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Vitamin D–Binding Protein Concentrations Quantified by Mass Spectrometry

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More than 50% of blacks carry a diagnosis of vitamin D deficiency, yet in blacks low 25-hydroxyvitamin D seems not to have a higher risk for fractures as it does in whites.^{1,2} One potential explanation for this centers around free or bioavailable 25-hydroxyvitamin D. Equations to estimate these concentrations include the amount of albumin and vitamin D binding protein. Recently, in community dwelling adults, concentrations of vitamin D binding protein were found to be lower in blacks. Consequently, the amount of bioavailable vitamin D was similar between blacks and whites,³ supporting the hypothesis that blacks are actually vitamin D sufficient in terms of bioavailable vitamin D.

Two major polymorphisms in the vitamin D binding protein gene give rise to three predominant haplotypes (GC1s, Gc1f, and Gc2) and blacks more often carry Gc1f.³ Importantly, the mean genotype-specific concentrations of vitamin D binding protein determined by the R&D Systems monoclonal sandwich immunoassay³ were quite different from those previously observed using a polyclonal nephelometric immunoassay.⁴ It seemed possible that one of the monoclonal antibodies recognizes an epitope near the polymorphic region of the protein and has different affinities for the different haplotypes of vitamin D binding protein, which would affect the assay. In contrast, a polyclonal antibody would have many epitopes with which to interact.

As an alternative method of quantification, we developed a liquid chromatography-tandem mass spectrometric assay. The assay proteolytically cleaves plasma proteins into peptides that can be specifically detected and quantified. Peptides common to all vitamin D binding

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protein isoforms are surrogates for the concentration of the protein in the sample. Calibrators are made from human serum.⁵ The coefficient of variation is <12%.

Using the new assay and the monoclonal immunoassay, we analyzed samples from 187 previously genotyped Atherosclerosis Risk in Communities (ARIC) Study participants. **Table 1** presents, for 3 assays, mean concentrations of vitamin D binding protein by genotype. When stratified (and ranked) by genotype, concentrations determined by liquid chromatography-tandem mass spectrometry were similar to the polyclonal immunoassay, while the monoclonal assay generated very different results. Specifically, concentrations by the monoclonal assay were much lower when Gc1f was present. While this does not provide definitive evidence that the monoclonal assay depends on the isoforms present, it strongly supports the hypothesis that the assay is affected by polymorphisms. Particularly since Gc1f is more common among blacks, it is unclear whether vitamin D binding protein levels differ by race.

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Table 1

Mean serum concentrations of vitamin D binding protein by genotype.*

Genotype	LC-MS/MS		Polyclonal [†]		Monoclonal [‡]		Monoclonal [§]	
Population	ARIC		DOPS		ARIC		HANDLS	
	Mean (SD) N	Rank	Mean (SD) N	Rank	Mean (SD) N	Rank	Mean (SD) N	Rank
Gc2/Gc2	242 (23) 15	1	227 (23) 40	1	157 (24) 15	3	190 (36) 80	2
Gc2/Gc1f	252 (15) 10	2	249 (24) 32	2	125 (13) 10	2	ND	ND
Gc2/Gc1s	254 (27) 33	3	250 (26) 196	3	204 (30) 33	5	ND	ND
Gc1f/Gc1f	264 (25) 35	4	269 (25) 17	5	85 (15) 35	1	93 (50) 632	1
Gc1f/Gc1s	269 (38) 41	5	268 (31) 111	4	174 (31) 41	4	285 (81) 413	3
Gc1s/Gc1s	277 (35) 53	6	274 (33) 188	6	262 (41) 53	6	468 (106) 313	4

LC-MS/MS, liquid chromatography-tandem mass spectrometry; **ARIC**, Atherosclerosis Risk in Communities Study; **DOPS**, Danish Osteoporosis Prevention Study; **HANDLS**, Healthy Aging in Neighborhoods of Diversity across the Life Span; **ND**, not determined.

* For each method, the mean concentration (SD) µg/mL is listed along with the number of samples analyzed for each genotype by each method. In addition, the genotype-specific rank from lowest to highest mean concentration is listed for each method.

[†] Mean concentrations (SD) are adapted from the figure previously published.⁴

[‡] Mean concentrations (SD) measured by the R&D Systems immunoassay.

[§] Mean concentrations (SD) are listed as previously published (R&D Systems immunoassay).³