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Letters

Problems with direct 25-Hydroxyvitamin D assays, and the target amount of vitamin D nutrition desirable for patients with osteoporosis

SIR—On the surface, we seem to have reached the end of what we need to know about vitamin D nutrition and osteoporosis. Based on the relationship between circulating parathyroid hormone (PTH) and 25-hydroxyvitamin D (25(OH)D) levels, there is now a consensus that serum 25(OH)D levels <100 nmol/l are 'desirable' [1–4]. As further support for this, one might note that consumption of approximately 20 μg/day of vitamin D_3 (700–900 IU/day) lowered fracture risk, and that this intake resulted in average 25(OH)D levels over 100 nmol/l [5,6]. McKenna and Freaney [4] and Kanis et al. [7] have both indicated that the desirable target for 25(OH)D is achievable with sunshine and the currently recommended intakes of vitamin D.

Look deeper, and the reports of Chapuy et al. [5] and Dawson-Hughes et al. [6] become an anomaly in terms of the serum 25(OH)D response. If I add newly published data [8,9] to what was summarized previously [10], there are at least 25 studies involving prolonged vitamin D intakes of 20–100 μg/day. Only two of the 25 reports average 25(OH)D levels higher than 100 nmol/l: Chapuy et al. [5] and Dawson-Hughes et al. [6]. What makes those studies different in terms of serum 25(OH)D levels?

The explanation for the anomaly became obvious upon reading the recent report by Lips et al. [11]. This compares 25(OH)D results for five facilities which have produced some of the most important work pertaining to vitamin D nutrition and osteoporosis. These laboratories used 'direct' measures of 25(OH)D. Four of the laboratories diluted the sample into ethanol, centrifuged and assayed directly with a competitive protein binding assay (CPBA), presumably with rat serum as the binding agent. Lips used the DiaSorin radioimmunoassay (RIA) for 25(OH)D, which binds with an antibody specific for the 25-hydroxyl-containing sidechain.

Based on the data in fig. 1 of Lips et al. [11], results obtained by the RIA are lower than direct extraction/CPBA by a ratio of 0.69. If I use this as a correction factor, it turns out that with a vitamin D intake of 20 μg/day, Chapuy et al. [5] achieved average serum 25(OH)D levels of 73 nmol/l, while subjects in the study of Dawson-Hughes et al. [6] achieved average levels of 77 nmol/l. These results are still relatively high, but now they fit the rest of the literature on the topic [10].

Direct CPBA is well known to produce spuriously high 25(OH)D results compared with either RIA or methods using chromatographic purification before doing the CPBA [12,13]. Modification of the direct CPBA method by altering the calibration procedure as suggested by Lips et al. [11], or by ultracentrifugation to remove lipoproteins, has been tried, and the approaches do not work [13]. As further support for the overestimation with direct CPBA three laboratories used direct CPBA in the latest DEQUAS proficiency survey: their average 25(OH)D results were 24% higher than those of the 49 other laboratories that used other assay methods [14].

The direct CPBA method is the least costly way to measure 25(OH)D and it does show a treatment response for larger studies in which 25(OH)D is not a primary outcome measure. One can understand the use of a minimalist assay for a secondary outcome measure. Investigators must direct resources toward their primary goal. However, because of the methodologic shortcomings relating to 25(OH)D, the studies of Chapuy et al. [5] and Dawson-Hughes et al. [6] cannot stand as valid evidence that 20 μg/day of vitamin D ensures the 'desirable' target of 25(OH)D >100 nmol/l.

The analytical issues addressed here lead to a re-emphasis of the concept that 20 μg/day (800 IU/day) of vitamin D may be suboptimal. If >100 nmol/l is desirable, it remains so only in theory. Practical evidence of desirability does not exist in terms of bone density preservation or fracture prevention. Vitamin D has not been studied in the way we expect any other treatment for osteoporosis to be. To do such trials properly, we must stop thinking that 20 μg/day (2000 IU/day) is the safety limit for vitamin D. Higher levels are needed to attain 25(OH)D >100 nmol/l, and they could turn out to be optimal for osteoporosis.

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Response to Dr Vieth

SIR—The letter by Dr Vieth points to two important issues: the 'desirable' serum 25-hydroxyvitamin D (25(OH)D) concentration and the shortcomings of the current assays for serum 25(OH)D. It is premature to conclude from the cited literature that there is a consensus that serum 25(OH)D should be more than 100 nmol/l. The cited studies suggest that serum 25(OH)D should probably be higher than previously indicated. Most authors suggest that serum 25(OH)D should be somewhere between 50 and 90 nmol/l [1–3].

When considering current routine assays for 25(OH)D, serum 25(OH)D may differ by 20–40% or more according to the chosen assay. As long as such variations are common, it is difficult to reach consensus on desired serum 25(OH)D concentrations and to develop guidelines on preventive treatment of vitamin D deficiency. Therefore it is still not possible to recommend either 10–20 μg vitamin D_3 per day (400–800 IU/day) or more and to specify to which populations it should be given. Standardization of assays and consensus development are necessary steps for effective prevention of vitamin D deficiency.

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Response to Dr Vieth

SIR—In our own studies, we have always used either the Haddad method of separation and chromatography prior to assay, or more recently a radioimmunoassay using an antibody specific for the 25(OH)D-containing side chain. We would agree with Dr Vieth that the mean level of serum 25(OH)D achieved by oral supplementation with 20 μg of vitamin D_3 is about 75–80 nmol/l [1]. Given the primacy of vitamin D supplementation over exposure to natural sunlight in preventing hypovitaminosis D, we favor policies that augment oral intake by fortification of milk [2] and by low-dose supplementation up to 20 μg daily for housebound individuals. Conceptually, the threshold for defining hypovitaminosis D should be considered the boundary between physiological need – that is achieved safely by continuous low-dose or intermittent high-dose supplementation in the elderly – and pharmacological intervention that would be the aim of higher doses of vitamin D or vitamin D analogues [3]. We consider that it is important to make such a distinction because recommendations for improving vitamin D status must be safe as well as efficacious.

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