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Calcium and Related Nutrients: Overview and Methods

OVERVIEW

This report focuses on five nutrients—calcium, phosphorus, magnesium, vitamin D, and fluoride, all of which play a key role in the development and maintenance of bone and other calcified tissues. Indeed, 99 percent of body calcium, 85 percent of body phosphorus, 50 to 60 percent of body magnesium, and 99 percent of body fluoride are found in bone or other calcified tissues. Vitamin D functions as the substrate for the synthesis of 1,25-dihydroxyvitamin D, which is the active hormone necessary for the regulation of calcium and phosphorus homeostasis.

Although the development and preservation of bone mass are key elements in the estimation of the needs for these five nutrients for the population of the United States and Canada, the evidence considered includes other biological roles for these nutrients and their possible relevance to human health and to decreasing risk of disease. For the most part, however, it is the functioning of these nutrients in bone and teeth that provided the most convincing criteria on which to base the new Dietary Reference Intakes (DRIs).

Other nutrients may be of biological significance to the development and maintenance of bone. These include the microminerals copper, zinc, manganese, and boron and the vitamins C and K. With the exception of boron, all these nutrients have been identified as required cofactors for enzymes that act in the synthesis or post-translational modification of constituents of bone matrix. Vitamin C is essential for collagen cross-linking, and vitamin K is essential for the gamma carboxylation of three bone matrix proteins (for example, osteocalcin, which facilitates calcium binding to hydroxyapatite). With the exception of boron, deficiencies of these nutrients in growing animals have been associated with bone lesions (Heaney, 1997). In humans, the primary biological function of these nutrients does not appear to be bone metabolism and maintenance of skeletal integrity. Bone fragility in humans related to deficiencies of these trace elements and vitamins has not been well described. Thus, these trace elements and vitamins will be covered in subsequent DRI reports that deal with their major functions.

The roles of boron in human health, in general, and in bone metabolism, in particular, are uncertain. Studies in animals suggest that boron contributes to the composition and strength characteristics of bone (Hunt and Nielsen, 1981). Boron may have an interactive role with vitamin D because it affects steroid hormone metabolism in humans (Nielsen, 1990; Nielsen et al., 1987). In studies in postmenopausal women, boron depletion was not consistently found to alter calcium and vitamin D homeostasis (Nielsen et al., 1987; Peace and Beattie, 1991). Epidemiological evidence of a relationship between dietary boron status and osteoporosis is not available. Thus, it was deemed premature to consider boron as a nutrient functionally related to bone health, and boron was not included in this report.

METHODOLOGICAL CONSIDERATIONS

The scientific data for developing the DRIs have come primarily from clinical, dose-response, balance, depletion-repletion, observational, and case-control studies. In general, only studies published in peer-reviewed journals have been utilized. However, studies published in other scientific journals or readily available reports were considered if they appeared to provide important information on health effects not documented elsewhere. In some cases they were used to estimate intakes. If applicable, original scientific studies were used for the critical determinants of endpoints for deriving the Estimated Average Requirement (EAR), Adequate Intake (AI), and Tolerable Upper Intake Level (UL) for each nutrient at each stage of the lifespan.

Based on a thorough review of the scientific literature, the possible criterion or outcomes of nutrient adequacy were identified for each nutrient and life stage or gender group. The choice of the indicator to utilize in determining the EAR or AI was based on scientific judgment. The strengths and weaknesses of each study considered in developing the EAR or AI were assessed. Chapters 4 through 8 describe the rationale for the inclusion or exclusion of evidence. Among the factors considered were the methods used to determine intake from food and supplements; methods used for measuring the indicator of adequacy; relationships among the indicator, dietary intake, and functional or physiologic outcome; any allowances for adaptation to changes in intake; and other aspects of the study design.

When applicable, the strength, consistency, and preponderance of the data and the degree of concordance among epidemiological, clinical, and laboratory evidence determined the strengths of the indicators that were used as the basis for EARs and AIs in each stage of the lifespan. As was adopted by the Surgeon General's *Report on Nutrition and Health* (DHHS, 1988) and the Food and Nutrition Board's *Diet and Health* (NRC, 1989b), the assessment of the strength of the data supporting a nutrient's role in decreasing risk of chronic debilitating disease or developmental abnormalities was based on the following criteria (Hill, 1971):

- strength of association, usually expressed as relative risk;
- dose-response relationship;
- temporally correct association, with exposure preceding the onset of disease;
- consistency of association;
- specificity of association; and
- biological plausibility.

