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Guest Editorial
Difficulties with vitamin D nutrition research: objective targets of adequacy, and assays for 25-hydroxyvitamin D

R Vieth[1]* and G Carter[2†]

[1]*Department of Laboratory Medicine and Pathobiology, University of Toronto, and Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Canada; and* [2]*Endocrine Laboratory, Charing Cross Hospital, London, UK*

Introduction

With the latest revision of North American nutritional guidelines, a record was probably set for an increase in a nutritional recommendation. For the elderly, the recommended vitamin D intake was tripled to 15 μg/day (600 IU/day) (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997; Heaney, 2000). Despite this, adult requirements for vitamin D continue to be the subject of controversy (Heaney, 2000; Vieth, 1999). There is a profound need for appropriate evidence about vitamin D nutritional needs in adults.

Circulating 25-hydroxyvitamin D (25(OH)D) has become the primary indicator of vitamin D adequacy (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1977; Heaney, 2000). Furthermore, since serum parathyroid hormone (PTH) correlates inversely with 25(OH)D, the problem of a desirable target involves the partial suppression of circulating PTH (Heaney, 2000; Chapuy *et al*, 1997).

Recent issues of this journal have seen two articles highlighting the nutritional need for vitamin D in adults (Schaafsma *et al*, 2000; Morabia *et al*, 2000). This editorial is a critical commentary with suggested strategies to make publications relating to vitamin D requirements of practical benefit to others.

Basic principle: ensure adequacy of the nutrient for practically all healthy persons

The working definition of recommended dietary allowances has been 'levels of intake of essential nutrients considered . . . to be adequate to meet the known nutritional needs of practically all healthy persons', (Yates, 1998). With the words, 'practically all healthy persons', the focus is on the extremes of nutritional requirement. By definition, if only average needs are ensured then half of the population may have a nutritional insufficiency. Unfortunately, most dietary surveys that report serum 25(OH)D simply report mean ± s.d., not the ensured concentration. Protection against deficiency is an entirely different issue from whether a dose changes the mean 25(OH)D level. That is, the key statistical question should be one that compares prevalence of insufficiency, not simply whether mean 25(OH)D is affected. There are examples of this more useful way to present the data. One recent report in this journal, by Lehtonen-Veromaa *et al* (1999), casts serious doubt on the value the vitamin D intake currently recommended beyond childhood. They asked whether 10 μg/day vitamin D given to 9 to 15-y-old girls would prevent them from developing 25(OH)D concentrations <37.5 nmol/l during winter. Their intervention study showed no preventive effect. Similar findings were obtained in a cross-sectional study by Glerup *et al* (2000) showing that immigrant women taking 5 to 15 μg/day vitamin D were not prevented from having serum 25(OH)D <40 nmol/l. Each study specified a 25(OH)D target, and the study outcome was a classification, that is, prevention of insufficiency.

Specify a suitable target for vitamin D nutritional adequacy

Circulating 25(OH)D was used by Schaafsma *et al* to determine whether the vitamin D status of women around 60 y of age could be improved if given 350–400 IU/day (8.75–10 μg/day). The study developed a major flaw when the research question focused on whether the vitamin D supplements might eliminate wintertime *percentage* declines in 25(OH)D. The reader should question why the authors expected a constant intake over the year to

*Correspondence: R Vieth, Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Ave, Toronto, Ontario, Canada M5G 1X5. E-mail: rvieth@mtsinai.on.ca
†Mr Carter is organizer of the Vitamin D External Quality Assessment Scheme (DEQAS), and can be contacted via e-mail, administrator@deqas.org
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eliminate the seasonal 25(OH)D fluctuation that will occur due to changing sun exposure. One should also question what the prevention of a seasonal decline (a percentage change) has to do with nutritional adequacy (an absolute value). Not only did Schaafsma *et al* (2000) fail to specify a target in terms of 25(OH)D concentration, they used one lab with one method for healthy subjects, and a different lab with a different method for those with osteoporosis. There was no validation that the two methods used for 25(OH)D assay could produce same result on the same sample. Consequently, the data are of marginal use to the international community.

Vitamin D intake at an established and appropriate level could be a meaningful target. The recent dietary survey of Morabia *et al* (2000) failed to specify an intake that they would have considered adequate. Morabia *et al* simply concluded that heavy smokers were more prone to vitamin D deficiency because their mean vitamin D intake of 1.92 μg/day was statistically less than the mean intake of 2.39 μg/day for non-smokers. We doubt anyone has ever shown that 5.0 μg/day of vitamin D has a detectable effect on mean serum 25(OH)D,[4] let alone prevent insufficiency. Therefore, it is far-fetched to imagine that the difference between 1.92 and 2.39 μg/day has any practical implication, even if the tiny difference between these numbers is statistically significant.

