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Update on free vitamin D

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Abstract

The lack of standardization of measurement of vitamin D, and unlike specificities of available techniques, according to the forms of vitamin D, had led to new approaches to establishing reference values for this hormone. It was suggested that the biological activity of a given hormone is dependent more at its free form rather than a bound form to serum proteins, what is known as "Free hormone hypothesis". The importance of the free form of vitamin D is demonstrated when it was observed that the concentration of free 25-hydroxyvitamin D (free 25 (OH)D) was similar among African Americans and whites, despite lower levels of total 25 (OH) D among Blacks. What gave the idea that free 25 (OH) D is a best marker of vitamin D status? This work is to put together the current literature data on free vitamin D, and try to find an answer to the question: Does the free vitamin D is a best marker of vitamin D status?

Keywords: Free vitamin D;25-Hydroxyvitamin D; Dosage; Status.

Introduction

Vitamin D plays an important role in bone health and growth. In addition to its classic effects on phosphocalcic metabolism, Vitamin D has increasingly known effects on other functions of the body. Vitamin D is essential for the calcium homeostasis and bone health, but also of importance for the immune function, muscles and the cardiovascular system. In humans, vitamin D is found in two forms, vitamin D3 or cholecalciferol, of animal origin, and vitamin D2 or ergocalciferol of plant origin. There are rare dietary sources of vitamin D3, especially marine fatty fish, Supplementation with vitamin D3 or vitamin D2. The skin can synthesize vitamin D3, from 7-dehydrocholesterol, under the action of UVB radiation which represents the main natural source of vitamin D (Holick et al., 2008; Adams et al., 2010; Souberbielle, 2013). Moreover, there is a possible association of 25(OH)D with certain types of cancer, infections, autoimmune diseases, cardiovascular disease, urinary stones, and hypercalciuria urolithiasis (Hu et al., 2017; Papadakiset al., 2017). The 25(OH)D levels vary with age, obesity, skin type, ethnicity, season, liver and kidney disease, medication, nutritional habits and others (Papadakis et al., 2017).

Vitamin D deficiency has been defined as a 25(OH)D of less than 20 ng/ml, and vitamin D insufficiency as a 25(OH)D of 21–29 ng/ml. It has been estimated that 20 –100% of U.S., Canadian, and European elderly men and women still living in the community are vitamin D deficient. Children and young and middle-aged adults are at equally high risk for vitamin D deficiency and insufficiency worldwide. Vitamin D deficiency is common in Australia, the Middle East, India, Africa, and South America (Holick et al., 2011). It was

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suggested that the biological activity of a given hormone is dependent more at its free form rather than a bound form to serum proteins, what is known as "Free hormone hypothesis". The importance of the free form of vitamin D is demonstrated when it was observed that the concentration of free 25-hydroxyvitamin D (free 25 (OH)D) was similar among African Americans and whites, despite lower levels of total 25 (OH) D among Blacks (Powe et al., 2013).

Physiology

Which is synthesized in the skin (vitamin D3) or absorbed in the small intestine by the chylomicrons (vitamins D2 and D3), The vitamin D is transported in the blood by a carrier protein, « Vitamin D-Binding Protein » (DBP), up to liver, where it is hydroxylated to form the 25-hydroxy-vitamin D (25(OH)D). This hepatic hydroxylation is very poorly regulated. The 25 (OH) D is hydroxylated a second time at the renal proximal tubule (Or in many other tissues) To make 1,25-dihydroxy-vitamin D (1,25(OH)2D); which represents the active metabolite of vitamin D (Figure1). Renal hydroxylation, which is regulated by phospho-calcium metabolism hormones such as parathormone (PTH) or the « Fibroblast growth factor 23 » (FGF23), allow production of 1,25 (OH) 2D. Whereas hydroxylation at the peripheral level is independent of phosphocalcium regulation and produces 1,25 (OH) 2D which acts locally "intracrin" and does not participate in phosphocalcium metabolism. The serum concentration of 25 (OH) D (Approximately 1000 times more than the serum concentration of 1,25 (OH) 2D) represents the vitamin status of an individual. Although 1,25 (OH) 2D is the active form of vitamin D (Souberbielle, 2013; Beaudart et al., 2014; Darling et al., 2014).