The greatest weight was given to studies, if available, that were directly related to a determination of nutrient needs and that used an appropriate experimental design and outcome measure. Less weight was given to studies in which observed levels of nutrient intake were related to a specific criterion or criteria of nutriture. Neither average dietary intake data nor indicator of adequacy data alone provided a sufficient basis for deriving an EAR, although this approach, of necessity, was applied to the development of AIs for infants and for fluoride. If necessary, a factorial model could be used as a basis for estimating the physiological requirement, which could then be used to estimate the dietary requirement for a nutrient. This process was used to estimate the phosphorus requirements for some life stage groups.

In developing estimates of average requirements for minerals such as calcium, phosphorus, and magnesium, the available literature until the late 1980s consisted primarily of balance studies in which normal subjects with somewhat similar age, body size, and gender characteristics were tested while consuming different dietary intakes. A key concern in such studies was the significant body store represented by the skeletal tissues for these nutrients and the fact that the balance method could easily fail to detect, due to systematic bias or error, small changes in mineral status. In addition, balance studies tend to err toward a positive balance since intake is usually overestimated and excretion is underestimated. With the advent of readily available noninvasive and fairly inexpensive methods to detect changes in bone mineral content and bone mineral density, additional information relative to small changes became available to augment information from balance studies.

In general, in order to account for possible errors introduced through the use of available balance data, criteria and methods for compiling balance data that could be used to develop DRIs for calcium and magnesium included the following:

- Preferential use was made of studies on self-selected calcium intakes in order to avoid the bone remodeling transient as described in Chapter 4.
- For every situation, data from more than one reported investigation of calcium balance were considered.
- Only balance studies whose dietary periods were thought to be long enough to assure a reasonable degree of adaptation to the diet via urinary and fecal losses were used.
- In some instances (as for children ages 9 through 13 years), comparable data from individual investigations were combined to create a larger sample size in order to facilitate use of a statistical model which describes the relationship between calcium intake and retention over a range of intakes. Using the equation derived from this model (see Appendix E), a prediction of calcium intake required to attain a desirable calcium retention could be obtained.
- When investigators did not measure or estimate miscellaneous losses of calcium in balance studies, an adjustment for this was made in predicting the desirable calcium retention. When rate of expected growth or change in tissue mass was not accounted for in individual studies (particularly related to magnesium in children), adjustments for growth were made to the available balance data.

NUTRIENT INTAKE ESTIMATES

Methodological Considerations

When examining data on an individual's requirement for any nutrient, it is essential to consider the quality of the intake data. The most valid intake data are those collected from the metabolic study protocols in which all food is provided by the researchers, amounts consumed are accurately measured, and the nutrient composition of the food is determined by laboratory analyses. Such protocols can be used for balance studies with a small number of subjects, but they are seldom possible for larger studies. Thus, intake data are often self-reported (for example, 24-hour recalls of food intake, diet records, or food frequency questionnaires), which have inherent limitations. Potential sources of error in self-reported intake data include over- or under-reporting of portion sizes, omission of foods, and inaccuracies in tables of food composition. These and other sources of dietary intake errors have been discussed in several reviews (Kohlmeier et al., 1997; LSRO/FASEB, 1986; Thompson and Byers, 1994; Willett, 1990) and at two recent conferences on dietary assessment methods (Buzzard and Willett, 1994; Willett and Sampson, 1997). The general conclusion is that self-reported dietary data are subject to a number of inaccuracies and biases. Therefore, the values reported by nationwide surveys or studies that rely on self reporting may be somewhat inaccurate and possibly biased.

