Supplementation Trials with Calcium Citrate Malate: Evidence in Favor of Increasing the Calcium RDA During Childhood and Adolescence[1,2]

MARK B. ANDON, TOM LLOYD* AND VELIMIR MATKOVIC[†]

The Procter & Gamble Company, Sharon Woods Technical Center, 11450 Grooms Rd., Cincinnati, OH 45242, *Department of Obstetrics and Gynecology, College of Medicine, The Pennsylvania State University, Milton S. Hershey Medical Center, Hershey, PA 17033, and [†]Bone & Mineral Metabolism Laboratory, Department of Physical Medicine, The Ohio State University, Columbus, OH 43210

ABSTRACT The vast majority of peak adult bone mass is accumulated by the time longitudinal growth is complete. As peak bone mass is an important determinant of future fracture risk, the goal of the current calcium recommended dietary allowance during youth is to provide a calcium intake that allows individuals to reach their full genetic potential for acquiring skeletal mass. The advent of controlled trials of calcium supplementation and total body bone mass measurements in children and adolescents provide the first direct way of determining the amount of calcium necessary to achieve optimal skeletal accretion. These studies indicate that the current RDAs are insufficient to support optimal bone mass gain during growth and development. Based on the recent intervention trials, recommendations are made for an RDA of 1250 mg during childhood and 1450 mg during adolescence. These values are consistent with established calcium balance intake thresholds for growth during pre-adolescence and adolescence. J. Nutr. 124: 1412S–1417S, 1994.

INDEXING KEY WORDS:

- bone mass • calcium • childhood
- recommended dietary allowance
- calcium requirement • citrate malate

RATIONALE FOR CURRENT RDA

Unlike most nutritional essential minerals, the distribution of calcium in the total body is heavily skewed to bony tissue with 99% residing in the skeleton and teeth. As such, the function of dietary calcium from a mass standpoint is largely defined in terms of bone mineralization and the resultant density and strength of the skeleton. Insufficient accretion of skeletal mass during growth, de-velopment and skeletal consolidation appears to predispose a person to fractures in adulthood. Thus, in the latest revision of the recommended dietary allowances (RDA) a greater emphasis was placed on the need for adequate calcium intake in early life (National Research Council 1989). The RDA subcommittee states that "the most promising nutritional approach to reduce the risk of osteoporosis in later life is to ensure a calcium intake that allows the development of each individual's genetically programmed peak bone mass." The spirit of this goal in setting the calcium RDA during childhood and adolescence is imminently reasonable. However, direct support of the appropriateness of the RDA via the conduct of controlled human intervention trials was not available when the 10th edition of the dietary allowance was published. Indeed, the RDA subcommittee qualifies the calcium allowance of 800 mg for children ages 1 to 10 y as being arbitrary due to a lack of data.

The allowance for adolescents is somewhat less arbitrary in that it is based on a model containing two assumptions. First, calcium absorption is efficient in youth and is conservatively estimated at 40%.

TABLE 1

Fractional calcium absorption in adolescents and adults[1]

Calcium source	Absorption		Subjects	Reference
	%	P		
Calcium citrate malate	36 ± 9		Male and female adolescents	Miller et al. (1988)
Calcium carbonate	26 ± 8	<0.03	age 9–17 y, n = 12 group	
Calcium citrate malate	41 ± 8		Male and female adolescents	Miller et al. (1989)
Calcium carbonate	27 ± 8	<0.05	age 11 to 17 y, n = 6/group	
Calcium citrate malate	37 ± 5		Female adults age 21 to 30 y,	Smith et al. (1987)
Calcium carbonate	30 ± 6	<0.01	n = 10/group	

[1] Values are means ± SD. Each study used a 250 mg oral calcium dose consumed with a standardized low calcium meal.

Second, the subcommittee recognizes that skeletal calcium accretion can be as high as 400 to 500 mg/d during puberty. Using these estimates, the rationale for the current RDA during adolescence is depicted by arithmetic statement *a*. This model is simple and appears to neatly account for calcium demands during rapid growth. However, it has several drawbacks. First, the model ignores losses of newly absorbed calcium through fecal, dermal, and renal routes and assumes that absorbed calcium equates on a one-to-one molar basis to calcium retained in the skeleton. Second, the model is very sensitive to the estimate for fractional absorption. For example, in arithmetic statement *b*, if a 30% absorption efficiency is used, then an intake of 1600 mg is required to produce the necessary skeletal calcium accretion.