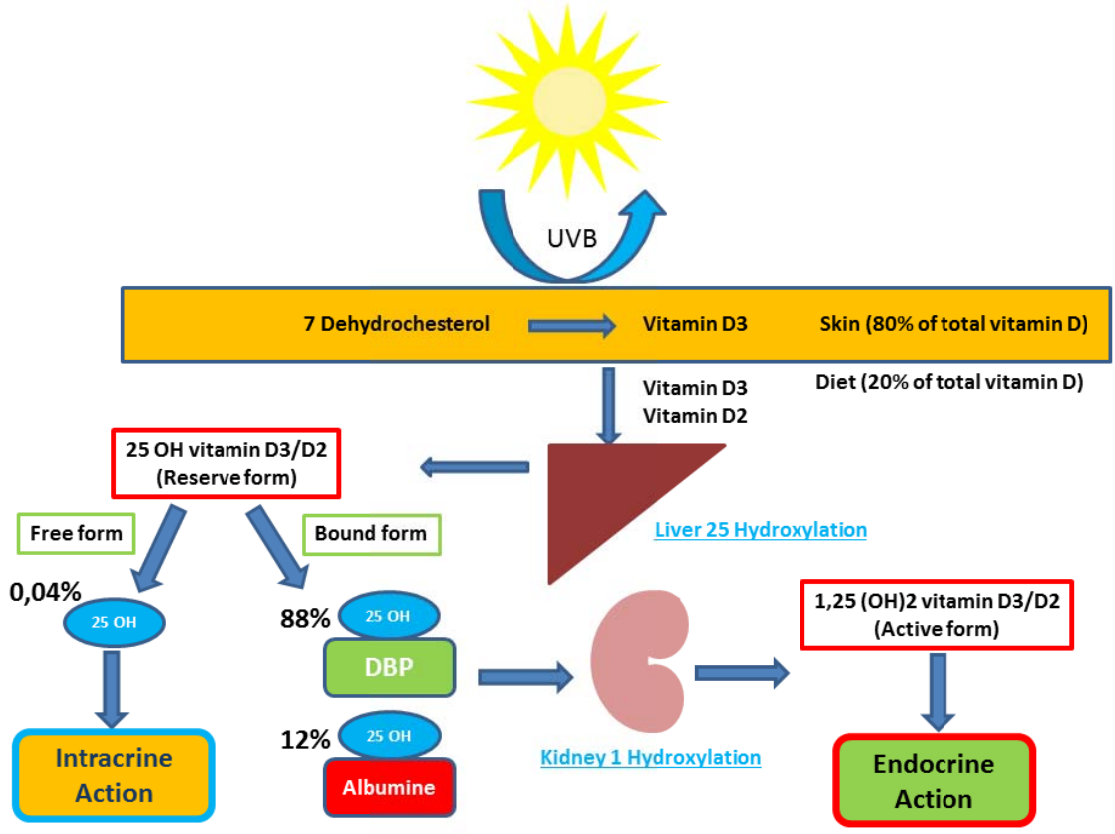

Figure 1. Metabolism of vitamin D.

Issues in the status of vitamin D

The status of vitamin D should reflect the body's vitamin content and the amount available for cellular use. The problem of the (OH) D reference values is just as important as the technical problems. Usually, the reference values of a biological parameter are based on its measurement in a large number of healthy subjects. This definition is not suitable for vitamin D because of the intervention of several factors such as season, latitude, altitude, skin pigmentation and age; Which can influence the concentrations of 25 (OH) D. This has led to new approaches for the establishment of these reference values which is based on:

- The study of the relationship between serum concentrations of 25 (OH) D and PTH.
- The study of concentrations of 25 (OH) D for which intestinal absorption of calcium is optimal.
- The study of the relationship between 25 (OH) D concentrations and the frequency of some diseases.

The study of mean concentrations of 25 (OH) D reached in intervention studies that showed positive effects of vitamin D (Cormier et al., 2010; Souberbielle, 2013).