Because of day-to-day variation in dietary intakes, the distribution of 1-day (or 2-day) intakes for a group is wider than the distribution of usual intakes, even though the mean of the intakes may be the same. Statistical adjustments have been developed (NRC, 1986; Nusser et al., 1996) that require at least 2 days of dietary data from a representative subsample of the population of interest. These adjustments have been made to the U.S. population intake data from the 1994 U.S. Department of Agriculture (USDA) Continuing Survey of Food Intake of Individuals (CSFII) (Cleveland et al., 1996), which are used in this report to more accurately estimate intakes of specific life stage and gender groups. However, this method does not adjust for the underreporting of intake, which may be as much as 20 percent (Mertz et al., 1991).

Finally, food composition databases that are used to calculate nutrient intake from self-reported and observed intake data introduce errors due to random variability, genetic variation in content, and use of poor analytical methods. In general, when estimating nutrient intakes for groups, the effect of errors in the composition data is probably considerably smaller than the effect of errors in the self-reported intake data (NRC, 1986).

DIETARY INTAKES IN THE UNITED STATES AND CANADA

Sources of Dietary Intake Data

The major sources of intake data for the U.S. population are the national surveys conducted by the U.S. Department of Health and Human Services (USDHHS) and by the USDA. Partial results of two surveys from phase I (1988–1991) of the Third National Health and Nutrition Examination Survey (NHANES III), which was conducted from 1988 to 1994 by USDHHS (Alaimo et al., 1994), and the first two years of the 1994 to 1996 CSFII, which was conducted by USDA (Cleveland et al., 1996) have been released recently. NHANES III examined 30,000 subjects aged 2 months and older. A single 24-hour diet recall was collected for all subjects, and a second recall was collected for a 5 percent subsample. The 1994 to 1996 CSFII collected two nonconsecutive 24-hour recalls from approximately 16,000 subjects of all ages. Both surveys used a food composition database developed by USDA to calculate nutrient intakes (Perloff et al., 1990).

National survey data for Canada are not currently available, although data have been collected from two provinces and should be available shortly. The data regarding nutrient intakes for individuals in the United States may be applicable to Canada, but until comparable databases are available, the degree of similarity of intakes is unknown.

When comparisons are made in this report between intake and DRIs (AIs, EARs, RDAs, and ULs), only intakes from the recent CSFII survey that have been adjusted for day-to-day variation in intake are presented. In many cases, values available from the NHANES III survey are similar, which is noted. Values reported by CSFII are for intake from food only.

Table 2-1 gives the fifth, median, and ninety-fifth percentiles of intakes of calcium, phosphorus, and magnesium by age in the United States from the first phase of the CSFII survey, as adjusted by the method of Nusser et al. (1996). Because food composition data are not readily available for vitamin D, neither of the U.S. national surveys has attempted to estimate intakes for this nutrient. An analysis using NHANES II data (collected from 1976 to 1980) has estimated median 1-day vitamin D intakes by young women at 2.9 μg (114 IU)/day from food (maximum of 49 μg [1,960 IU]/day) (Mur-

TABLE 2-1 1994 CSFII Daily Intakes of Calcium, Phosphorus, and Magnesium by Life Stage and Gender

	Calcium (mg) (Percentiles)			Phosphorus (mg) (Percentiles)			Magnesium (mg) (Percentiles)		
Life Stage[a]	5th	50th	95th	5th	50th	95th	5th	50th	95th
All (5,576)	349	742	1,429	620	1,164	2,020	128	248	451
Males and Females									
0 to 6 months (69)	191	457	745	131	322	532	21	55	105
7 to 12 months (45)	351	703	1,177	302	612	1,086	61	109	171
1 through 3 years (702)	399	766	1,276	552	926	1,396	106	180	274
4 through 8 years (666)	455	808	1,325	677	1,059	1,596	128	205	315
Males, 9 + years (2,053)	431	865	1,620	874	1,445	2,282	177	310	516
9 through 13 years (180)	499	980	1,702	771	1,359	2,203	140	258	445
14 through 18 years (191)	554	1,094	2,039	956	1,582	2,534	166	298	522
19 through 30 years (328)	484	954	1,746	1,002	1,613	2,472	185	328	553
31 through 50 years (627)	429	857	1,588	907	1,484	2,312	194	329	529
51 through 70 years (490)	362	708	1,268	769	1,274	1,956	169	295	474
> 70 years (237)	368	702	1,185	721	1,176	1,712	160	275	429
Females, 9 + years (1,992)	316	625	1,109	620	1,001	1,510	134	222	342
9 through 13 years (200)	486	889	1,452	768	1,178	1,725	142	223	339
14 through 18 years (169)	348	713	1,293	632	1,097	1,727	123	217	352
19 through 30 years (302)	300	612	1,116	560	1,005	1,571	110	205	322
31 through 50 years (590)	297	606	1,082	593	990	1,516	133	229	363
51 through 70 years (510)	294	571	1,001	599	966	1,444	141	231	362
> 70 years (221)	277	517	860	521	859	1,282	118	206	314
Pregnancy (33)[b]	656	1,154	1,729	1,012	1,581	2,108	187	292	399
Lactation (16)[b]	794	1,050	1,324	1,211	1,483	1,822	244	315	396

