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## *PERSPECTIVES*

# Adolescence: How Do We Increase Intestinal Calcium Absorption to Allow for Bone Mineral Mass Accumulation?

**Steven A. Abrams[1] and Gordon J. Strewler[2]**

***[1]USDA/ARS Children's Nutrition Research Center, and Texas Children's Hospital, Department of Pediatrics, Baylor College of Medicine, Houston, Texas, USA***
***[2]Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts, USA***

**Abstract**

An increase in calcium absorptive efficiency (fractional absorption of dietary calcium) during adolescence is associated with a rapid increase in total body bone mineral mass (BMM) accumulation. This increase occurs across a range of calcium intakes. It appears to be principally mediated by hormonal changes of puberty including increases in insulin-like growth factor-1 (IGF-1), luteinizing hormone (LH) and estrogen. Calcium supplementation during adolescence has led to short-term increases in measures of BMM, but has not had a consistent long-term benefit. This is likely due to the efficient nature of catch-up of mineralization throughout adolescence and early adulthood as long as calcium intake is not severely deficient and other dietary factors are adequate. Vitamin D is needed for active (transcellular) calcium absorption of calcium. However, in contrast to adults, no close relationship between serum 25-hydroxyvitamin D (25-OHD) concentration and calcium absorption in non-vitamin D-deficient adolescents has been demonstrated. No data are available to identify an optimal 25-OHD concentration in adolescents, although avoidance of very low levels is necessary. The routine supplementation of adolescents with high dose vitamin D (e.g., more than about 400 IU/d) is not justified based on currently available data unless specific risk factors for low vitamin D status or malabsorption of vitamin D exist. Other interventions that may enhance the absorption of calcium require further evaluation but may be of importance. For example, prebiotics have been demonstrated to enhance calcium absorption and BMM acquisition, but longer-term studies are needed. For most adolescents, a combination of avoiding a very low calcium intake (< 600-800 mg/d), and maintaining a diet with adequate amounts of other essential bone nutrients will lead to adequate calcium absorption and BMM accumulation during adolescence. Revision of dietary guidelines for calcium and vitamin D to create a full range of recommendations is urgently needed. *BoneKEy-Osteovision*. 2007 May;4(5):147-157.


*Keywords*: Calcium absorption; Bone mineral; Adolescence; Peak bone mass

## Introduction: Adolescence and Peak Bone Mineral Mass

Adolescence is a critical time for the accumulation of bone mineral mass (BMM). Close to half of all total body bone mineral content (BMC) acquisition occurs in girls over a narrow window of 4 to 5 years beginning with the onset of pubertal development (1-4). Achieving the genetically "preprogrammed" rate of bone mineral accumulation and thus optimal "peak bone mass" during adolescence is a critical part of public policy to decrease the rate of postmenopausal osteoporosis (3;5;6). It may also be important in decreasing adolescent fractures, although the data to support this effect are not compelling at this time (7-9). A meta-analysis has recommended that further data are needed from controlled intervention trials related to fracture prevention and BMM in children (10).

The optimal method for achieving the genetically determined peak bone mass for an individual is uncertain. The most obvious approach, increasing calcium intake during adolescence, has not been convincingly shown to increase long-term BMM accumulation. Over a dozen well-conducted, blinded, controlled intervention trials have demonstrated a *short-term* increase in some measure of BMM or density, usually total body bone mineral content (BMC) or bone mineral density (BMD). However, several of these trials included a follow-up period after supplementation or a long-term supplementation. These studies have shown little if any maintenance of a benefit to BMM or density after calcium supplementation was stopped. In the longest supplementation study in adolescents, very little benefit was demonstrated for 7 years of supplementation (7;11-14). Some of this is due to substantial limitations in study design, especially regarding whether studies had adequate power to follow this effect over time. However, it appears that adolescents and young adults are able to catch-up in BMM as long as they are not severely deficient in calcium intake or vitamin D status.

Therefore, efforts have centered on a variety of strategies other than promoting high calcium intakes or calcium supplementation to support BMM accumulation. Further physiological data have focused on the interaction of hormones, genes, exercise and diet in promoting BMM accrual (12;15).