Vitamin D-Binding Protein (DBP)

Most circulating 25(OH)D is bound either to vitamin D binding protein (DBP) (88%) or to albumin (12%) and only a small fraction, less than 1%, circulates in a free un-bound form . The albumin bound fraction of 25(OH)D plus free fraction has therefore been referred to as the bioavailable fraction of 25(OH)D (GöranOleröd et al, 2017). The 25 (OH) D is transported into the bloodstream by « Vitamin D-Binding Protein » (DBP), also called « group-specific component » (Gc-globulin). It ensures their solubilisation, their transport and their bioavailability for the target organs. DBP is synthesized by the liver with a high plasma concentration (400 mg/L; 4 à 8 µM). First of all DBP is a carrier protein for the circulating vitamin D metabolites, but other roles for the protein have been described, such as the binding of fatty acids and endotoxins, chemotactic effects on neutrophil granulocytes, activating of macrophages and the sequestration of actin upon tissue damage. It is coded by a unique gene located on the chromosome 4q12-q13. It is a serum polymorphic protein (More than 120 variants detected in humans) of approximately 52-58 kDa comprising 458 amino acids, whose including 28 cysteines, all forming disulfide bridges. It is part of the same family as albumin (ALB) and fetoprotein (AFP) with which it has a strong homology of primary structure. It consists of three independent domains of similar structures: 1-191, 192-378 and 379-458. Like other proteins in this family, DBP has two binding sites, one for vitamin D in domain I and one for actin in domain III (White et al., 2000; Hazell et al., 2015; Sollid et al., 2016; Oleröd et al., 2017).

DBP binds 25 (OH) D and also 1, 25 (OH) 2D. It binds the following derivatives of vitamin D in order of decreasing affinity: 25-hydroxy-vitamin D3 = 24,25-dihydroxy-vitamin D3 = 25,26 dihydroxy-vitamin D3> 1,25-dihydroxy-vitamin D3> vitamin D3. It circulates in plasma at concentrations 20 times the concentration of all metabolites of vitamin D. The DBP binds 88% of (OH) D with a high affinity constant ($Ka = 5 \times 10^{8}$ M) and 85% of the 1.25 (OH) 2D with an affinity constant 10 times lower ($Ka = 4 \times 10^{7}$ M). Only 0.40% of the vitamin D metabolites are in free form. The rest is linked to other serum proteins such as albumin and lipoproteins (White et al., 2000; Hazell et al., 2015; Sollid et al., 2016; Oleröd et al., 2017).

Several physiological and pathological conditions may have an

influence on DBP levels: Genotypic variations of DBP initially called Gc1F, Gc1S and GC2 may be associated with changes in affinity or serum DBP concentration, phenotypic variations in sequence of DBP amino acids are distinguished by simple nucleotide polymorphisms (SNPs) of rs7041 and rs4588. Blacks and Asians are more likely to carry DBP / Gc1F, which has a higher affinity for 25 (OH) D and associated with low DBP levels. Whites are more likely to carry DBP / GC1S, GC2, which has a lower affinity for 25 (OH) D and associated with high levels of DBP (Yousefzadeh et al., 2014).

Age seems to have an impact on DBP concentration, vitamin D deficiency is common in the elderly but no association is demonstrated. It was found that women had higher DBP levels than men, but also higher levels during pregnancy, which may be due to the effects of estrogens on BPD. Some diseases such as liver disease, kidney disease and diabetes may be associated with a decrease in DBP level (Yousefzadeh et al., 2014).

The Free hormone hypothesis

The Free hormone hypothesis indicates that the biological activity of a given hormone is dominated by its free form rather than its concentration related to serum proteins. The free form of the hormone can diffuse freely through the plasma membrane of the target cell and bind to intra-cytoplasmic or intranuclear receptors. There are many examples to support the idea that the free (or bioavailable) form of hormones is physiologically more relevant than its total concentration, taking the example of using thyroxine T4 measurements and free triiodothyronine T3 Rather than the total concentrations for the diagnosis of thyroid disorders, the same finding for testosterone, and cortisol (Chun et *al*., 2014) (Lai et al., 2014).