Calcium intake	Fraction absorbed		Net skeletal calcium retained
a) 1200 mg/d ×	0.4	=	480 mg/d
b) 1600 mg/d ×	0.3	=	480 mg/d

The sparse amount of absorption data available for adolescents is summarized in **Table 1** and shows that adolescents are no more efficient at absorbing calcium than adults. Furthermore, these data indicated that an assumed absorption efficiency of ~40% is greater than that observed experimentally. The meta-analysis of calcium balance studies compiled by Matkovic and Heaney (1992) also suggest that an assumed absorption efficiency of 40% is an overestimate. Mean net calcium absorption values calculated from 88 balance studies in preadolescents and 112 balance studies in adolescents were 27 and 30%, respectively.

With respect to the RDA subcommittee's approach of estimating skeletal calcium accretion during growth as a means of estimating the calcium allowance, a number of models have been proposed for this purpose (**Table 2**). The most direct information comes from measurements of total body bone mineral content and indicates that the mean gain in skeletal calcium content of girls is about 340 mg/d during the rapid growth of puberty. Since half of the population

TABLE 2

Calculated peak skeletal calcium accretion rates during adolescence

Reference	Skeletal calcium accretion	Sex	Basis for calculation
	mg/d		
Mitchell et al. (1945)	361	Male	Growth curve for body weight
Leitch and Aiken (1959)	394	Male	Growth curve for body weight
Peacock (1991)	310	Male	Dimensions of second metacarpal
	285	Female	
	210	Male	Midshaft radius bone mineral density
	165	Female	
Matkovic et al. (1994)	194	Female	Total body bone mineral content by age
	340	Female	Total body bone mineral content by pubertal stage

TABLE 3

Baseline characteristics of identical twin children who completed a controlled trial of calcium citrate malate (CCM) supplementation[1]

| | Prepubertal $n = 22$ pairs Age = 6.9 ± 1.4 y | | Pubertal $n = 23$ pairs Age = 10.6 ± 2.0 y | |
	CCM	Placebo	CCM	Placebo
Weight, *kg*	22.5 ± 3.3	23.0 ± 3.7	38.4 ± 11.2	39.3 ± 13.6
Height, *cm*	120 ± 8	121 ± 8	145 ± 13	145 ± 13
Pubertal stage	1	1	2.3 ± 1.4	2.2 ± 1.4
Dietary calcium intake, *mg/d*	896 ± 194	888 ± 173	888 ± 202	924 ± 179
Sex, %				
Male		36		22
Female		64		78

[1]Table values are means ± SD from the data of Johnston et al. (1992).

is above the mean and accretion rates are greater for males, then the subcommittee's assumption of 400 to 500 mg Ca/d would appear to be adequate to cover the majority of the adolescent population. However, there is a major flaw in this approach. The model assumes that the study population used to calculate skeletal calcium accretion is optimal with respect to acquisition of bone mass. Thus, although the rate of calcium accretion needed to produce a given bone mass can be calculated, the level of calcium needed to produce optimal bone mass accumulation remains unknown. A more direct approach to answering this question is the conduct of controlled intervention trials of calcium supplementation and bone mass gain during growth and development.

CONTROLLED INTERVENTION TRIALS WITH CALCIUM CITRATE MALATE

Results from double-blind, placebo controlled, calcium supplementation studies in children and adolescents are described below. In all cases, calcium citrate malate was the supplemental source of calcium. At equimolar calcium doses, this source of calcium has been shown to yield greater fractional absorption than milk or calcium carbonate (Miller et al. 1988 and 1989, Smith et al. 1987) and to retard bone loss in older postmenopausal women to a greater extent than calcium carbonate (Dawson-Hughes et al. 1990). Calcium citrate malate (trade name FruitCal) is used commercially to fortify certain fruit juice drinks (Sunny Delight Plus Calcium and Hawaiian Punch Plus Calcium, Procter & Gamble, Cincinnati).