NOTE: 1994 CSFII data from Cleveland et al. (1996) adjusted using the method developed by Nusser et al. (1996). Grouped data do not include pregnant or lactating women.

[a] Number of subjects measured given in parentheses.
[b] Estimates are less reliable than other life stage groups due to extremely small sample size.

phy and Calloway, 1986). A smaller study of older women estimated median food intakes of vitamin D at 2.3 μg (90 IU)/day, with a maximum of 12.5 μg (500 IU)/day (Krall et al., 1989). No estimates of the extent to which exposure to sunlight met part of the individual's requirements for vitamin D are available in these studies. Intakes of fluoride from foods are difficult to estimate due to wide variations in the fluoride content of local water supplies and inadvertent consumption of fluoride through dental products. As a result, none of the U.S. national surveys has attempted to estimate fluoride intake. Some data are available from relatively small samples of individuals, and these are provided in Chapter 8 which discusses fluoride.

Estimates of intakes for some of these nutrients can be made from per-capita food availability (disappearance data). For the United States, the most recent disappearance data (USDA, 1997) show 900 mg (22.5 mmol) of calcium per person, 1,420 mg (45.8 mmol) of phosphorus per person (which excludes the phosphorus used as a food additive in processed foods and beverages such as in soft drinks), and 320 mg (13.3 mmol) of magnesium per person. These daily average figures are lower than those shown in Table 2-1 for adults because intakes of all age groups are combined. In addition, they overestimate actual intakes of the population because spoilage, trimming, and plate waste are not subtracted from the per-capita estimates. However, the general magnitude of the intake estimates is confirmed by this alternative source of food consumption data.

Finally, drinking water may be an important source of some minerals other than fluoride. Although accounted for in most balance studies conducted in modern metabolic units, the contribution of calcium, magnesium, and phosphorus from water to the total estimated intake of subjects is frequently not included or estimated.

Sources of Supplement Intake Data

Although NHANES and CSFII ask subjects about the use of dietary supplements, neither collects quantified information on intakes from supplements. In 1986, the National Health Interview Survey (NHIS) queried 11,558 adults and 1,877 children on their intake of supplements during the previous 2 weeks (Moss et al., 1989). The composition of the supplement was obtained directly from the product label whenever possible. These data indicated that almost 25 percent of adults took a vitamin D supplement during the previous 2 weeks, 20 percent took a calcium supplement, and 15 percent took a magnesium supplement. Use of phosphorus

TABLE 2-2 Percentage Use of Supplements in the United States by Children and Adults: Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride

	Percentage Who Reported Use in Previous 2 Weeks	Intake (mg/day) of Nutrient Supplements by Those Reporting Use in Previous 2 Weeks (Percentiles)		
Nutrient	Median	50th	95th	99th
Children				
Calcium (mg)	7.5	88	160	304
Phosphorus (mg)	6.2	48	128	200
Magnesium (mg)	7.9	23	70	117
Vitamin D (μg)	38.2	10	10	10
Fluoride	2 .5	ND[a]	ND	ND
Men				
Calcium (mg)	14.0	160	624	928
Phosphorus (mg)	9.2	120	264	448
Magnesium (mg)	13.5	102	200	350
Vitamin D (μg)	19.9	10	12	20
Fluoride	0	ND	ND	ND
Women				
Calcium (mg)	24.7	248	904	1,200
Phosphorus (mg)	11.2	128	264	448
Magnesium (mg)	17.1	100	240	400
Vitamin D (μg)	27.6	10	13	17
Fluoride	0.1	ND	ND	ND

[a] ND = not determined.

