Free 25OHD and Vitamin D Binding Proteins: physiology and assay aspects amongst in studies of multi-ethnic populations Inez Schoenmakers. MRC Human Nutrition Research, Cambridge, CB1 9NL, UK.

There is controversy about the appropriateness of uniform markers and thresholds for the assessment of vitamin D status and adequacy, particularly across different ethnic groups. Markers other than 25OHD have been proposed to provide additional or better measures of tissue availability and utilisation. These include free 25OHD (either directly measured or calculated), 1,25 and 24,25 dihydroxyvitamin D, PTH, vitamin D binding protein (DBP) and/or their ratios.

Recently free 25OHD has received considerable interest, particularly in the investigation of racial disparities in the relationship of 25OHD with diverse health outcomes. Some reports, while others do not, demonstrate stronger associations for free 25OHD compared to total 25OHD with PTH, BMD and various non-skeletal or calcaemic outcomes, including the risk of various types of cancer and complications of renal disease. Recently, we showed that some of these findings were confounded by methodological issues in one of the most commonly used DBP assay [1, 2]. This monoclonal ELISA provides pronounced differences in DBP concentrations between races and GC-genotypes. These racial disparities were not present with other methods. Since calculated free 25OHD is derived from the concentration of DBP, the validity of methodology to measure DBP is critical. We demonstrated that when free 25OHD was calculated on the basis of DBP values derived from the monoclonal assay, this resulted in artificially elevated free 25OHD concentrations. With methods unbiased by GC-genotype, we demonstrated that measured and calculated plasma free 25OHD in a healthy population, are highly correlated irrespective of race, DBP genotype or 25OHD concentration. Studies reporting racial disparities between total and calculated free 25OHD and their relationship to health outcomes should be critically assessed, since many of these were based multi-ethnic cohorts and the results of the monoclonal ELISA for DBP.

DBP is a multifunctional protein; it is the major carrier of vitamin D metabolites, but is also involved in other physiological processes such as immune function. It's plasma concentration is influenced by several physiological and pathological factors and further, small differences in concentrations are associated with genotype. This may influence plasma 25OHD concentration and metabolism. The impact of variations in the plasma concentration of DBP on tissue 25OHD availability are unclear. It is biologically plausible that this depends on the tissue type and the predominant internalisation pathway of 25OHD and/or the 25OHD-DBP complex, i.e., mediated by megalin (kidney and possibly muscle) or via passive diffusion and therefore dependent on the free 25OHD fraction.

I will review the physiological relevance of free 25OHD, DBP, their methodological issues and recent evidence on associations total and free 25OHD and functional outcomes.

1. Nielson CM, Jones KS, Chun RF, Jacobs J, Wang Y, Hewison M, Adams JS, Swanson CM, Lee CG, Vanderschueren D, Pauwels S, Prentice A, Smith RD, Shi T, Gao Y, Zmuda JM, Lapidus J, Cauley JA, Bouillon R, Schoenmakers I, Orwoll E. Free 25-hydroxyvitamin D: impact of vitamin D binding protein assays on racial-genotypic associations. JCEM. 2016;In press.

2. Nielson CM, Jones KS, Chun RF, Jacobs J, Wang Y, Hewison M, Adams JS, Swanson CM, Lee CG, Vanderschueren D, Pauwels S, Prentice A, Smith RD, Shi T, Gao Y, Zmuda JM, Lapidus J, Cauley JA, Bouillon R, Schoenmakers I, Orwoll E. Free vitamin D concentrations reflect racial and geographic differences in total 25OHD Bone Research Society; Liverpool2016.