Investigators must avoid the ambiguities that result from an unstated nutritional target. Depending on the kind of study, the target could be circulating 25(OH)D or vitamin D intake. In the statistical analysis, authors should consider whether comparisons among group means (parametric statistics), or of prevalence in a defined group category (non-parametric statistics), or both, are most relevant to readers.

How to measure 25(OH)D, and make the results meaningful to others

The Vitamin D External Quality Assessment Scheme (DEQAS) has been monitoring performance of 25(OH)D assays since 1990. Over 75 laboratories in 13 countries take part. Agreement among 25OHD assays is assessed four times a year, from sets of five samples distributed to each participant. Inaccuracies can arise because all vitamin D assay methods require some form of extraction or purification, the 25(OH)D is poorly soluble in the assay reagent, or the calibrator can be wrong. Lastly, these methods are faced with an unusual expectation, to detect two different compounds, 25(OH)D2 and 25(OH)D3, simultaneously and identically. Obviously, 25(OH)D assays are challenging laboratory procedures.

With the DEQAS survey, we now have a tool by which any author can make data for 25(OH)D useful internationally. Participation either reassures us of the validity of results, or it highlights technical issues that need to be addressed before publication. In fact, it was poor performance in proficiency surveys like DEQAS that resulted in discontinuation of the inferior methodology of 'direct' 25(OH)D assays (competitive binding but without chromatography to purify the sample; Preece *et al*, 1975; Vieth, 2000 and removal from the market of 25(OH)D kits by at least one well respected manufacturer. The editor of this journal asked us to recommend appropriate methods for 25(OH)D assay. Since the technology continues to evolve, there is no way to provide a simple answer, other than to re-emphasize that, whatever the method, results must be validated against those of other laboratories.

Closing comments

A recommended intake for vitamin D must ensure the needs of the most nutritionally needy of healthy adults—those without sun exposure. The statistical expedient that a nutritional intake 2 s.d. above the average requirement will ensure adequacy for 'practically all healthy adults' (Yates, 1998) is not applicable to vitamin D because it is the one 'nutrient' acquired through non-dietary means. Therefore, a non-parametric approach would be more appropriate for presenting the 25(OH)D levels attained with specific vitamin D intakes. The question we need to answer is, 'How much vitamin D must a healthy adult consume to be ensured of having at least a specific, target concentration of serum 25(OH)D?'

References

Chapuy MC, Preziosi P, Maamer M *et al* (1997): Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporosis Int.* **7**, 439–443.

Glerup H, Mikkelsen K, Poulsen L *et al* (2000): Hypovitaminosis D myopathy without biochemical signs of osteomalacic bone involvement. *Calcif. Tissue Int.* **66**, 419–424.

Heaney RP (2000): Vitamin D: how much do we need, and how much is too much? *Osteoporosis Int.* **11**, 553–555.

Lehtonen-Veromaa M, Mottonen T, Irjala K *et al* (1999): Vitamin D intake is low and hypovitaminosis D common in healthy 9- to 15-year-old Finnish girls. *Eur. J. Clin. Nutr.* **53**, 746–751.

Morabia A, Bernstein MS & Antonini S (2000): Smoking, dietary calcium and vitamin D deficiency in women: a population-based study. *Eur. J. Clin. Nutr.* **54**, 684–689.

Preece MA, Tomlinson S, Ribot CA *et al* (1975): Studies of vitamin D deficiency in man. *Q. J. Med.* **44**, 575–589.

Schaafsma A, Muskiet FA, Storm H, Hofstede GJ, Pakan I & der Veer EV (2000): Vitamin D3 and vitamin K1 supplementation of Dutch postmenopausal women with normal and low bone mineral densities: effects on serum 25-hydroxyvitamin D and carboxylated osteocalcin. *Eur. J. Clin. Nutr.* **54**, 626–631.

Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (1997): *Dietary Reference Intakes: Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. National Academy Press.

Vieth R (1999): Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am. J. Clin. Nutr.* **69**, 842–856.

Vieth R (2000): Problems with direct 25-hydroxyvitamin D assays and the target amount of vitamin D nutrition desirable for patients with osteoporosis. *Osteoporosis Int.* **11**, 635–636.

Yates AA (1998): Process and development of dietary reference intakes: basis, need, and application of recommended dietary allowances. *Nutr. Rev.* **56**, S5–S9.