Current Controversies

Are Free Vitamin Metabolite Levels a More Accurate Assessment of Vitamin D Status than Total Levels?

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KEYWORDS

• Vitamin D • Free hormone hypothesis • Free vitamin D • Vitamin D metabolism • Pregnancy • Liver disease

KEY POINTS

- Vitamin D and its metabolites are tightly bound to serum proteins, of which the vitamin D binding protein (DBP) is the most important, such that less than 1% of the total concentration of vitamin D and its metabolites are free in the circulation.
- For most tissues, the vitamin D metabolites enter the cell as the free hormone presumably by diffusion (the free hormone hypothesis), although a few tissues such as the kidney express megalin/cubilin enabling by endocytosis vitamin D metabolites bound to DBP to enter the cell.
- Measuring the free levels of the vitamin D metabolites may provide a better measure of the true vitamin D status than measuring the total levels.
- Early methods to determine the free levels of 25-hydroxyvitamin D (25(OH)D) and 1,25 dihydroxyvitamin D ($1,25(OH)_2D$) demonstrated that the free levels were normal in patients with liver disease despite low total levels and that free levels were elevated in pregnant women in the third trimester more than would be predicted based on total levels.
- Newer methods for measuring free 25(OH)D have been developed that are easier to perform, and their widespread application should help determine the clinical value of determining free 25(OH)D in addition to and/or instead of total 25(OH)D in the evaluation of vitamin D status.

Disclosures: Dr D.D. Bikle is currently or recently funded by grants from the NIH (RO1 AR050023) and VA (IBX001066). Dr S. Malmstroem is supported by a fellowship from the Lundbeck Foundation for Clinical Research. Dr J. Schwartz has received research funds from NIA (R21 AG041660), Future Diagnostics Solutions B.V., and DiaSource and has consulted for Amgen and Pfizer.

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Endocrinol Metab Clin N Am 46 (2017) 901–918
http://dx.doi.org/10.1016/j.ecl.2017.07.013
0889-8529/17/Published by Elsevier Inc.

INTRODUCTION

Circulating levels of 25-hydroxyvitamin D (25(OH)D) are the most commonly used marker for the assessment of vitamin D nutritional status. The main reason why 25(OH)D levels are used to assess vitamin D nutritional status is because its concentration in blood is higher than all other vitamin D metabolites, making it easier to measure, and because its conversion from vitamin D is substrate dependent with minimal regulation. The liver is the major source of this conversion, performed by several enzymes with 25-hydroxylase activity, the most specific of which is CYP2R1. However, 25OHD is not the most biologically active metabolite of vitamin D. Instead, 25(OH)D must be further metabolized to 1,25 dihydroxyvitamin D ($1,25(OH)_2D$) for vitamin D to achieve its full biologic potential. $1,25(OH)_2D$ is the ligand for a nuclear transcription factor, the vitamin D receptor (VDR), that mediates the genomic and at least some of the nongenomic actions of vitamin D within the cell. Nearly all if not all cells express the VDR at some stage in their development or activation. The kidney produces most of the circulating $1,25(OH)_2D$ through the enzyme CYP27B1, but many cells also express CYP27B1 and so are able to form their own $1,25(OH)_2D$. As the appreciation that vitamin D and its metabolites affect numerous physiologic processes and not just bone and mineral metabolism, and that these physiologic processes may have different requirements for these vitamin D metabolites,[1] interest in determining optimal levels of the vitamin D metabolites to effect these different biologic processes has grown. Complicating this determination is the fact that all the vitamin D metabolites circulate in blood tightly bound to proteins, of which the vitamin D binding protein (DBP) plays the major role. For most cells, these binding proteins limit the flux of the vitamin D metabolites from blood into the cell, where they exert their biologic activity. This observation raises the issue then of what should be measured to determine vitamin D status: the total levels of these metabolites or the free levels. Before considering this subject directly, a brief review of vitamin D production and metabolism is undertaken by way of introducing the key players in the vitamin D endocrine system.

VITAMIN D PRODUCTION AND METABOLISM

Vitamin D Production

Vitamin D_3 (D_3) (cholecalciferol) is produced from 7-dehydrocholesterol in the skin through a 2-step process in which the B ring is broken by UV light (UV-B spectrum 280–320 nm), forming pre-D_3 that isomerizes to D_3 in a thermosensitive but noncatalytic process. Vitamin D is also obtained from the diet. Most foods with the exception of fatty fish contain little vitamin D unless fortified. The vitamin D in fish is D_3, whereas that used for fortification is often D_2 (ergocalciferol). D_2 is produced by UV-B irradiation of ergosterol in plants and fungi (eg, mushrooms). It differs from D_3 in having a double bond between C22 and C23 and a methyl group at C24 in the side chain. These differences from D_3 in the side chain lower its affinity for DBP, resulting in a higher ratio of free to total vitamin D metabolite concentration as well as faster clearance from the circulation and altered catabolism by the 24-hydroxyase (CYP24A1).[2–4] Moreover, several immunoassays do not recognize the D_2 metabolites as well as the D_3 metabolites, a problem addressed in later discussion. However, the biologic activity of D_2 and D_3 metabolites is comparable, and if no subscript is used, both forms are meant.

Vitamin D Metabolism

The 3 main steps in vitamin D metabolism, 25-hydroxylation, 1α-hydroxylation, and 24-hydroxylation, are all performed by cytochrome P450 mixed function oxidases (CYPs) located either in the endoplasmic reticulum (eg, CYP2R1) or in the mitochondrion (eg, CYP27A1, CYP27B1, and CYP24A1).