Indeed, renal tubular cells release 25 (OH) D by its serous membrane, but also 25 (OH) D linked to DBP, filtered in the glomerulus, with the aid of the megalin-cubulin receptor expressed on the luminal site. Megalin is also expressed in the lungs, thyroid, mammary gland, gall bladder and thyroid, while most other cells are not or minimally express such absorption mechanism, and therefore will depend on 25 (OH) D as a substrate for the local production and paracrine action of 1,25 (OH) 2D (Marzolo et al., 2011; Aloia et al., 2015).

In addition, DBP appears to slow down the action of vitamin D in the intestine and reduces liver consumption; DBP has been shown to decrease the action of vitamin D on target tissues such as monocytes and keratinocytes (White et al., 2000). These results supported the application of the "free hormone hypothesis" on vitamin D.

Is free vitamin D a good marker for vitamin D status?

It is unclear whether free or bioavailable 25 (OH) D is a better marker of vitamin D status than total (OH) D, the bioavailable (OH) D is the sum of free (OH) D and (OH) D weakly bound to albumin, which represents a very small fraction of the total concentration.

The study of (Powe et al., 2013) was found that the concentration of free 25 (OH) D in Americans of African origin was similar to that of white Americans, although their total 25OHD concentrations were much lower. It was also found that the concentration of DBP was lower in Blacks compared to American whites. The DBP measurement was based on an ELISA using monoclonal antibodies. This difference in DBP concentration was due to genetic variation in the DBP (protein / GC) structures. Indeed, most African Americans express DBP / Gc1F, while most Whites produce DBP / Gc1S or DBP

/ GC2. These proteins differ by one or two amino acids (Bouillon, 2011). Therefore the authors suggested that free 25 (OH) D is the good marker for the status of vitamin D. Several other publications using the same methodology for DBP measurements confirmed a much lower concentration of DBP in African Americans (Bouillon et al., 2014).

In a study of Denburg et al. (2016), it has been found that serum DBP in African Americans is approximately 50% lower than in Whites American with chronic renal insufficiency, using monoclonal assay, but DBP did not differ not between races when measured by polyclonal antibody assay or mass spectrometry. They also confirmed that the monoclonal DBP assay poorly detects DBP / Gc1F. While, polyclonal immunoassays or mass spectrometry do not detect a large racial or genetic difference. Mass spectrometry allows measuring the peptides of the DBP region common to all the genetic variants separately from the specific sequences of the genotype (Denburg et al., 2016). On the basis of this technique, other studies confirmed the absence of racial or genetic differences in the concentration of DBP (Yousefzadeh et al., 2014; Srikanth et al., 2016). Thus the DBP assay by monoclonal antibodies shows a bias in the measurement of DBP / Gc1F, and cannot be considered as a reliable method in the genetic mix of the population. Polyclonal antibodies do not exhibit this bias, but the absolute concentration of DBP differs widely according to the assay technique.

The study of Sollid et al. (2016) found that the concentration of DBP, as measured by polyclonal antibodies, was significantly lower in the Gc2 / Gc2 DBP phenotype than in other phenotypes. So no final response on the racial or genetic differences of measured free (OH) D.

The status of vitamin D is closely related to PTH, calcium, and bone mineral density (BMD). It was found that the relationship of free 25 (OH) D measured with calcium and PTH is more significant than the relationship of total 25 (OH) D with these two biomarkers (Schwartz et al., 2014). Some studies have shown a strong relationship of free (OH) D with PTH (Bhan et al., 2012; Schwartz et al., 2014) and with BMD (Powe et al., 2011; Srikanth et al., 2016), while other studies did not find this correlation either with PTH (Dastani et al., 2014; Johnsen et al., 2014; Sollid et al., 2016) or with the BMD (Jemielita et al., 2016). So the data is contradictory. Most studies indicating a better correlation of free 25 (OH) D with biomarkers (PTH, DMO) are based on DBP measurements by monoclonal antibodies (Bouillon et al., 2016).

