D and percent body fat (Figure 2, r = –0.52, p = .013) in the male subjects; no such correlations were observed in the female subjects. The 25-hydroxyvitamin D levels in neither the male nor the female subjects correlated with age.

DISCUSSION

The most significant result of our study was the finding that the vitamin D status of most of the Fulani women was poor: 83% of the women had a serum 25-hydroxy-vitamin D concentration in the insufficient of deficient range. In contrast, only 45% of the Fulani males had a serum 25-hydroxyvitamin D value below 30 ng/mL. What might account for this gender difference?

Table 2. Percentage of Fulani Men and Women With Serum Vitamin D Concentrations in the Insufficient and Deficient Ranges

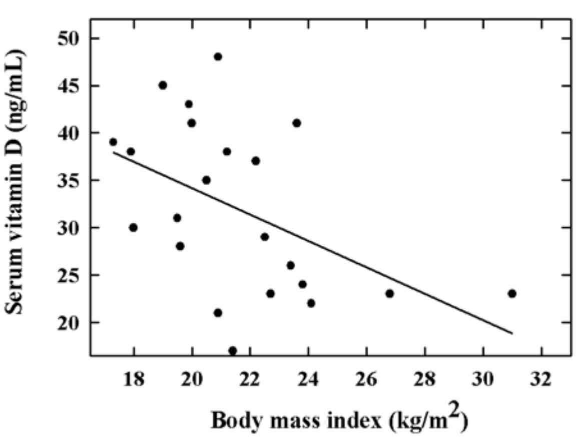
Serum Vitamin D Concentration	Males (n = 22)	Females (n = 29)	*P* Value
<30 ng/mL	10 (45%)[a]	24 (83%)	.007
21-29 ng/mL (insufficient)	9(40%)	14 (48%)	.78
≤20 ng/mL (deficient)	1 (5%)	10 (34%)	.015

[a] The number in parentheses indicates percent of total male and total female subjects.

Since Fulani men and women are both dark skinned and share the same diet[14] and neither group was taking vitamin supplements, the answer probably lies with differences in the extent of skin exposure to sunlight between Fulani men and women. Whereas Fulani men tend to spend most of the day out of doors tending to their cattle, thereby benefitting from extensive exposure of their skin to sunlight, Muslim custom dictates that their female counterparts cover most of their skin surface when they are in public, therefore depriving the women of UV light-generated vitamin D_3.

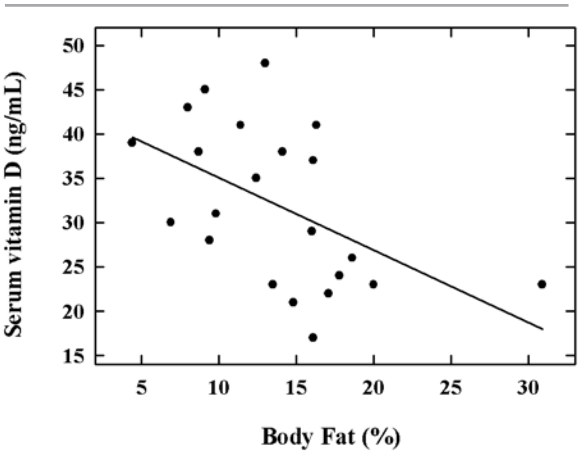
Another explanation for the low vitamin D concentrations obtained for the Fulani subjects may be the increased conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D in the presence of low intake of dietary calcium. Using a rat model, Clements and coworkers[22] demonstrated increased catabolism of 25-hydroxyvitamin D when animals were fed a low-calcium diet. It was therefore proposed by Clements[23] that the rickets observed in an Asian community in the United Kingdom could be attributed to a high-cereal, low-calcium diet resulting in mild hyperparathyroidism and elevation of 1,25-dihydroxyvitamin D concentrations with a concomitant reduction in serum 25-hydroxyvitamin D levels.

Figure 1. Relationship Between Serum Vitamin D_3 Concentration and Percent Body Fat in the Male Subjects



r = –0.48, *p* = .022.

Figure 2. Relationship Between Serum Vitamin D_3 Concentration and Body Mass Index in the Male Subjects



r = –0.52, *p* = .013.

