INTRODUCTION

Vitamin D is best known for its role in maintaining calcium homeostasis and skeletal integrity, although vitamin D is also critical for maintaining the health and function of the immune, reproductive, and muscular systems. Vitamin D status is largely determined by cutaneous synthesis from solar exposure and dietary sources and is assessed by measuring circulating levels of 25-hydroxyvitamin D (25(OH)D). However, in free-living individuals, the majority of circulating 25(OH)D originates from UVB exposure. Epidemiologic studies have linked low 25(OH)D with obesity, type 2 diabetes, cancer, cardiovascular disease, and infectious and autoimmune diseases.

The mean concentration of 25(OH)D is generally lower among the elderly and among dark skinned individuals. Individuals of African ancestry living in the United States typically have lower levels of serum 25(OH)D than Caucasians across all age groups and both sexes due at least in part to differences in skin pigmentation and dietary vitamin D intake. Vitamin D status in African ancestry individuals has been primarily assessed in healthy younger individuals, pregnant and lactating women, healthy children and those with rickets, and clinical studies of tuberculosis and pneumonia patients. A recent review of studies in the African continent has shown that vitamin D status varies considerably depending on geography, climate, and other factors. However, there is insufficient data in African ancestry individuals living in other geographic regions, particularly in tropical climates where there is high sun exposure, and among elderly men.

The validity of earlier studies of 25(OH)D have been questioned due to the use of insensitive and unreliable radioimmunoassay-based methods which were fraught with inaccuracies due in part to protein binding artifacts. Improvements in assay methods, such as liquid chromatography tandem mass-spectrometry (LC-MS/MS) and high performance liquid chromatography, have enabled the direct detection of the D2 (ergocalciferol) and D3 (cholecalciferol) metabolites and are considered preferred techniques for assessing nutritional vitamin D status.

In our study, we measured 25(OH)D, 25(OH)D2 and 25(OH)D3 using LC-MS/MS in elderly African ancestry men living in Tobago. Tobago (latitude 11°N) is a Caribbean island, located in the West Indies, between the Caribbean Sea and the North Atlantic Ocean, northeast of Venezuela. We examined the prevalence of vitamin D deficiency and also determined the impact of several potential correlates of serum 25(OH)D in this population.

DESIGN AND METHODS

Study Population

Between 1997 and 2003, 3170 previously unscreened men were recruited for a population-based prostate cancer screening study on the Caribbean Island of Tobago, Trinidad and Tobago. To be eligible, men had to be ambulatory, non-institutionalized and not terminally ill. Recruitment for the survey was accomplished by flyers, public service announcements, posters,
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Informing health care workers at local hospital and health centers, and word of mouth. Approximately 60% of all age-eligible men on the island participated and participation was similar across the island Parishes. All men were invited to participate in a follow-up clinic exam between 2004 and 2007 and 2,031 men in the cohort (70% of survivors) and 451 new participants completed the visit. Approximately 89% of the men at the follow-up visit reported that both paternal and maternal grandparents were of African ethnicity. There were 618 Afro-Caribbean men aged ≥65 years (with all 4 grandparents of African ancestry). Vitamin D was measured in serum 25(OH)D and 25(OH)D3 were performed at the Mayo Clinic using samples that had not been previously thawed. Deuterated stable isotope (d3-25-hydroxyvitamin D) was added to a .2-mL serum sample as an internal standard. 25 (OH)D3 and 25 (OH)D2 and the internal standard were extracted using acetonitrile precipitation. The extracts were then further purified on-line utilizing high turbulence liquid chromatography (HTLC). This was followed by conventional liquid chromatography and analysis on a tandem mass spectrometer (LC-MS/MS) equipped with a heated nebulizer ion source and operated in the multiple-reaction monitoring positive mode. The calibration utilized a six point standard curve over a concentration range of 0–200 ng/mL. The minimum detectable limit for 25(OH)D2 was 4 ng/mL and for 25(OH)D3 was 2 ng/mL. 25(OH)D2 and 25(OH)D3 were quantified, reported individually and summed for total 25(OH)D. Using the pooled serum, the inter-assay CV for 25(OH)D2 was 6.1% and the intra-assay CV was 4.4%, whereas the inter-assay CV for 25(OH)D3 was 6.4% and the intra-assay CV was 3.8%. Deficiency was defined as total 25(OH)D <20 ng/mL, insufficiency as 20–29 ng/mL and sufficiency as 30–149 ng/mL. No participant had toxic levels (> 150 ng/mL).