Comparison between measured and calculated free vitamin D

To calculate the free concentrations of vitamin D metabolites, a valid estimate of the affinity of these DBP ligands at 37 ° C is required; In a study (Ying et *al*., 2015), calculated free 25 (OH) D, calculated bioavailable 25 (OH) D and directly measured 25 (OH) D did not show a significant difference between the different DBP phenotypes. Free 25 (OH) D and free 1,25 (OH) 2D are more difficult to measure because their concentrations are much lower. In the absence of standardization of 25 (OH) D assays, most estimates of free 25 (OH) D or free 1,25 (OH) 2D are calculated using formulas using DBP, albumin, and of the affinity constants of these proteins for 25 (OH) D (Denburg et al., 2016), but which can be influenced by the phenotypic difference of DBP (Sollid et al., 2016).

In principle, measured and calculated free vitamin D should provide similar data, but this is not always the case in reality. In study (Sollid et al., 2016), it was found that the calculated free 25 (OH) D dosage was overestimated with respect to the direct assay; contrariwise the absolute values of free 25 (OH) D were

about twice higher in the direct assay than the calculated values (Nielson et al., 2016). In Oleröd et al. (2017) study, directly measured free 25(OH)D in serum was strongly correlated with total 25(OH)D and followed the same seasonal variation, whereas the serum concentrations of DBP and albumin were stable during the year. The variation in directly measured free 25(OH)D was however damped with an increase in the percentage of free 25(OH)D in situations of vitamin D deficiency and a decrease in vitamin D abundance. The present data are confused by the differences in the analytical techniques.

For both sexes, the calculated free 25 (OH) D and calculated bioavailable 25 (OH) D showed a decreased difference, for the directly measured free 25 (OH) D, concentrations were equal in both sexes (Sollid et al., 2016). In the study of Schwartz et al. (2014), the measured free 25 (OH) D exhibited an inversely significant correlation with PTH but not for the calculated free 25 (OH) D. In the same study (Schwartz et al., 2014) a significant association was found between the free 25 (OH) D measured with calcium, but this relationship was found with calculated free 25 (OH) D. In Oleröd et al. (2017) study, it is reported that both directly measured and total 25(OH)D were negatively correlated with serum PTH, but calculated free 25(OH)D was only weakly associated with PTH. Therefore, these results favor the use of measured free 25 (OH) D over calculated free 25 (OH) D using the current techniques and equations.

In the situation of the differentiation of DBP phenotypes, as in the case of Whites and Blacks Americans, or under conditions associated with a low concentration of DBP such as cirrhosis of the liver or nephrotic syndrome, or in cases associated with Concentrations of DBP such as pregnancy and estrogen therapy, direct measurement of serum free 25 (OH) D should be recommended (Nielson et al., 2016).

Remarks and suggestions

The evaluation of the status of vitamin D still presents a problem, concerning markers and serum thresholds for evaluation, especially in the racial heterogeneity of studied populations. The free 25 (OH) D have been proposed to offer additional or better measures of vitamin D status. It has received considerable interest, particularly in the exploration of racial disparities in the association of 25OHD with diverse health effects. A problematic with the calculation of the free 25 (OH) D concentration is that the DBP levels are not constant, but can vary in different physiological and pathological conditions such as pregnancy, liver cirrhosis, kidney disease and malnutrition. The levels of DBP also vary among different populations depending on polymorphism in the expression of the three different DBP isotypes. The confused results found in different studies can be explained by the technical methodology in the monoclonal ELISA that offers distinct differences in DBP concentrations between races and GC-genotypes. These racial differences were not present with other methods.

Consequently, pending resolution of the vitamin D status issue, total 25 (OH) D can be used in the general population as a marker of vitamin D status, irrespective of race or DBP genotype.

Conclusion

These contradictions in these measurements of free vitamin D between the different studies make it difficult to answer the interest of this metabolite. The racial differences in DBP concentration are probable due to monoclonal assay bias, as there was no racial difference in DBP concentration by other methods. This

confirms the utility of total 25 (OH) D in measuring vitamin D in the general population. The methodological problems limit comparability between studies, hence the need to carry out studies on the methodology of the dosage to ensure its standardization.

Disclosure of interest

The authors declare that they have no competing interest.

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