Our finding of a strong negative correlation between serum vitamin concentration and BMI and percent body fat in the Fulani males was not surprising since such a relationship has been documented previously in other populations.[24-28] However, we have no explanation for why similar correlations were not observed in the Fulani women since other investigators have consistently found a negative correlation between vitamin D status and BMI or body fat in women.[24,26-28] It is worth noting that the mean BMI of the women in our study(21.6 kg/m^2) was considerably lower than the mean BMI reported by Bischof and coworkers[24] for Austrian women (mean, 32.7 ± 8.2 kg/m^2) for whom they observed a significant inverse correlation between BMI and serum vitamin D. In addition, the body fat content and percent body fat of the Fulani women were low, 8.7 kg and 13.8%, respectively. Since one of the explanations for the relation between body fat and circulating vitamin D levels is deposition of vitamin D in fat tissue,[29] the lack of correlation between body fat and serum vitamin D levels in our study suggests that there is a threshold for uptake of vitamin D by adipose tissue and the Fulani women in our study have a fat content below that threshold.

The main implication of our findings is that Fulani pastoralists, females in particular, may be at increased risk of bone fracture. In a previous study in which we assessed the bone quality of adults in Nigeria using portable calcaneal ultrasound,[30] we found that the stiffness index-based *T* scores of Nigerian women were similar to the *T* scores of Caucasian women in the United States that were based on dual x-ray absorptiometry (DEXA) measurements. A progressive decline in bone ultrasound parameters beginning at about 40 years of age occurred in both male and female Nigerians. In a study of the dietary habits of the rural Fulani of northern Nigeria,[14] we found that the calcium intakes of Fulani men and women were below the recommended daily allowances for European and North American populations. A less-than-ideal intake of calcium coupled with suboptimal vitamin D levels could contribute to the precipitous decline in bone quality that occurs in Fulani men and women after the age of 40 years.

There is evidence that in certain parts of the world where sunlight is abundant and could be advantageous with regard to the vitamin D status of women, cultural and religious practices deprive them of this benefit. For example, in a study of healthy Saudi women, Ghannam and associates[31] found that the bone density of healthy women was lower than in their counterparts in the United States and that this deficit could be accounted in part by the high prevalence of vitamin D deficiency. In a study of 119 Muslim women in Australia,[32] it was found that nearly 70% of them had severe vitamin D deficiency and high rates of bone turnover that placed them at increased risk of osteoporosis or osteomalacia and ultimately fracture. In their study of 682 women and young girls in Iran, Ghazi and colleagues[33] found that, unlike their male counterparts who exhibited seasonal changes in their serum vitamin D levels, the females showed no such variation in their vitamin D levels. This difference was attributed to the different patterns of lifestyle between males and females in that country. A subsequent study in Iran[34] reported that a high prevalence of vitamin D deficiency in maternal serum at the time of delivery (66.8%) and in the cord blood samples (93.3%), thereby illustrating the importance of adequate vitamin D intake during pregnancy in a traditional Muslim population.

How might the vitamin D status of Fulani pastoralists be improved? In economically advanced countries, the obvious answer would be vitamin D supplements. However, in practical terms, in the region of the world where the present study was conducted, the relatively high cost of supplements would preclude this solution. Therefore, it is imperative to identify locally available and affordable sources of vitamin D. A potential source of vitamin D for the Fulani would be chicken eggs, which contain reasonable amounts of vitamin D. The yolk of 1 large egg contains 25 IU of vitamin D.[35] The adequate intake of vitamin D for adults between the ages of 31 and 50 years is 5 μg/day (200 IU).[36] Although seafood in general is rich in vitamin D, most Fulani pastoralists live hundreds of miles from the Atlantic Ocean and do not have access to such foods. In a future study, we plan to investigate the content of vitamin D in local freshwater fish in northern Nigeria.

The results of our study are particularly relevant to pregnant and lactating Fulani women. Since the concentration of vitamin D in human milk is correlated strongly and positively with the mother's serum vitamin D concentration,[11] it is likely that infants of Fulani women in the community where this study was conducted and who rely on breastmilk for a significant part of their nutrition will, like their mothers, be vitamin D deficient. Thus, ensuring adequate vitamin D nutrition for pregnant and lactating women in the region should be a priority.

This study was limited by the relatively small number of subjects and by the fact that serum 1,25-dihydroxyvitamin D levels were not determined. In addition, it would have been helpful to know the subjects' calcium intake since this information would have permitted us to assess the correlation between dietary calcium and serum vitamin D levels.

The results of this study should be of interest to public health officials at the federal and local levels in Nigeria, and should prompt indigenous research that would provide knowledge regarding the scope and significance of vitamin D deficiency in that country. Finally, the finding that hypovitaminosis D seems to be common in at least 1 ethnic group in Nigeria should encourage similar studies of the prevalence of poor vitamin D nutrition in ethnic groups elsewhere in Nigeria and other parts of sub-Saharan Africa as well.

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