Setting Dietary Reference Intakes with the use of bioavailability data: calcium[1–5]

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ABSTRACT

The determination of Dietary Reference Intakes (DRIs) for calcium, especially in children, has relied in significant part on the evaluation of the relation between calcium intake and calcium absorption and retention. At present, most of these studies are conducted with the use of dual-tracer stable isotope, although mass balance or other isotope methods are still used occasionally. Studies carried out to evaluate DRI values need to be conducted under the most controlled conditions possible. However, the achievement of such conditions can be difficult, especially in studies in small children, because strict, long-term dietary monitoring and sample collections are not well tolerated. Other dietary factors, which include vitamin D status and the presence of enhancers and inhibitors of calcium absorption, may have to be considered. However, for most healthy populations who do not have very low calcium intakes or serum 25-hydroxyvitamin D concentrations, other dietary factors will not be major determinants of the net calcium absorption or retention that will be used for the establishment of DRI values. Ultimately, DRI values must be chosen based on an attempt to achieve some targeted value for calcium absorption/retention or to maximize, within constraints, the overall calcium absorbed and retained. In children, it is important to use data obtained at the age and pubertal status being evaluated rather than to interpolate from data performed in other age groups. *Am J Clin Nutr* 2010;91(suppl):1474S–7S.

INTRODUCTION

In 1997, Dietary Reference Intake (DRI) values for calcium were released by the Institute of Medicine and remained in use in the United States and Canada in 2009. The values ultimately published were limited to Adequate Intake and Tolerable Upper Limit (1). Those DRI values are in the process of reevaluation, with expected release of updated values in 2010.

A number of endpoints were used to determine the Adequate Intake values in the establishment of the 1997 DRI values. In adults, these included randomized, controlled supplementation trials and nutrient metabolism studies. In children there were very few available supplementation studies. The primary endpoints used were metabolic studies, especially those that assessed calcium absorption and retention. Unfortunately, even these data were significantly limited in some groups of children.

Since 1997 there have been very few additional long-term controlled trials of calcium supplementation in children, especially those who are prepubertal. Therefore, it will be necessary to continue to use studies of calcium metabolism, especially calcium absorption and retention, to establish DRI values. The purpose of this article is to describe some of the issues involved in the design and interpretation of such metabolic balance studies and to provide an example of how these data can be used in the revaluation of possible DRI values.

METHODS FOR ASSESSMENT OF BIOAVAILABILITY

In this article, the term *bioavailability* is used to describe the use of dietary calcium primarily by the skeleton. In this case, based on common usage, it refers specifically to the net retention of calcium from a given dietary intake. This review does not consider the details of the specific chemical form of calcium as a variable in the determination of bioavailability because such information has not been used, and is unlikely to be widely used, in the establishment of DRI values for calcium.

Bioavailability of calcium can be assessed with multiple techniques (**Table 1**) (2, 3), most commonly mass balance or isotope techniques. In rapidly growing children, the rate of change in total body bone mineral content may also be used to estimate long-term dietary calcium retention. Isotope techniques involve the use of stable or radioactive tracers and either single- or dual-tracer methods. Currently, the ready availability of both tracers and analytic laboratories has led to a shift such that most studies are performed with the use of stable isotopes of calcium to avoid any radiation exposure. Single oral tracer methods require fecal collections or the use of mathematic correction for calcium body pool determinations. In general, these estimations are less accurate and

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TABLE 1
Methods for measurement of calcium absorption or retention[1]

Method	Advantages	Disadvantages
Mass balance	No tracers needed	Prolonged fecal collections needed
Single oral tracer	No IV tracer needed	Fecal collection needed, may be less accurate
Bone mineral change	No tracers or collections needed	More suitable for children, long-time period of observation needed
Dual tracer	Accurate, rapid, no stools	IV infusion needed

[1] IV, intravenous.

are more practical for the evaluation of groups of adults. They are less useful in children or for small groups of subjects.

In the dual-tracer method, one isotope is given orally and a second is given intravenously. With stable isotopes, different minor isotopes are almost always used for the oral and intravenous tracers (**Table 2**). This technique has been applied widely to a range of nutrients, especially minerals, in recent years. Some of the key methodologic considerations in such studies of calcium bioavailability are described below.

The first decision is how to distribute both the isotope given orally and the dietary calcium in the study. For whole-diet studies, such as those used to evaluate DRI values, extrinsically provided calcium tracers are usually given with a calcium source such as milk or calcium-fortified orange juice. The tracer can be given with a single meal or divided across the meals for a day. Although it is theoretically preferable to dose the tracer proportionally to the daily calcium intake, this is relatively impractical and much more often an attempt is made to balance the calcium intake across multiple meals during the day and to give a single dose with a small meal at breakfast.

Although very small calcium loads may be used to determine the bioavailability of a particular single food, in the evaluation of whole diets for DRI purposes, it is best to use a typical dietary calcium load with the meal. It is common for a meal load of 200–400 mg calcium to be given with the tracer. With the use of currently available analytic techniques, the actual tracer dose of a given stable isotope of calcium is somewhat small relative to the meal calcium. For example, if ^{44}Ca is given orally, a dose of 10–20 mg would generally suffice and this can be calculated readily as part of the total calcium intake from the whole meal. As such, consideration of the effect of the actual tracer on the overall meal intake is relatively minimal.

