2003 W. O. Atwater Memorial Lecture: Defining Nutrient Requirements from a Perspective of Bone-Related Nutrients

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The U.S. Department of Agriculture, Agricultural Research Service has a valued tradition of sponsoring an annual lecture in honor of W. O. Atwater. I will always treasure the wonderful experience of sharing my thoughts on nutrient requirements and the lively reception that followed with so many supportive colleagues, students and friends at the annual meeting of the American Society for Nutritional Sciences at Experimental Biology. Atwater studied food composition, nutrient availability from the diet, nutrient intake of American families and nutrient requirements. It is not surprising then that related topics are often the focus of Atwater lectures. Elsie Widdowson claimed in her Atwater lecture that “Atwater contributed more to our knowledge about the energy value of human foods than anyone who has ever lived, either before or since his time” (1). The most recently published Atwater lecture by Vernon Young advanced Atwater’s legacy by reflecting on the role that the sequencing of the human genome would have on determining human nutrient requirements (2).

My participation on the Dietary Reference Intake (DRI) Panel for calcium and bone-related nutrients (3) acquainted me with the process of weighing the science to set nutrient requirements and Upper Levels (UL). The tremendous amount of information that had accumulated on calcium requirements was gathered in an NIH Consensus Conference on Defining Optimal Calcium Intakes (4), which became one of the motivating factors for embarking on the new iteration of nutrient requirements, the DRI, which were released in 1997 for calcium, vitamin D, phosphorus, magnesium and fluoride. The Atwater Lecture gave me the opportunity to tell the story of my own research contributions to the increased body of literature available for establishing calcium requirements in adolescents and screening most of the calcium sources in the human diet for bioavailability with my long time co-investigators, Robert Heaney, Munro Peacock, George McCabe, Meryl Wastney and my capable research group. The volume of science generated on vitamin D since 1997 is calling into question both the Adequate Intakes (AI) and UL our panel set for vitamin D. The Atwater Lecture was a chance to review the science, discuss the remaining gaps, and speculate that this nutrient will be a driving force for the next iteration of setting nutrient requirements. Finally, the Atwater Lecture was an opportunity to express the importance of considering the inter- action of nutrients on defining nutrient requirements. The interdependency of calcium and vitamin D requirements provides a classic example.

Defining requirements and upper levels

The most important data to have in setting nutrient requirements concern the relationship of intake to a functional measure of inadequacy or to optimal health and reduced risk of disease. It is also important to know the variation in response to nutrient intake and to understand what population characteristics alter the relationships. Of course, this information is often inadequate because we rarely have functional outcome measures that are completely satisfactory. Therefore, we often set requirements based on intakes that relate to nutrient stores, although nutrient stores are typically not functional themselves. Alternatively, we use a factorial approach to replace losses modified by absorption and accretion rather than optimal status. Sometimes we lack the data even to use indirect biomarkers or to calculate absorption or need for growth for the factorial approach and are left to adopt usual intakes of a healthy population. Adopting usual intakes of our population is inappropriate for calcium and vitamin D given the high incidence of osteoporosis in our population.

Data on the functional consequences of excess nutrient intakes for setting UL are even more difficult to acquire. The ethical issues of dose-response studies to observe harmful effects, especially in children, make intervention studies in healthy populations unrealistic except for very mild adverse effects.

Increasingly, we are realizing that more than one outcome measure is appropriate for defining nutrient requirements or UL and that the most relevant one for one population is not the most relevant for another. These points will be illustrated below using calcium and vitamin D as examples.

Calcium

Calcium is an example of a nutrient for which the threshold (plateau) intake for maximal retention can be used to set the requirement. Calcium is unique among nutrients in that its storage is a functional reserve; >99% of the body’s calcium is in the skeleton as a consistent proportion of bone mineral. Calcium retention, reflective of bone mass, increases linearly with calcium intake below the threshold intake, but is unre-
lated to intake above the threshold intake. Maximizing bone mass reduces risk of fracture in both adults (4) and children (5). Bone densitometry is readily available for measurements of bone mineral content (BMC) or bone mineral density (BMD). Bone strength would be a better surrogate for fracture risk than bone mass or BMD because changes in BMD with treatment do not always predict fracture effects (6). However, we cannot yet easily measure fracture risk in humans.

To determine the calcium intake for maximal retention requires measuring an outcome over a range of calcium intakes. Calcium retention can be determined from total body calcium derived from BMD or balance. Epidemiologic approaches to determining the relationship between dietary calcium and maximal retention can measure BMC, which is quite accurate and has a long-term precision of <2%. A range of calcium intakes are available in most cohorts consuming a self-selected diet. The difficulty with this approach comes with accurately assessing calcium intake and determining the relationship with minimal confounders. The goal is to determine the role of dietary calcium and optimize peak bone mass within the full genetic potential or the consequences when only calcium is in insufficient supply. Randomized controlled trials could be used to determine the relationship between calcium intake and calcium retention. They have the advantage of determining reasonably accurately an increase in calcium with treatment over a placebo control. However, the calcium content of the underlying self-selected diets of trial participants is as poorly known as with observational studies. Furthermore, randomized controlled trials typically use only two levels of calcium, the treatment and the placebo; this is insufficient to determine maximal retention. Randomized controlled trials have been useful in showing the effect of calcium intake on bone mass gains or retention and reduction in fracture risk (7).