**Factors Affecting Calcium Absorption and Peak Bone Mass**

To consider these, we will initially note genetic and hormonal effects on peak BMM and then consider intervention strategies and the status of these interventions on supporting BMM accumulation. Finally, we will try to summarize these factors into a current perspective on this issue.

It is clear that the single largest determinant of peak bone mass and BMM accumulation during adolescence is genetic. Traditionally recognized genetic factors are gender and race. More recently, a series of genetic polymorphisms associated with the vitamin D receptor have been recognized as affecting BMM. Data, however, are minimal in adolescents compared to adults. We found a relationship between calcium absorption and *Fok1* polymorphisms of the vitamin D receptor gene. Other polymorphisms associated with bone mineral density in adults are less clearly associated with BMM in adolescents (16). It is likely that this distinction is due to variable timing of the effects of different genes in relationship to their effects on calcium absorption and bone mineral metabolism. However, little is actually known regarding these factors and we are far from identifying any specific genes that are a risk factor for low BMM accumulation during adolescence.

An additional genetic factor that has been identified in determining BMM is stature. In both adolescents and adults, there is a small but significant relationship between height and calcium absorption (17;18). Rationally, one could expect that the relationship is such that providing more calcium and absorbing more calcium will lead to an increase in height. However, except in the case of truly deficient intake of calcium as has been described in parts of Africa (19) or extremely low absorption (as in severe vitamin D deficiency), this is unlikely. Rather, it is more likely that the genetically programmed ultimate height affects the rate at which calcium is absorbed in order to provide substrate for this growth. However, we are far from establishing or understanding these effects or even the etiology of different levels of calcium absorption from the diet of African-Americans compared with Caucasians (20). Of note is that in one controlled trial of calcium supplementation, supplementation was only significantly beneficial in those who had a height greater than the mean height of the study group (14). However, translating these types of findings into dietary guidelines or clinical practices will be difficult.

A difficult factor to assess has been the effects of reproductive and growth hormones on the pattern and accumulation of BMM. Bone calcium deposition peaks 6-12 months

prior to puberty (21), near the peak of linear growth. Clear relationships between bone formation and skeletal calcium utilization and serum concentrations of luteinizing hormone (LH), IGF-1 and estrogen have been described (22-24). We reported that changes in calcium metabolism and utilization for bone formation were associated with maturation of the hypothalamic-pituitary axis, as measured by the GnRH-simulated LH level, and occurred after initial increases in estradiol in most girls (22). Small increases in estradiol are probably the earliest marker of pubertal changes before physical changes are observed, as estradiol stimulates these physical changes. Pubertal increases in calcium gain and bone calcium deposition were paralleled by changes in IGF-I and biochemical markers of bone formation including alkaline phosphatase activity and serum osteocalcin.

Studies of hormone administration in normal subjects and disease states are consistent with important effects of sex steroids on calcium absorption. Administration of testosterone to prepubertal boys induced large changes in calcium absorption (25;26). Growth hormone and IGF-1 levels were also increased by testosterone therapy; hence, the increase in calcium absorption could be either a direct effect of testosterone (possibly after conversion to estrogen) or an indirect effect mediated by somatotrophic hormones. Treatment of GH-deficient adults for eight weeks with growth hormone or IGF-1 had no significant effect on calcium absorption, however (27). Some information in adolescents is available from disease “models” (28). For example, adolescent girls with anorexia nervosa have lower rates of calcium absorption and bone formation than girls who are not anorectic (29). They have increased levels of serum cortisol and lower serum estrogen levels. Girls with Turner syndrome responded to a regimen of growth hormone and low dose estrogen with increased bone formation rates determined using calcium stable isotopic methods. Estrogen treatment of girls with Turner syndrome produced significant changes in bone formation rate (30;31), but growth hormone alone did not (30).

The adolescent growth spurt itself depends on both growth hormone and estradiol in normal girls and boys. Several lines of evidence support the primacy of estradiol in the control of the growth spurt. Hypopituitary patients deficient in both growth hormone and gonadotropins do not have an adolescent growth spurt when growth hormone alone is replaced (32;33); gonadal steroids must also be given. In addition, after treatment with an LHRH analog for sexual precocity, patients with growth hormone deficiency and true precocious puberty have a significant decrease in growth velocity (34). Estradiol is the prime driver of the pubertal growth spurt in boys as well as girls. A pubertal growth spurt occurs in individuals with complete androgen resistance (35). In prepubertal boys, serum estrogen concentrations are correlated with peak growth velocity but not with serum growth hormone levels (36).

