INVITED COMMENTARY

# Vitamin D and the Free Hormone Hypothesis

**Sushmita Banerjee,[1,2] Rajiv Sinha[1*]**

[1]*Division of Pediatric Nephrology, Institute of Child Health, Kolkata, West Bengal, India*
[2]*Department of Pediatrics, Calcutta Medical Research Institute, Kolkata, West Bengal, India*
**rajivsinha_in@yahoo.com*

Vitamin D has been shown to have multiple utilities including a very important role in bone metabolism. Serum 25-hydroxy-cholecalciferol or 25(OH)D level is commonly measured to assess the vitamin D status. Multiple reports from cohorts with nephrotic syndrome (NS) have documented very low levels of 25(OH)D [1]. This is not surprising as 85-90% of circulating 25(OH)D is tightly bound to vitamin D binding protein (VDBP) while 10-15% is bound to albumin albeit more loosely, and both of these fractions are lost in urine during episodes of massive proteinuria [1,2]. Surprisingly however, despite such low 25(OH)D levels, the majority of NS patients do not have biochemical abnormalities linked with significant hypovitaminosis D and usually have normal 'corrected' calcium, phosphate, alkaline phosphatase and parathyroid hormone (PTH) levels [3,4]. This phenomenon may be explained by the "free hormone hypothesis" which states that where hormones are extensively protein bound, it is the free circulating fraction and not the protein bound fraction that enters cells and exerts biological effects. A similar phenomenon has been demonstrated with thyroid, cortisol and sex hormones [5].

Osteoporosis in NS has been ascribed to steroid exposure, and appears to occur maximally when high doses of steroids are given on a daily or prolonged basis. While a few studies have shown improved bone health with vitamin D supplementation other studies have not confirmed this effect [4]. Indeed, a randomized controlled trial (RCT) failed to show any significant change in bone mineral density (BMD) over 6 months of calcium and vitamin D supplementation in steroid sensitive NS, despite significant increases in 25(OH)D levels in the supplemented group [4]. This suggests that serum 25(OH)D levels may not be the ideal target for determining the need for supplementation of vitamin D in proteinuric states. The levels of free and bioavailable 25(OH)D may be more relevant to assess the biologically active fraction and guide therapeutic decisions in the presence of hypoproteinemia.

It is to be noted that there is clinical evidence in support of the free hormone hypothesis for vitamin D [5]. A patient with homozygous VDBP mutation, had prolonged, nearly undetectable levels of total 25(OH)D but free 25(OH)D, calcium, phosphate and PTH levels were normal. Similarly in animal studies, VDBP knockout mice did not reveal any evidence of vitamin D deficiency despite very low levels of total 25(OH)D, as long as normal daily calcium and vitamin D intake were maintained. Bioavailable 25(OH)D is likely to be the combination of free 25(OH)D [usually only 0.03 to 0.04% of the total 25(OH)D] and the albumin bound fraction. The latter, because of its lower affinity to albumin may be considered as "easily transformable into the free state", although robust evidence for this is lacking. This albumin bound fraction will be low in a hypoalbuminemic states like NS, as is the VDBP bound fraction. VDBP, having a small molecular weight, is lost in the urine in NS, and the small amount of VDBP-25(OH)D complex usually reabsorbed by the megalin cubilin pathway in proximal tubule is likely to be rapidly saturated, and insufficient to compensate for the losses occurring during heavy proteinuria [5].

Although current evidence increasingly supports the need to estimate free 25(OH)D levels in proteinuric and hypoproteinemic diseases, the methods and ability to do so remains sub-optimal [5]. Originally performed by centrifugal ultrafiltration method, the procedure has been replaced by kits based on enzyme-linked immunosorbent assay (ELISA) which are expensive, and only available as research tools till date. Mean (SD) serum concentrations obtained from healthy adults have been reported around 4.3 (1.9) pg/mL [6]. Measurement by liquid chromatography-tandem mass spectrometry from saliva samples has also been described.

To overcome the difficulties associated with direct measurement of free 25(OH)D, alternatives have also been explored. Formulae for calculating free 25(OH)D were previously used which entailed measuring serum total 25(OH)D, VDBP and albumin concentrations along with

experimentally derived affinity constants. However current research indicates that because of the poly-morphism of VDBP gene, the binding capacity may be different between races and VDBP levels can vary with disease and type of assay used [5]. This could to some extent explain the significant difference, which has been noted between calculated measurements when compared to direct assays, in different ethnic groups and disease states in both children and adults [7,8]. Thus currently direct assays are recommended for estimating free 25(OH)D concentrations and for calculating bioavailable levels.

Keeping the need for further study on the "free hormone" hypothesis the current study by Sai Charan et al is relevant [9]. It examines free and bioavailable 25(OH)D levels in patients with first episode of NS, at onset and at 4 weeks of standard steroid therapy, and compares them with healthy controls. Instead of actually estimating the free 25(OH)D serum concentration, the authors calculated free and bioavailable 25(OH)D levels, by using an adapted formula, originally used for free testosterone calculation. As mentioned earlier there are limitations of calculated vs directly measured levels of free 25(OH)D and this has been well described in several papers [5-7]. It is noted in the index study that even healthy controls had significantly lower levels of free 25(OH)D (0.62 pg/mL, IQR 0.5, 0.7) compared to previous reports [6,8]. The finding of low calculated free 25(OH)D levels in pediatric NS contrasts with another Indian study in which the concentrations measured by direct assay, were similar in patients and controls, despite significantly lower levels of total 25(OH)D in the NS patient group [10]. A definite possibility is that these differences may be because of the difference in the estimation method. Unfortunately, we were unable to find any study for comparison, which calculated bioavailable 25(OH)D from *directly measured* concentrations of free 25(OH)D and albumin in kidney diseases.

Given all the current evidence available, we completely agree with Sai Charan et al, that free and possibly also bioavailable 25(OH)D concentrations may be better markers for therapeutically significant hypovitaminosis D in proteinuric kidney diseases. There is a need to develop economical, freely available kits for direct assay of free 25(OH)D and further studies to validate their reproducibility in kidney diseases, correlation with bone health, and to ascertain the benefits of supplementation based on free 25(OH)D targets.

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