Johnston et al. (1992) reported the effects of three years of calcium citrate malate supplementation on bone density gain in identical twin children. One twin from each of 45 pairs that completed the study

received a supplement of 1000 mg elemental Ca/d while the co-twin control received placebo. The study cohort was partitioned into subjects who remained prepubertal throughout the study and those who were postpubertal at base line or entered puberty during the study (**Table 3**). Total calcium intake in the prepubertal calcium citrate malate supplemented group exceeded that of the placebo group (1615 ± 288 vs. 888 ± 173 mg/d) and was accompanied by greater mean increases in bone mineral density at all six skeletal sites measured (**Fig. 1**). In contrast, increased calcium intake was not associated with significantly greater bone density gains in pubertal subjects even though supplementation raised total calcium intake from 924 ± 179 to 1593 ± 185 mg/d. Johnston et al. (1992) suggested that the effects of puberty may so dominate mineral accretion that the more modest effects of dietary intervention are difficult to detect. This, coupled with the relatively small number of subjects studied, may account for the failure to observe an effect of supplementation in these pubertal subjects.

Further assessment of the effects of calcium supplementation on bone mass accretion during puberty has been performed in healthy adolescent females recruited from the Hershey, PA, and Columbus, OH, areas. Study protocols were approved by the appropriate institutional review boards at the Pennsylvania State and Ohio State Universities, and informed consents were obtained from the participants and their parent(s) or legal guardians. All participants were premenarchal Caucasian females in the early stages of reproductive development (mean pubertal stage = 2).

Total body bone mineral content via dual energy X-ray absorptiometry (Lunar Radiation Corp., Madison, WI and Hologic Inc., Waltham, MA) was measured at base line and after 6 mo of calcium intervention.
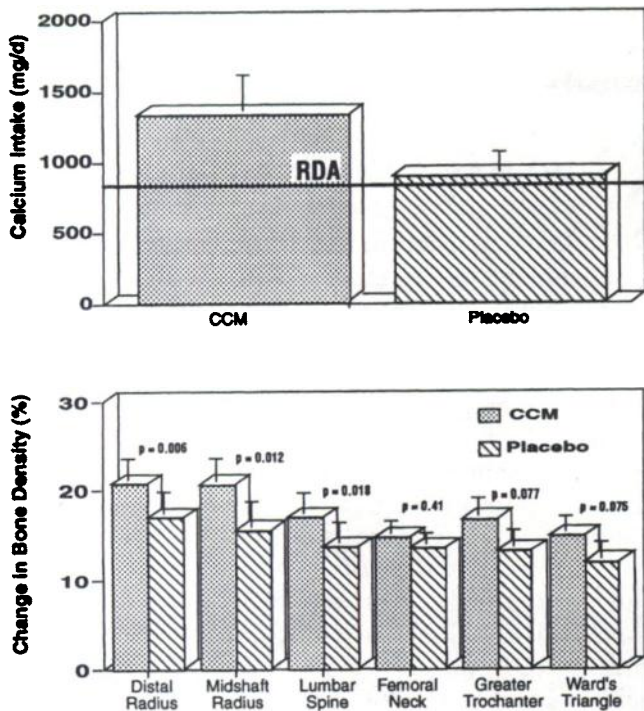
FIGURE 1 Calcium intake from diet and calcium supplementation in prepubertal twins from the study by Johnston et al. (1992). Subjects consumed their self-selected diets for a 3 y period and received placebo or 1000 mg supplemental calcium per day as calcium citrate malate (CCM). Values are the means ± SD intakes calculated from 39 dietary records per subject and supplement compliance records collected on a monthly basis. B) Mean ± SEM gain in bone mineral density from base line in the prepubertal twins described in A. P values were calculated by paired, two-tailed, t tests.

TABLE 4

Clinical characteristics at enrollment of adolescent girls who received placebo or calcium supplementation[1]

	Placebo		Calcium	
n	128		120	
Age, y	11.3 ±	0.8	11.4 ±	0.8
Height, cm	147 ±	7	147 ±	7
Weight, kg	41 ±	8	41 ±	7
Pubertal stage	2.1 ±	0.6	2.1 ±	0.6
Dietary calcium intake, mg/d	884	± 290	884	± 297
Total body bone mineral content, g	1311	± 246	1299	± 261

[1]Table values are means ± SD.

During the intervention period, subjects at Hershey were randomly assigned to receive placebo or 500 mg elemental Ca/d as calcium citrate malate. Subjects at Columbus received placebo or 1000 mg elemental Ca/d as calcium citrate malate. All participants continued consuming their normal self-selected diets and received no specific counseling related to food intake. Dietary calcium intake at entry was measured by collecting three 24-h dietary records. The records were completed by the subjects and their parents and then reviewed by a dietitian or trained research assistant. Supplemental calcium intake was measured by counting the number of returned tablets. Gain in total body bone mineral content in the supplemented and placebo groups was compared by t tests. In addition, each subject's gain in total body bone mineral content was indexed to the mean gain of their respective placebo group. In this way, three treatments—placebo, 500 mg supplemental Ca/d and 1000 mg supplemental Ca/d were compared by ANOVA and a multiple range comparison test.