Potential Correlates of 25(OH)D

We tested several potential correlates of 25(OH)D that were available in our study. Body mass index was calculated from height and weight (kg/m²). Height was measured to the nearest .1 cm using a wall-mounted stadiometer. Body weight was recorded to the nearest .1 kg without shoes on a balance beam scale. Waist circumference was measured at the narrowest point of the waist using an inelastic fiberglass tape. Information on lifestyle habits, demographic information, medical conditions (type 2 diabetes and hypertension), and medication use were assessed using interviewer administered questionnaires. Obesity was defined as BMI ≥30, and type 2 diabetes was defined as fasting serum glucose ≥126mg/dL or currently taking anti-diabetic medication. Alcohol drinking frequency (never, less than one drink per week, 1–3, 4–7, 8–14, 15–21, 22–27, ≥28, drinks per week) and hours of TV watching, as a measure of sedentary lifestyle, (0, 1–6, 7–13, 14–20, 21–27, ≥28, hours of TV watching per week) were self-reported in predefined categories. Fish and milk intake frequency were also self-reported in predefined categories (never, a few times per year, 1 time per month, 2–3 times per month, 1 time per week, 2 times per week, 3–4 times per week, 5–6 times per week, every day). We arbitrarily created two categories of fish, milk and alcohol use (≤1 and >1 once per week) and two categories of TV viewing (<14 hours/week).

Statistical Analyses

The distributions of 25(OH)D, 25(OH)D3 and detectable 25(OH)D2 levels were approximately normal. Season of visit was coded as winter (January–March), spring (April–June), summer (July–September), and fall (October–December). Using linear regression analysis, we first evaluated the age-adjusted association of each measured risk factor with 25(OH)D. The relationships between potential correlates and 25(OH)D were expressed as one unit for categorical variables or 1 SD for continuous variables, along with 95% confidence intervals (CIs). To identify the independent correlates of 25(OH)D, multiple linear regression analysis with backwards elimination of the least significant variable was performed separately. Variables with \( P < .10 \) in the age-adjusted univariate linear regression model were entered into the multiple variable model. The Statistical Analysis System (SAS, version 9.1.2; SAS Institute, Cary, NC) and the Statistical Package for the Social Sciences (SPSS, version 16; Chicago, IL) were used for statistical analysis.
**RESULTS**

The mean age of the men was 72 years, range 65–92 years (Table 1). The average serum total 25(OH)D level was 35.1 ± 8.9 ng/mL. Almost all circulating 25(OH)D was derived from vitamin D3; only 8% had 25(OH)D2 in the detectable range (>4 ng/mL), and mean 25(OH)D2 levels were low (5.7 ± 2.9 ng/mL) among men with detectable levels. Vitamin D insufficiency was observed in 24% whereas vitamin D deficiency was observed in only 2.8% of the men. Fish intake was relatively frequent with almost 29% reporting that they ate fish (with meat and bones) more than once per week and 17.5% reporting that they ate fish 3 or more times per week. The daily use of vitamin D supplements was very low (8.1%). 18.6% of the men were obese and 36% had type 2 diabetes.

In age-adjusted regression analyses each SD (4.3 kg/m2) increase in BMI was associated with 1.8% lower 25(OH)D (Table 1). In addition, every 11.5 cm increase in waist circumference was associated with 1.2% lower 25(OH)D. The daily use of vitamin D supplements was also negatively associated with 25(OH)D levels. No other variables were significantly correlated with 25(OH)D. To identify the independent correlates of 25(OH)D, we further tested age, BMI, waist circumference, and daily vitamin D supplement use in the multiple linear regression model. Age, BMI and daily vitamin D supplement use remained significant, and BMI-adjusted P = .01, adjusted for age, BMI, and daily vitamin D supplement use was also negatively associated with vitamin D deficiency. To identify the independent correlates of 25(OH)D deficiency between vitamin D supplement users and non-users (age- and BMI-adjusted P = .085), and vitamin D deficiency between vitamin D supplement users and non-users (age- and BMI-adjusted P = .98).

The difference in serum 25(OH)D levels between those who reported vitamin D supplement intake and those who did not (age- and BMI-adjusted P = .08), or in the prevalence of vitamin D insufficiency (age- and BMI-adjusted P = .055) and vitamin D deficiency between vitamin D supplement users and non-users (age- and BMI-adjusted P = .98).

Although fish intake was not associated with mean levels of 25(OH)D, none of the men who ate fish ≥1 times per week had vitamin D deficiency, whereas the 4% who consumed fish less than once per week had vitamin D deficiency (P = .01, adjusted for age, BMI, and daily vitamin D supplement). The prevalence of vitamin D insufficiency was similar in both groups (P = .80, adjusted for age, BMI, and daily vitamin D supplement). No difference in prevalence of deficiency and insufficiency was observed among those who drank more milk, currently smoked, drank more than one alcoholic drink per week, watched TV for 14 or more hours per week (data not shown).

There was no significant difference in serum 25(OH)D levels between those who reported vitamin D supplement intake and those who did not (age- and BMI-adjusted P = .08), or in the prevalence of vitamin D insufficiency (age- and BMI-adjusted P = .055) and vitamin D deficiency between vitamin D supplement users and non-users (age- and BMI-adjusted P = .98).

The difference in serum 25(OH)D levels between seasons was small and not statistically significant (age- and BMI-adjusted P = .25). Mean total 25(OH)D levels were highest in fall (36.2 ± 8.7 ng/mL) compared to winter (34.6 ± 9.6 ng/mL), spring (34.5 ± 8.8 ng/mL) and summer (34.4 ± 8.1 ng/mL).