The only data collected to date on a sufficient range of calcium intakes to determine the threshold intake are from calcium retention as measured in metabolic balance studies. Data were available to determine the calcium intake for maximal retention for most age groups in setting AI for the 1997 DRI (3). The data for adolescents were generated from my laboratory (8). Each adolescent was studied at two calcium intakes for a period of 3 wk for each intake. We developed a nonlinear regression model as illustrated in Figure 1. Even with appropriate data, decisions must be made on how to use the model to determine requirements. Because the point at which the mean crosses the 100% maximal retention line has a CI that is dependent on the sample size, the intake cannot be known precisely. Similar nonlinear regression models were developed for several age groups, and the intake at which the CI crossed the 100% maximal retention line was used to set the AI. Although increasing calcium retention, i.e., bone mass, is associated with decreased fracture risk (9), some would argue that an aim for 100% maximal retention is not practical. Figure 1 shows how a decision to use 70% maximal retention would influence the calcium requirement.

The mechanism whereby increasing calcium intake results in increased calcium retention is best understood with the use of isotopic calcium tracers. By administering oral and intravenous stable isotopes of calcium to adolescents in steady state consuming a controlled diet and following the fate of the isotopes and total calcium in the blood, urine and stools, the effect of increasing calcium intake on calcium homeostasis can be determined. We found that increasing calcium intake increased the amount of absorbed calcium and suppressed bone resorption with no change in bone formation rate (9). The decrease in bone resorption equaled the increase in absorbed calcium mg for mg, thus sparing bone to supply obligatory losses, via endogenous secretion and the kidney. These changes were not related to traditional regulators of calcium homeostasis, i.e., parathyroid hormone (PTH) and vitamin D metabolites, whose fasting values were unchanged by calcium intake. Hormones that regulate bone accretion may be involved, although insulin-like growth factor-I was also unchanged.

The magnitude of the effect of increasing calcium intake on bone accretion depends greatly on the age of the subject and starting calcium intake. The pubertal growth spurt is associated with large increases in bone gain over a very narrow window of time. Approximately one fourth of the adult skeleton is accumulated in 2 y during the adolescent period (10). The potential of increases in bone gain with adequate calcium intake during this time is greater than at any other. Bone formation rates fall off exponentially with years postmenarche in females (11). By young adulthood, calcium retention is unaffected by increased calcium intake > 800 mg/d due to decreased fractional calcium absorption and increased endogenous secretion (12). Postmenopausal, osteoporotic women increase calcium retention when calcium intakes are increased from 500 to 2500 mg/d through decreased bone resorption (13), as for adolescents.

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The relationship of optimal calcium intake to health may require different end points for different populations. The focus on Caucasians has been bone, but it may be more appropriate to measure blood pressure in African Americans. Regardless of the disease endpoint, the calcium intake for maximal retention may accomplish what is possible for protecting against any disease. Future work should focus on determining threshold intakes for various populations.

**Vitamin D**

Requirements for vitamin D cannot be based on intakes to achieve maximal retention as for calcium for several reasons.
The major source of vitamin D for humans is not diet, but rather is produced through photosynthesis of vitamin D from cutaneous 7-dehydrocholesterol upon sunlight exposure to UV B photons with energies between 290 and 315 nm (15). Furthermore, vitamin D is converted to various metabolites in the body. The 1997 DRI committee used serum 25 (OH)D as the criterion for determining adequacy because its production is not regulated; therefore, it reflects both absorption from the diet and cutaneous synthesis. Reference ranges for serum 25 (OH)D were used, but the lower limit of various populations ranged from 20 to 50 nmol/L, likely reflecting available sunlight of the appropriate energy in their environments. In older individuals, AI for vitamin D were based on observed values to prevent seasonal variation in PTH, a marker for bone resorption (3).

Recently, several researchers have questioned the adequacy of current vitamin D recommendations and suggested that daily inputs from diet and cutaneous origin should be 1000 IU or even 3000–5000 IU (75–125 μg) (16–18). Healthy adults can achieve this level of input primarily through cutaneous production. Early humans, evolving at the equator in East Africa, would have had cutaneous vitamin D synthesis sufficient to produce serum 25-hydroxyvitamin D levels at ~150 nmol/L (18). The elderly who have reduced sun exposure and efficiency of conversion of 7-dehydrocholesterol to vitamin D-3 cannot.

As for calcium, there may be different health end points more relevant to different populations for vitamin D; <50 nmol/L of 25 (OH)D may increase risk of secondary hyperparathyroidism enhancing the risk for osteoporosis, rickets or osteomalacia. Holick (19) proposed that higher levels of serum 25 (OH)D (75 nmol/L) may be necessary to maximize cellular health.