Clinical characteristics of the participants assigned to placebo or calcium citrate malate treatment did not differ at base line (Table 4). Total body mineral content at entry was not significantly different between the calcium citrate malate and placebo groups within each investigative site or between sites. During the 6 mo intervention period there was no difference between the calcium citrate malate and placebo supplemented subjects with respect to gain in height (3.4 vs. 3.3 cm), weight (2.8 vs. 2.9 kg), or pubertal progression (0.5 vs. 0.5 pubertal stage units).

As expected for healthy growing children, total body bone mineral content increased significantly from base line in both the placebo and supplemented groups ($P < 0.0001$). The gain in total body bone mineral content was not significantly different between the placebo groups at the two sites and averaged (mean ± SEM) 125 ± 6 g. In contrast, subjects who received 1000 mg of supplemental calcium had significantly greater increases in total body bone mineral content than those supplemented with only 500 mg Ca/d ($P < 0.05$). The mean ± SEM additional gain in skeletal mass beyond that experienced by the respective placebo treated subjects was 13 ± 7 g for subjects supplemented with 500 mg Ca/d and 29 ± 7 g for subjects supplemented with 1000 mg Ca/d.

In further analyses, the data from both institutions were combined to yield a pooled placebo group, an intermediate calcium supplemented group (500 mg Ca/d) and a high calcium supplemented group (1000 mg Ca/d). Mean ± SD total calcium intakes from diet and supplementation for these subjects were 888 ± 290, 1315 ± 287, and 1618 ± 288 mg/d, respectively. The effect of calcium supplementation was significant ($P < 0.02$) with the increase in total body bone mineral content lowest for placebo, intermediate for subjects supplemented with 500 mg Ca/d, and greatest for those receiving 1000 mg supplemental Ca/d (Fig. 2).

Reasons for the difference in results from these adolescents and the pubertal twins studied by Johnston et al. (1992) may have been caused by a
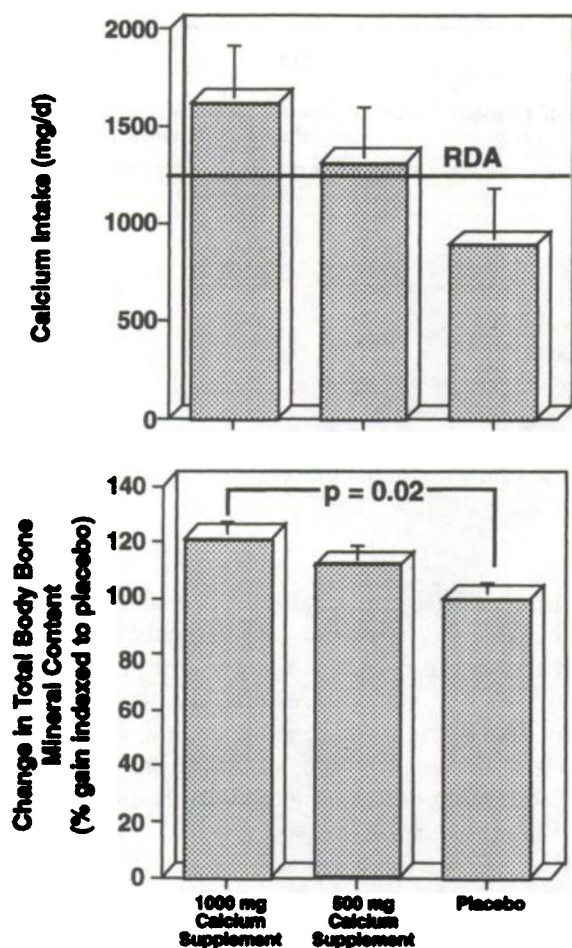
FIGURE 2 *A)* Total calcium intake from dietary and supplemental sources in adolescent females. For 6 mo, subjects consumed their self-selected diets and were randomly assigned to placebo, 500 mg supplemental calcium per day as calcium citrate malate (CCM), or 1000 mg supplemental Ca/d as CCM. Values (mean ± SD) were calculated from three 24-hr dietary records collected at base line and by counting the number of returned supplement tablets. *B)* Increase (mean ± SEM) in total body bone mineral content in adolescent girls who received the treatments described in *A.* The change in total body bone mineral content for each subject was indexed to the mean gain in the placebo group which was set at 100%. A one-way ANOVA for the effect of calcium supplementation was significant, $P < 0.02$. The placebo group differed significantly from the 1000 mg calcium group when analyzed by Tukey's multiple range comparison test ($P = 0.02$).