SOURCE: Moss et al. (1989).

supplements was less common (10 percent); virtually no adults used fluoride supplements. Children aged 2 to 6 years were less likely to take supplements of calcium, phosphorus, or magnesium (6 to 8 percent took these supplements) but they were more likely to take vitamin D (38 percent). The percentages of supplement use for adults and children, as well as the median, ninety-fifth, and ninety-ninth percentile of intake, are shown in Table 2-2 for calcium, phosphorus, magnesium, vitamin D, and fluoride.

The lack of accurate estimates of a population's intake from supplements plus food prevents accurate examination of the upper end of the nutrient intake distribution. This, in turn, limits the ability to identify intakes that approach or exceed the UL.

Use of Intake Data in This Report

Intake data from food, water, and, when available, supplements and some over-the-counter medications are used for several purposes in this report: to estimate the average requirement of a nutrient, to determine the lowest-observed-adverse-effect level (LOAEL) or the no-observed-adverse-effect level (NOAEL) of a nutrient, and to characterize the risk of exceeding the UL for a nutrient. They are also used in a few examples of applying DRIs to specific situations.

Food Sources of Calcium and Related Nutrients

Availability of nutrients from a range of foods provides useful information when setting nutrient requirements and ULs. Calcium in the United States and Canada is obtained primarily from dairy products (Cleveland et al., 1996; NIN, 1995). Household consumption data show that individuals in the United States consume the equivalent of 2 cups of milk per day (based on the calcium content of various dairy products), while Canadians consume approximately 1.6 cups per day (Cleveland et al., 1996; NIN, 1995).

Cross-Cultural Differences in Dietary Intake and Bioavailability

For this report, consideration of the dietary practices associated with intakes of calcium and related nutrients has been limited to observations within U.S. and Canadian populations. The recommendations for the DRIs may not be generalizable globally, especially where food intake and indigent dietary practices may result in very different bioavailability of mineral elements from sources not considered in traditional diets of Canadians and Americans. For example, both the consumption of bones from fish and meat foods and the practice of geophagia are more common in developing countries than in the United States or Canada. Population variations in the consumption of other diet components such as protein and sodium may significantly affect population calcium and magnesium needs and ULs.

With regard to the need for calcium and related nutrients for bone health, cross-cultural comparisons must also consider variability among populations in activity, weight-bearing practices, and sun exposure. Differences in hip axis length or other structural features may vary across cultures; hip axis length is directly associated with hip fracture risk (Cummings et al., 1993). Until more is learned about the prevalence of osteoporosis and hip fracture risk in other countries (Cooper et al., 1992), and about habitual intakes of calcium and related nutrients according to specific cultural dietary practices, the implementation of the published DRIs should be used with caution outside the United States and Canada.

USE OF ADEQUATE INTAKE RATHER THAN ESTIMATED AVERAGE REQUIREMENT

As defined in Chapter 1, the AI is used as a reference value when sufficient data are not available to estimate an average requirement. In this report, AIs rather than EARs and RDAs are developed for all nutrients for infants to age 1 year, and for calcium, vitamin D, and fluoride for all life stages. The method used to derive the AIs differs for infants and for each nutrient as follows.

Infants: Ages 0 through 6 Months

The AI is the intake by healthy breast-fed infants as obtained from average human milk nutrient composition and average milk volume. Since infants self-regulate milk intake from the breast, it is presumed that larger infants, who may require more milk than the average population intake, will achieve this by increasing milk intake volume.