**DISCUSSION**

We found a very low prevalence of vitamin D deficiency among older men of African ancestry in Tobago. Tobago, located at 11 degrees north latitude,
experiences a tropical climate and is sunny all year. The average annual daytime temperature is 29°C (83°F). There is a short rainy season from June until the end of October, but there are periods of sunshine between the episodes of very short but heavy rainfall. The adult dress does not restrict sunshine exposure of the arms, face, or head. The high year-round sun exposure is a likely explanation for the low prevalence of vitamin D deficiency in this population.

Previous studies have shown that African Americans typically have lower levels of serum 25(OH)D than Caucasian Americans.26-31 The mean levels of total 25(OH)D among Afro-Caribbean men in our study (35 ng/mL) were considerably higher than the levels among older African American men in the Third National Health and Nutrition Examination Survey (NHANES III) (17 ng/mL) and among older African American men in the Study of Osteoporotic Fractures in Men (MrOS) (18.5 ng/mL).20,21 Additionally, in MrOS, 64% of African American men and 23.3% of older Caucasian American men aged ≥65 years had vitamin D deficiency based on the same assay methods and laboratory that we used. These prevalences are considerably higher than the prevalence (2.8%) among the Afro-Caribbean men from our study.

The lower values of 25(OH)D in black African ancestry individuals compared with Caucasians have been attributed primarily to their darker skin pigmentation and insufficient cutaneous synthesis of 25(OH)D.22 Lower consumption of dairy products and other foods fortified with vitamin D and lower sun exposure are frequently cited as additional causes of the high prevalence of vitamin D deficiency in black African ancestry individuals.23 Epidemiologic data on vitamin D in African countries has been limited and mainly derived from populations at high risk for deficiency, such as undernourished children, women, and tuberculosis and pneumonia patients.14 However, the prevalence of vitamin D deficiency in the African continent varies widely, in line with a geographical gradient, and 25(OH)D seems to be much lower in North African countries and in South Africa compared with tropical African countries.14 Interestingly, in the northwestern African country of Gambia with latitude similar as Tobago (13°N), the mean 25(OH)D level (25.7 ng/mL) among healthy men aged 60–64 years, was higher than reported levels for African-Americans, but still slightly lower than levels in our study.24

Levels of 25(OH)D2 were undetectable in most of the men in our study suggesting that many older Afro-Caribbean men have minimal exposure to vitamin D2. Although we did not attempt to assess dietary intake of vitamin D from food, very few men (8.1%) in our study reported daily use of vitamin D supplements. We found that daily use of vitamin D supplements is inversely associated with 25(OH)D in our study, and insufficiency was higher in supplement users than in non-users. However, typical vitamin D supplement doses are not generally thought to be adequate to ensure sufficient serum 25(OH)D levels.25 Holick et al recently reported that for every 100 IU intake of vitamin D2 or vitamin D3, there is a very small increase in circulating 25(OH)D levels of only 1 ng/mL.26 This may explain in part why in our study men reporting supplement use have lower serum 25(OH)D levels.

We observed a negative and independent association between BMI and 25(OH)D. Few studies have examined whether obesity is linked to lower serum 25(OH)D levels in African-Americans.27,28 Some studies have shown a weaker inverse correlation between 25(OH)D and obesity in African-Americans compared to Caucasians of the same age.27 Reduced serum levels of 25(OH)D in obesity has been attributed to sequestration of fat soluble vitamin D in adipocytes.29 Enhanced lipogenesis and reduced lipolysis in African ancestry individuals may also lead to their greater ability to sequester vitamin D in adipocytes than Caucasians.30 Although the possible mechanisms linking vitamin D and accumulation of fat are still unclear, a recently study has proposed a novel aspect of vitamin D biology in regulation of energy metabolism.31 Wong et al have reported that in animals, vitamin D is involved in energy metabolism at least in part through the regulation of β-oxidation in white adipose tissue and direct suppression of the expression of UCP1 and UCP3 in brown adipose tissue.31

We identified a positive, but weak association between fish intake and vitamin D deficiency in our population sample. Fish consumption is a significant factor in maintaining adequate levels of serum 25(OH)D, and a positive association between fish intake and 25(OH)D was observed among active elderly Japanese men and women, and healthy middle-aged Asian men.32,33 We are unaware of studies reporting an association between fish intake and vitamin D status in African ancestry individuals, mainly because of their very low fish intake.34

The present study has several potential limitations. Demographic and lifestyle information was self-reported and may be subject to misclassification. Other potentially important factors associated with 25(OH)D levels were not assessed in our study including

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direct estimates of sunlight exposure, sun protection behaviors and dietary intake of vitamin D from food sources other than fish and milk intake. However, our study also had notable strengths including its reliance on a population-based sample of Afro-Caribbean men and the use of an accurate and reliable LC-MS/MS method to measure 25(OH)D2 and 25(OH)D3 that was free of the artifacts that have affected earlier radioimmunoassay based methods.16

In conclusion, vitamin D deficiency is very uncommon in the Afro-Caribbean male population of Tobago. Future longitudinal studies are needed to delineate the possible effects of high serum 25(OH)D levels in this population on vitamin D related outcomes.

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