number of factors. They studied a substantially smaller group. Moreover, the range in age and pubertal stage in the current study was far narrower than in the study of pubertal twins. For example, although the mean pubertal stage at base line in the studies were comparable (2.2 compared with 2.1) the SD about the mean for the pubertal twins was 2.5-times greater (1.4 vs. 0.6). Because puberty is a strong determinant of bone mass gain, it seems likely that relatively large groups of subjects closely matched with respect to reproductive development

may be required in order to examine the effect of nutritional influences on bone mass gain during adolescence.

SUMMARY AND CONCLUSIONS

The latest revision to the RDA occurred before the establishment of the threshold intake of calcium for growth and the advent of controlled trials of calcium intervention and bone mass acquisition during childhood and adolescence. In the intervention studies, the magnitude of increase in bone acquisition afforded by calcium supplementation above the RDA equates to approximately 10% of 1 SD in bone mass per year. From prospective studies in adults, the relative risk of fracture is known to change by as much as 100% for every 1 SD change in bone mass (Hui et al. 1989). Thus, the additional gain in bone associated with long-term calcium intakes above the current RDA during youth would be predicted to yield a significant reduction in lifetime fracture risk. The fact that calcium citrate malate was the supplemental source of calcium used in these studies strengthens the evidence that intakes substantially above the current RDA are required to optimize bone mass acquisition during childhood and adolescence (Matkovic and Ilich 1993). Although additional calcium intake from any source would presumably be beneficial, greater intakes may be required if less well absorbed calcium sources are ingested. This situation is analogous to correcting iron deficiency anemia by ingesting hydrolytically reduced iron compared to iron sulfate, two iron salts with differing bioavailabilities. Clearly, less total iron would be required if supplied as the sulfate salt. Calcium absorption from calcium citrate malate is known to be high (Miller et al. 1988 and 1989, Smith et al. 1987) and Dawson-Hughes et al. (1990) reported that at equal molar calcium doses calcium citrate malate was more effective for reducing bone loss in postmenopausal women than $CaCO_3$. Calcium absorption from dairy products, the primary source of calcium in the U.S. diet, has consistently been shown to be equivalent to $CaCO_3$ (Recker et al. 1988, Sheikh et al. 1987, Smith et al. 1987). Thus, calcium bioavailability can be important in interpreting the results of intervention studies and their potential impact on selecting appropriate RDA values.

An important question that cannot be addressed with the current data is whether increased calcium intake during youth provides for a sustained increase in bone mass in adulthood. It is possible that increasing calcium intake during growth and development may simply accelerate the attainment of peak bone mass. A direct and unequivocal answer to this question may never be provided because of the logistical requirements that such a study would

entail. However, cross-sectional and retrospective studies of adult bone mass among individuals with varying calcium intakes during youth suggest that the differences in bone mass obtained during childhood do persist into adulthood (Halioua and Anderson 1989, Matkovic et al. 1979, Sandler et al. 1985). Thus, a prudent approach at this time is to use the intervention trials as a framework to revise the calcium RDA to more appropriate values. For preadolescents, intakes of the placebo and supplemented groups averaged 888 and 1618 mg Ca/d, respectively. Given that the current RDA for this age group has no empirical basis, it seems reasonable to recommend a calcium intake for preadolescents at the midpoint, namely, 1250 mg. This value is similar to the calcium intake threshold of 1380 mg calculated by Matkovic and Heaney (1992) for pre-adolescents. For adolescents, increasing calcium intakes from a mean of 1315 to 1618 mg was associated with continued improvement in total body bone mineral accretion. Again, a conservative approach would be to select the midpoint of 1450 mg. Once more, this value is in good agreement with the threshold intake of 1480 mg during adolescence calculated by Matkovic and Heaney (1992) from their meta-analysis of the calcium balance literature.

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