Calcium

In this report, three major approaches were considered in deriving the AIs for calcium—calcium balance studies of subjects consuming variable amounts of calcium, a factorial model using calcium accretion based on bone mineral accretion data, and clinical trials which investigated the response of change in bone mineral content/density or fracture rate to varying calcium intakes. The prepublication version of this report estimated per cent maximal calcium retention derived from calcium balance data as one of the three major approaches considered to develop the recommended intakes for calcium. Subsequent comments received following the report's release in prepublication form indicated concerns with the statistical methodology used to obtain such estimates from the available balance data. In response to the technical issues raised, the DRI Committee determined for this final printed version that it would estimate *desirable* calcium retention in place of estimating the percent of maximal retention, using the same data and statistical methodology as was included in the prepublication version (see Appendix E).

Where sufficient data were available, values from balance studies for individual subjects within specific age groups were applied to a nonlinear mathematical model recently used by Jackman et al. (1997) which describes the relationship between varying calcium intakes and retention. The equation derived from this model was then solved to determine the calcium intake required to achieve retention of the desirable amount of calcium. The *desirable* retention varied by age group but for the most part reflected accretion of calcium in bone based on bone mineral accretion data available for some of the age groups.

Another major approach considered by the Committee to estimating intake needed to maintain calcium adequacy was the factorial method. This is based on combining estimates of losses of calcium via various routes by apparently healthy individuals and then assuming that these represent the degree to which calcium intake, as corrected by estimated absorption, will balance these losses. The weakness of using this approach alone is that the data come from different studies, in different subjects, and the variation in absorption, particularly depending on previous intake, may be significant. The third approach derives calcium requirements from the few available clinical trials in which addditional calcium was given and changes in bone mineral content or density or in fracture rate were measured over time.

Comparison of the intakes needed to achieve desirable calcium retention or maintain minimal calcium loss using each of these three methods gave reasonable confidence and concordance to the levels of intake recommended as AIs. Thus the recommended AI for each life stage group is an approximation of the calcium intake that would appear to be sufficient to maintain calcium nutriture for almost all the individuals in the specific group. It is also recognized that the ability to maximize calcium retention may not be limited by calcium intake alone since there are many other factors that affect calcium retention, such as growth velocity (in children), hormonal status, gender and ethnic backgrounds, other diet components, and genetic patterns. Evidence to support this is cited in the study by Jackman et al. (1997), which demonstrated that the further into puberty the teenage girls were, the lower their relative calcium retention was even though calcium intake remained the same. In addition, calcium retention would be expected to oscillate above and below a mean value at the calcium intake levels tested, which often were intended to approximate or exceed the subjects' usual intakes. Additional consideration of the approach used is included in Chapter 4.

The decision to set AIs rather than EARs and thus RDAs for calcium was based on the following concerns: (1) uncertainties in the methods inherent in and the precise nutritional significance of values obtained from the balance studies that form the basis of the desirable retention model, (2) the lack of concordance between observational and experimental data (mean calcium intakes in the United States and Canada are much lower than are the experimentally derived values required to achieve average desirable calcium retention), and (3) the lack of longitudinal data that could be used to verify the association of the experimentally derived calcium intakes for achieving a predetermining calcium retention with the rate and extent of long-term bone loss and its clinical sequelae, such as fracture. Taking all of these factors into consideration it was determined that an EAR for calcium could not be established at the present time. The recommended AI represents an approximation of the calcium intake that, in the opinion of the DRI Committee and its Panel on Calcium and Related Nutrients, would appear to be sufficient to maintain calcium nutriture while recognizing that lower intakes may be adequate for many; however, this evaluation will have to await additional studies on calcium balance over broad ranges of intakes and/or of long-term measures of calcium sufficiency.

Vitamin D

The AI is the intake value that appears to be needed to maintain, in a defined group of individuals with limited but uncertain sun exposure and stores, serum 25-hydroxyvitamin D concentration above a defined amount. The latter is that concentration below which vitamin D deficiency rickets or osteomalacia occurs. The intake value was rounded to the nearest 50 IU, and then doubled as a safety factor to cover the needs of all, regardless of their sun exposure.

Fluoride

The AI is the intake value that reduces the occurrence of dental caries maximally in a group of individuals without causing unwanted side effects. For fluoride, the data are strong on risk reduction, but the evidence on which to base an actual requirement is scant, thus driving the decision to adopt an AI as the reference value.