Much research is required to identify appropriate functional end points for vitamin D. Serum 25 (OH)D is an indicator for status, but we have a poor understanding of the relationship of this marker to ultimate health outcomes. Only recently have we had good information available on the relationship of vitamin D intake and serum 25 (OH)D (17,18). Serum 25 (OH)D levels have been related to serum PTH (20). Although PTH does promote bone resorption, serum PTH is only a surrogate for bone resorption. Additional studies must be conducted to directly relate vitamin D intake to bone loss. Relating vitamin D intakes to measures of optimal cellular health is in its infancy.

**Interdependency of calcium and vitamin D requirements**

The roles of calcium and vitamin D are so intertwined that it makes sense that the requirements of either one of these nutrients depend on the adequacy of the other. The role of vitamin D metabolites in calcium metabolism is depicted in Figure 2.

Maintaining serum calcium concentrations within the normal range is a biological priority and the major biological function associated with vitamin D (21). When dietary calcium is inadequate and serum [Ca^{2+}] falls, PTH is released, which promotes conversion of vitamin D to its most potent form, 1,25-dihydroxyvitamin D (calcitriol). One recent study showed that in postmenopausal women, PTH is most effective in maintaining serum 1,25-dihydroxyvitamin D levels if serum 25-hydroxyvitamin D levels are <40 nmol/L, whereas the substrate, 25-hydroxyvitamin D, is more important above this cut-off point (22). Calcitriol enhances transcellular calcium absorption by upregulating synthesis of a calcium-binding transport protein, increasing renal tubule reabsorption of calcium and increasing bone resorption by mobilizing monocyctic stem cells in the bone marrow to become mature osteoclasts (23). However, 25-hydroxyvitamin D can also directly stimulate calcium absorption, and serum levels of this metabolite were found to be more related to intestinal calcium absorption than calcitriol in premenopausal and elderly women (24,25).

Testing only one level of calcium and vitamin D is insufficient. Calcium absorption efficiency rose substantially as 25-hydroxyvitamin D levels increased from 50 to 80 nmol/L, but not above 80–90 nmol/L (26). Treating postmenopausal women of average age 56 ± 7 with 20 μg 25-hydroxyvitamin D on alternate days for 3 wk resulted in mean serum 25-hydroxyvitamin D levels of 86.5 nmol/L compared with 50.2 nmol/L in a group of women of age 64 ± 9 y. The group pretreated with 25-hydroxyvitamin D had a 65% higher calcium absorption efficiency. Fourteen of the 34 women participated in both groups. This study measured calcium absorption as area under the curve of load-induced rise in serum calcium, which is not very sensitive and likely successful only because of the large loads (500 mg) employed. Nevertheless, this represents the most progress toward linking vitamin D with a functional outcome measure to date. Low fractional 45Ca absorption was associated with increased risk of hip fractures in elderly women participating in the Study for Osteoporosis Fractures; these women had low calcium intakes regardless of use of vitamin D supplementation (27). This is an indication that vitamin D alone is insufficient to protect against hip fracture, at least at common usage levels.

In vitamin D deficiency, calcium is absorbed dominantly by the passive, extracellular route. In this condition, >2000 mg calcium/d would be required to prevent losses due to net intestinal losses and cutaneous and renal losses (28). Thus, calcium requirements for optimal retention likely depend on vitamin D status. On the other hand, calcium supplementation was more effective than vitamin D supplementation on mitigating age-related bone loss in the elderly (29).

Interestingly, to increase serum 25 (OH) D to 80 nmol/L from ≤30 nmol/L could require vitamin D intakes that exceed the current UL of 2000 IU (50 μg)/d (30). This applies to more than one third of women >60 y old (30). The effect of serum 25 (OH)D is age dependent. Vieth et al. (31) showed that vitamin D supplementation increased serum 25 (OH) D equally in young and old subjects, but higher serum 25 (OH) D levels were required in older subjects to suppress serum PTH.
Summary and recommendations

Research gaps on both optimal intakes based on functional outcome measures and risk of excess exist for calcium and especially vitamin D. Too little is known about how the adequacy of one bone nutrient influences the requirements of another nutrient for optimizing health. This will require development of sensitive, short-term assessment tools so that studying nutrient requirement interdependencies is practical. Likely, multiple health outcomes must be studied because insufficiencies affect different populations in different ways. In the future, we may be able to determine how a nutrient alters gene patterns on a chip favorably to lower risk for a given disease and allow recommendations to be made for an individual.

For now, the evidence to support the role of adequate calcium and vitamin D for bones is strong for the general population. Thus, the first line of treatment by clinicians for treating osteoporosis is to give calcium and vitamin D supplements. Nutritionists have stood back and allowed the concept to be adopted that pills are sufficient to address nutritional needs. If an isolated nutrient is all that is deficient, it may be adequate. But we have to care enough about our students, clients and patients to assess their entire diet and give complete nutritional advice. It is a critical time for nutritionists to provide leadership for raising nutritional awareness. We can do this in many ways from teaching audiences to simply raising a challenge every time a prescription is given or a grant or manuscript is reviewed that has considered supplementation of only one or two nutrients. If nutritionists do not take responsibility for increasing the awareness of total diet, who will?

LITERATURE CITED