EFFECTS OF FOOD MATRIX ON ABSORPTION (eg, PREBIOTICS)

An extensive literature describes the effects of various dietary conditions on calcium absorption (1, 3–7). The key issue is to determine at the outset whether one is attempting to evaluate a specific component or effect, or to evaluate the calcium bioavailability of the whole diet. The latter is usually what is intended in studies that evaluate possible DRI values. In the former case, multitracer studies that use ≥2 oral tracers can be used on the same or successive days, with fixed calcium dietary loads, to evaluate relative bioavailability. The direct effect of a dietary enhancer on the meal can be identified with this approach.

TABLE 2
Calcium stable isotopes frequently used in nutritional research[1]

Isotope	Natural abundance	Typical dose for adults	Typical dose for children > 5 kg
	%		
^{42}Ca	0.65	1–2 mg IV	0.05 mg/kg IV
^{44}Ca	2.08	10–15 mg PO	0.2 mg/kg PO
^{46}Ca	0.0032	20 μg PO	2 μg/kg PO

[1] IV, intravenous; PO, by mouth.

However, when the primary goal is to evaluate whole diets, an adaptation period is required, and evaluation of the effects of the nutritional intervention should be carried out soon after the intervention and then again at a much later time. A relevant example is the effect of prebiotics on calcium absorption (7). In this case, because the prebiotic effect requires several weeks to change the gut flora and affect calcium absorption, a simple comparison of 2 labeled meals given within a day of each other is not helpful. Instead, it is necessary to perform a baseline study and then compare results after adaptation to the prebiotic intervention. Evaluation of prebiotic interventions should be done 3–6 wk after the initial intervention and then 1 y later. This allows for the determination of both the initial effect and whether such an effect would persist.

In studies of a dietary intervention on calcium bioavailability, one has to consider whether a placebo intervention should also be studied. It can be difficult to obtain ethical approval to conduct placebo interventions in children because of the lack of any possible benefit to the placebo group. This is especially true if one plans a pre- and posttest on a nonintervention group. Nonetheless, it is general practice to include a placebo group for several reasons. First, in children and adolescents, growth and pubertal changes over the period of time of a long-term intervention (eg, the use of prebiotics for a whole year) would lead to difficulty in the identification of the effect related to the intervention itself. Only with the comparison of changes over a long period of time with a placebo intervention can long-term dietary effects to be evaluated. The second reason is that any effects on subject diet or behavior of participation in the research study would be mitigated. That is, although we can attempt to control the diet at home, the nature of being part of a research study might affect dietary or other patterns over a long period of time. Therefore, a placebo control group is optimal for long-term studies of changes in diet that may be considered as part of evaluations of the DRI or of other dietary recommendations.

Ultimately, however, although we can identify enhancers and inhibitors of calcium absorption, it is unlikely that these will be central factors in the determination of DRI values for healthy populations. It is true that certain types of diets, such as those with high sodium or those with very low (or high) protein intake, will lead to lower net calcium retention (8, 9). It might be possible to evaluate calcium requirements based on the amount of enhancers and inhibitors in the diet. However, this evaluation has not been done in the past and would be extremely difficult to implement practically in calcium DRI values.

EFFECTS OF VITAMIN D ON CALCIUM BIOAVAILABILITY

One unique challenge in conducting calcium bioavailability studies is the effect of vitamin D on calcium absorption. Vitamin D is necessary for transcellular ("active") calcium absorption and

its deficiency is associated with the clinical disease of rickets. Because of the large variation in vitamin D status, as reflected by serum 25-hydroxyvitamin D concentration [25(OH)D], together with hard-to-control factors such as season, ethnicity, clothing, sunshine exposure, and dietary vitamin D intake, it can be very difficult to determine a value of calcium absorption. Nonetheless, if these factors are an important part of calcium bioavailability, then they may need to be considered in the establishment of dietary requirements for calcium.

The relation between vitamin D status and calcium absorption is complex and not completely understood. Although some reports indicate optimization at specific concentrations of serum 25(OH)D above those commonly found in the population, others do not (10–12). Data from several reports in children have also shown that it is difficult to relate any specific season or serum 25(OH)D to maximum calcium absorption (13, 14). It is undoubtedly true that extremely low serum 25(OH)D values are associated, in both children and adults, with decreased calcium absorption. However, the establishment of calcium DRI values based on vitamin D status would be difficult based on the limited data that relates outcomes such as bone mineral content to the relative amounts of calcium and vitamin D in the diet. This relation issue may be handled most readily through specific dietary guidelines to provide adequate vitamin D, and not by the creation of calcium guidelines stratified by serum 25(OH)D concentrations.

