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CORRESPONDENCE

Role of Assay Type in Determining Free 25-Hydroxyvitamin D Levels in Diverse Populations

TO THE EDITOR: It is unclear whether circulating free or bioavailable 25-hydroxyvitamin D is a better marker of vitamin D status than is total 25-hydroxyvitamin D, especially in racially diverse populations. Until recently, the only method to compare the levels was to estimate the level of free or bioavailable 25-hydroxyvitamin D from total 25-hydroxyvitamin D, vitamin D–binding protein (also known as gc-globulin, encoded by the *GC* gene), and albumin, with or without the *GC* genotype. Powe et al. reported that levels of vitamin D–binding protein, as measured on a monoclonal enzyme-linked immunosorbent assay (ELISA, R&D Systems), were lower in black participants than in white participants in the United States.[1] Consequently, calculated levels of bioavailable 25-hydroxyvitamin D were shown to be similar in black participants and white participants, despite a lower mean level of total 25-hydroxyvitamin D in black participants.[2] This finding gained widespread attention and was suggested to have implications for screening for 25-hydroxyvitamin D and for health policy.[3]

Here we present evidence that the use of a monoclonal ELISA to measure vitamin D–binding protein in persons of African ancestry introduces a critical flaw into the calculation of free or bioavailable 25-hydroxyvitamin D,[4] a limitation that influenced the conclusions of Powe et al. and other investigators. In addition, we present racial and geographic comparisons of directly measured free 25-hydroxyvitamin D.

In two studies involving a total of 1057 black men and non-Hispanic white men in the United States (the Osteoporotic Fractures in Men study) and in the United Kingdom and Gambia (the Medical Research Council study), we compared circulating levels of total 25-hydroxyvitamin D on liquid chromatography–tandem mass spectrometry with directly measured free 25-hydroxyvitamin D (ELISA, Future Diagnostics) and calculated levels of free 25-hydroxyvitamin D, using levels of 25-hydroxyvitamin D and vitamin D–binding protein as measured by means of monoclonal ELISA, polyclonal radial immunodiffusion, and two polyclonal ELISAs (Table 1).

The median level of vitamin D–binding protein on monoclonal ELISA in men of African ancestry was approximately 65% lower than the level in white men, whereas the level of vitamin D–binding protein was nearly identical in all groups when measured by means of polyclonal antibodies against vitamin D–binding protein. The fact that levels of vitamin D–binding protein do not vary between races was confirmed by measurements of two nonvariant and five *GC*-variant peptides of vitamin D–binding protein by means of proteomic methods (data not shown), which support the findings of recent mass spectrometry studies.[5] Racial and geographic differences in levels of total 25-hydroxyvitamin D were reflected in directly measured levels of free 25-hydroxyvitamin D, with lower levels in black men than in white men in the United States (Table 1).

Levels of free and total 25-hydroxyvitamin D were strongly correlated (overall Spearman's correlation coefficient, 0.84). Similarly, levels of total 25-hydroxyvitamin D were highly correlated with calculated levels of free 25-hydroxyvitamin D derived from the two polyclonal measures of vitamin D–binding protein ($r \geq 0.93$) but not from the monoclonal assay ($r = 0.54$). Levels of measured free 25-hydroxyvitamin D and levels that were calculated on the basis of polyclonal

Table 1. Levels of Total and Free 25-Hydroxyvitamin D and Vitamin D–Binding Protein, According to Race and Geographic Region.*

Substance and Type of Assay	MrOS Study		MRC Study	
	White Men (N=919)	Black Men (N=101)	White Men (N=18)	Black Men (N=19)
	median (interquartile range)			
Total 25-hydroxyvitamin D (nmol/liter)†	62.2 (50.4–73.4)	38.4 (24.5–53.9)	25.6 (22.8–36.2)	65.1 (57.1–78.3)
Vitamin D–binding protein (μmol/liter)				
Polyclonal radial immunodiffusion	5.11 (4.69–5.48)	5.08 (4.67–5.48)	5.22 (4.79–5.48)	4.79 (4.46–5.31)
Polyclonal ELISA: GenWay‡	4.79 (4.29–5.31)	5.33 (5.00–5.69)	ND	ND
Polyclonal ELISA: Immundiagnostik‡	ND	ND	6.77 (6.60–7.58)	6.65 (6.10–7.17)
Monoclonal ELISA: R&D Systems†	4.86 (3.94–6.15)	1.77 (1.34–3.25)	5.50 (4.04–6.19)	1.94 (1.64–3.85)
Calculated free 25-hydroxyvitamin D (pmol/liter)§				
Polyclonal radial immunodiffusion†	27.2 (22.5–32.5)	17.3 (10.3–23.1)	11.2 (9.4–18.1)	31.4 (24.7–37.7)
Polyclonal ELISA: GenWay†	29.0 (23.3–34.5)	16.8 (9.7–22.1)	ND	ND
Polyclonal ELISA: Immundiagnostik†	ND	ND	8.7 (6.7–13.7)	23.8 (20.4–27.9)
Monoclonal ELISA: R&D Systems¶	28.1 (21.7–35.8)	31.3 (19.8–50.6)	12.0 (8.5–18.7)	56.9 (45.1–69.2)
Directly measured free 25-hydroxyvitamin D: Future Diagnostics and DIAsource (pmol/liter)†‖	13.2 (11.0–16.5)	9.0 (7.5–11.5)	7.1 (5.5–8.3)	16.0 (14.1–20.4)

* The Osteoporotic Fractures in Men (MrOS) study involved men 65 years of age or older in the United States, and the Medical Research Council (MRC) study involved men between the ages of 25 and 39 years in the United Kingdom and Gambia. P values for all listed comparisons in each study were performed by means of the Wilcoxon rank-sum test. P>0.10 for all comparisons with no P value indicated. To convert the values for total 25-hydroxyvitamin D to nanograms per milliliter, divide by 2.496. ND denotes not done.
† P<0.001 for the comparisons between the two groups in both studies.
‡ P<0.001 in the MrOS study and P=0.30 in the MRC study.
§ Findings with respect to free 25-hydroxyvitamin D were similar to those for calculated bioavailable 25-hydroxyvitamin D (r=0.99).
¶ P=0.04 in the MrOS study and P<0.001 in the MRC study.
‖ For directly measured free 25-hydroxyvitamin D in the MrOS study, the evaluations were performed in 194 non-Hispanic white men and 80 black men.

antibodies against vitamin D–binding protein were strongly correlated (r≥0.80), whereas measured levels and levels that were calculated on the basis of monoclonal antibodies against vitamin D–binding protein were only moderately correlated (r=0.56).

In conclusion, median levels of free 25-hydroxyvitamin D were significantly lower in black men than in white men in the United States when the levels were measured directly or calculated on the basis of polyclonal antibodies against vitamin D–binding protein. However, both measured and calculated median levels of free 25-hydroxyvitamin D were lower in white men in the United Kingdom than in black men in Gambia. These findings are consistent with the median levels of total 25-hydroxyvitamin D in all the study participants. These results contradict previous reports of similar levels of free or bioavailable 25-hydroxyvitamin D between races. Our results underscore the importance of the choice of assay for vitamin D–binding protein in the calculation of free 25-hydroxyvitamin D in diverse populations and support the measurement of total 25-hydroxyvitamin D in the general population as a marker of vitamin D status, regardless of race or *GC* genotype.

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