Assessing Vitamin D Status:
Time for a Rethink?

Graham D. Carter1* and Karen W. Phinney2

Vitamin D status is universally assessed by measuring circulating total 25-hydroxyvitamin D \([25(OH)D]\)^3, the sum of \(25(OH)D_3\) and \(25(OH)D_2\). However, despite the widespread acceptance of this biomarker of vitamin D status, studies have suggested that measurement of \(25(OH)D\) alone may not be sufficient to understand the relationship between vitamin D exposure and certain health outcomes. As a result, attention has turned to other aspects of vitamin D biology. Approximately 90%–95% of \(25(OH)D\) is tightly bound to a specific \(\alpha\) globulin, vitamin D binding protein (VDBP), which is structurally related to serum albumin. Of the remaining \(25(OH)D\), about 1% is unbound and the rest loosely bound to serum albumin. A current hypothesis is that the free and albumin-bound moieties are responsible for delivering \(25(OH)D\) to the cell, with the VDBP-bound \(25(OH)D\) acting as a reservoir of the metabolite. In humans, common genetic polymorphisms produce 3 major circulating variants of the VDBP (Gc1F, Gc2, and Gc1S), which differ in their affinity for \(25(OH)D\). The prevalence of these polymorphs has been shown to vary across different ethnicities and populations, with the Gc1F variant generally more common among individuals of African ancestry. In a recent study reported in the *New England Journal of Medicine* (1), Powe et al. investigated the prevalence of the different VDBP phenotypes (Gc1F, Gc2, and Gc1S) in a cohort of black and white Americans and examined the relationship with \(25(OH)D\). Their results showed no significant correlation with those given by an alternative VDBP assay (R&D Systems) used in this work, and further research is clearly warranted. The VDBP concentrations found in black Americans result in concentrations of bioavailable \(25(OH)D\) that are similar to those found in whites (calculated values 2.9 and 3.1 ng/mL, respectively). This might explain why black Americans can have apparently good bone health in the presence of low total \(25(OH)D\) concentrations.

The Powe et al. study does have some limitations, and further research is clearly warranted. The VDBP assay used in this work (R&D Systems) is intended only for research purposes and has not been validated to the standard required for diagnostic use. Worryingly, results of the R&D assay showed no significant correlation with those given by an alternative VDBP assay (Alpco Diagnostics) that was also investigated in the Powe et al. study. Bioavailable \(25(OH)D\) calculated using the VDBP concentration (R&D kit) and previously published affinity constants for Gc1F and Gc1S did correlate with values obtained with a radioligand-binding assay. Although these results are encouraging, no procedure currently exists to evaluate the accuracy of these methods. Furthermore, standardization of such assays is currently problematic because the available calibration materials tend to contain either a single

---

1 Imperial College Healthcare NHS Trust, Oncology/Endocrine Laboratory, Charing Cross Hospital, London, UK; 2 Biomolecular Measurement Division, NIST, Gaithersburg, MD

* Address correspondence to this author at: Imperial College Healthcare NHS Trust, Oncology/Endocrine Laboratory Charing Cross Hospital, Fulham Palace Rd., London W6 8RF, UK. Fax +44-(0)20-8846-7007; e-mail b.carter1@which.net.

Disclaimer: Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Received January 30, 2014; accepted February 4, 2014.

Previously published online at DOI: 10.1373/clinchem.2013.219386

Nonstandard abbreviations: \(25(OH)D\), 25-hydroxyvitamin D; VDBP, vitamin D binding protein; LC-MS/MS, liquid chromatography–tandem mass spectrometry.

Perspective

Clinical Chemistry 60:6
809–811 (2014)
VDBP variant or a mix of variants of unknown composition. Similarly, no details were given of the standardization or specificity of the liquid chromatography–tandem mass spectrometry (LC-MS/MS) method used for 25(OH)D measurements.

In some respects the problems potentially limiting the utility of serum VDBP measurements mirror those that historically affected 25(OH)D assays. The situation for VDBP is particularly complex because of the existence of several variants with different binding affinities. The impact of these differing affinities on assay performance has not yet been fully investigated. Additionally, the variants have distinct patterns of glycosylation (2) which could contribute to discrepancies in the results given by different assays. In trying to establish the basis for an accurate assay, it might be necessary to know which protein variant was present (perhaps from genetic information) and then ensure that the assay was calibrated with the appropriate form of the protein or some known mixture of the variants. In particular, methods will need to be cospecific for the variants likely to be encountered in a particular set of samples. In practice, this could be immensely challenging and it might prove easier and clinically more useful to develop a method for measuring the bioactive fraction of 25(OH)D, perhaps by LC-MS/MS as has been done for certain steroid and thyroid hormones.

The conclusions reached by Powe et al. on the basis of their study may also have broader implications for 25(OH)D measurements because of the integral role of VDBP in many automated assays. Great strides have been made in recent years to improve the accuracy of 25(OH)D assays, not least through the activities of the Vitamin D Standardization Program (3), the basic aim of which is to standardize the results of different measurement techniques to those of the reference measurement procedures developed by NIST and the University of Ghent. Nevertheless, well-documented differences between methods remain, particularly those for routine clinical chemistry analyzers. Ironically, VDBP might itself be the source of some of these differences. The fully automated methods have necessarily abandoned solvent extraction of the analyte and its chromatographic separation from potential interfering molecules. In these automated assays, 25(OH)D must be liberated from VDBP and its rebinding prevented. The assumption is that whatever approach is used (usually it involves a pH change), either 100% of the 25(OH)D is removed from the VDBP or, if not, the percentage removed is the same in all samples. Manufacturers of assay kits have been reluctant to publish data on this aspect, but a study done in 2012 (4) using the same VDBP method adopted in the Powe et al. study revealed that, for 4 commercial non-extraction methods, the inactivation of VDBP is inconsistent and 25(OH)D results are influenced by the VDBP concentration in the sample. These results have not been confirmed and, like those of Powe et al., would need to be reinvestigated when a reference method became available.

The work of Powe et al. and other researchers is advancing our knowledge of vitamin D biology and the assessment of vitamin D status. The importance of accurate methods in this fundamental research, whether for 25(OH)D, VDBP, or other analytes, cannot be overstated. The potential problems of measuring 25(OH)D parallel those experienced in direct immunoassays for other steroid hormones such as testosterone. Indeed, the limitations of direct immunoassays of sex hormones were recognized in a report commissioned by the Endocrine Society. From January 2015, the Journal of Clinical Endocrinology and Metabolism will no longer accept research manuscripts reporting studies in which sex steroid assays are important end points, unless the assays are based on mass spectrometry (5). From a scientific standpoint, it will be increasingly difficult to defend the use of nonextraction immunoassays in fundamental vitamin D research unless the accuracy and comparability of these methods can be demonstrated. Hospital laboratories will undoubtedly continue to use fully automated immunoassays for financial and logistical reasons, but as LC-MS/MS develops and we see the arrival of fully automated, compliant systems, this technology will become accessible to most medium-sized laboratories with suitably trained staff. However, robust and reliable methods for VDBP or bioavailable 25(OH)D are likely to remain elusive, with measurements confined to research laboratories for the foreseeable future. Meanwhile, when interpreting total 25(OH)D results, clinical laboratories should be mindful of possible ethnic differences in circulating VDBP. Further research is likely to lead ultimately to more meaningful measures of vitamin D status and perhaps a reassessment of the most appropriate biomarkers.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: No authors declared any potential conflicts of interest.
References