EFFECTS OF GENOTYPE ON ABSORPTION AND METABOLISM

It is well recognized that genetics plays a central role in the determination of bone mineral mass and, ultimately, the risk of osteoporosis. Readily identifiable genetic factors for bone mineral mass include sex and race. These factors also affect calcium bioavailability in terms of both absorption and retention of calcium. However, the stratification of DRI values for calcium based on sex or race is not usually determined and was not determined in the 1997 US and Canadian DRI guidelines because of challenges in the identification of individual racial groups and because of the general perception that dietary guidelines should optimize calcium bioavailability for all subgroups of populations. That is, although calcium absorption or urinary retention, as well as response to dietary factors (eg, sodium), may differ by sex or race, guidelines specific to either would not present a useful public health message unless clear differences in intake-outcome relations were identified based on race or sex, such that different calcium intakes would lead to different outcomes (eg, bone mineral content) in selected groups. This has not been shown convincingly to date.

It is uncertain whether the use of any genetic factors to categorize DRI values for calcium is a good idea. Ultimately, however, consideration may be given to the identification of genetic characteristics that would serve as modifiers of intake recommendations. Among those factors are the vitamin D receptor genotypes of the individual and the known relation between these genotypes and functional outcomes such as bone mineral content. Current data related to this topic are complex and generally mixed in terms of the effects of various genes on markers of calcium and bone mineral status.

We have reported differences in calcium bioavailability based on different polymorphisms of the vitamin D receptor gene *Fok1* (15). This gene may also be important in the determination of the risks of some malignancies. Guidance will be needed to determine whether populations with specific vitamin D receptor (or other) genotypes should alter their dietary calcium or vitamin D intake based on these risks. However, as improvements arrive in the understanding of specific genetic factors related to calcium bioavailability, the use of genetic information to determine dietary requirements remains a possibility for the future.

USE OF BIOAVAILABILITY DATA FOR DRI DETERMINATION

To determine possible DRI values, one approach is to evaluate the amount of dietary calcium that would lead to a predetermined "target" net calcium retention (1, 16). Most often, in children and young adults, this target is the net calcium retention expected to provide age-appropriate bone mineral increment. In older adults, it is more often "zero" balance, that is, the intake at which no bone is lost. We used a targeted calcium balance approach to evaluate possible Estimated Average Requirement (EAR) and Recommended Dietary Allowance values in children aged 2–4 y (17).

In this study, calcium absorption was measured in 14 boys and 14 girls whose mean (±SD) calcium intake averaged 551 ± 219 mg/d. We then fitted the data for absorption with the use of an S-shaped curve (**Figure 1**). From this relation we determined an estimate for the amount of calcium in the diet that would be needed to achieve various levels of calcium retention. This amount of calcium ranged from 332 mg/d for a retention of 100 mg/d, up to 857 mg/d for a retention of 200 mg/d. With the use of available data for the rate of skeletal mineralization, we concluded that 140 mg/d calcium retention was the average accretion during this time period. This accretion rate would be achieved with ≈500 mg calcium/d, which means this can be considered a possible EAR.

Ultimately, the development of intake–retention relation data is necessary for any population group to evaluate DRI values. However, note that this approach is based on the assumption that the usual rate of bone growth (or zero balance) is optimal. This assumption is generally reasonable and parallels similar assumptions for biochemical and growth data. However, with the use of newer techniques that evaluate bone strength and geometry as well as net bone mineral content, it may also be reasonable to

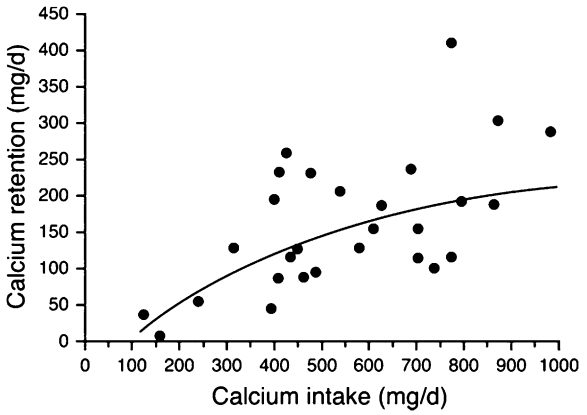

FIGURE 1. Relation between calcium intake and retention fitted in a linear model. Reproduced with permission from reference 17.

ask whether different data sets for bone mineral accumulation should be considered as endpoints.

CONCLUSIONS

The current DRI values for calcium in the United States and Canada, as set by the Institute of Medicine in 1997, do not provide for an EAR. A new panel is currently considering these values. In children, calcium supplementation trials are mostly short term or difficult to use for the establishment of DRI values. Therefore, bioavailability studies are an important aspect of this process.

Factors that may affect bioavailability, which include calcium source, dietary intake, genetic factors, other dietary factors, and vitamin D status, may all eventually be taken into consideration in the determination and individualization of dietary guidance. However, for now, it is likely that targeting outcomes for calcium balance (and bone mineral content) from broad population-based studies will be necessary. In this regard, one can use data, especially from properly performed dual-tracer calcium balance studies, to determine potential EAR values.

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