How would sex steroids or growth hormones effect a substantial increase in calcium absorption during puberty? One possibility is that they may directly increase the efficiency of intestinal calcium absorption. Calcium absorption takes place by a passive paracellular route and an active transcellular transport mechanism (37). The rate-limiting step in transcellular intestinal calcium transport is entry across the brush border, which takes place via the vitamin D-responsive TRPV6 calcium channel. Estrogen treatment of rodents leads to large increases in the number of TRPV6 calcium channels on the enterocyte, an effect that is independent of vitamin D (38;39). Estrogen treatment also induces smaller increases in calbindin-9K, which ferries calcium across the cell, and in expression of the plasma membrane calcium pump PMCA1b, which extrudes calcium from the basolateral side of the enterocyte into the blood (39). Estrogen treatment induces marked vitamin D-independent increases in intestinal calcium absorption (40-42). Whether testosterone, growth hormone or IGF-1 directly affects the levels of these calcium transporters is unknown.

An estrogen- or growth hormone-responsive increase in the number of calcium

transporters would be predicted to increase the efficiency of calcium transport at any given level of calcium intake and vitamin D, and such a change could in theory account for much of the increase in calcium absorption that is required to support rapid bone growth in the peripubertal period. Bone calcium deposition falls off after menarche, however, in the face of continued high estradiol levels (21), suggesting that additional factors are involved in controlling calcium absorption. As noted above (42;43), there is also a significant relationship between height and calcium absorption, indicating that the extent of genetically programmed calcium accretion into bone affects the rate at which calcium is absorbed.

The increased rate of calcium accretion into bone during the pubertal growth spurt, by reducing serum calcium, could induce an increase in intestinal calcium absorption via PTH and vitamin D. In cross-sectional studies, the serum 1,25-dihydroxyvitamin D concentration increases in the peripubertal period, but the changes are relatively small (43). In a longitudinal study of a relatively vitamin D-replete population of young American adolescents, serum PTH levels were indeed correlated with fractional calcium absorption but accounted for only 9.6% of the variance (24), and the serum 1,25-dihydroxyvitamin D concentration was more weakly correlated with fractional calcium absorption. Although increased calcium deposition in bone, as mediated by the PTH/vitamin D system, may not be the primary regulator of the adolescent increase in calcium absorption, it is likely that effects of vitamin D, sex hormones and growth hormone are synergistic.

To unravel further these hormonal effects, simultaneous determinations of the associations of serum estrogen, IGF-1, PTH and serum 1,25-dihydroxyvitamin D concentrations with calcium absorption and bone formation in longitudinal studies of puberty would be useful. If calciotropic hormones couple the demands of bone growth to intestinal calcium absorption, it might be predicted that adult height, as a surrogate of the extent of bone growth, is a predictor of the PTH and serum 1,25-dihydroxyvitamin D concentrations during puberty. Absent the stimulatory effect of estrogen on calcium absorption, growth hormone therapy of growth hormone-deficient prepubertal children might be expected to cause larger increases in PTH and 1,25-dihydroxyvitamin D than normal puberty; some evidence to suggest this has been reported (44;45). To clarify which of the pubertal hormones directly increase intestinal calcium absorption, it will also be important to determine whether growth hormone or IGF-1 has direct effects on transporter levels or intestinal calcium absorption, and to ascertain the relative efficacy of androgens versus estrogen as inducers of intestinal calcium transport.

**Intervention Strategies to Increase Absorbed Calcium**

Intervention strategies to increase the total amount of absorbed calcium and thus total body BMM have generally focused on the following; increases in calcium intake, increase in vitamin D intake (or sun exposure), and increasing the bioavailability of dietary calcium using other dietary factors such as prebiotics or by providing more soluble salts of calcium (46). This last method has minimally been applied to healthy children and adolescents after infancy, as the solubility of most calcium salts, including calcium carbonate, should be adequate. Other dietary factors related to bone have had more limited evaluation in pediatrics, including increasing intakes of vitamin K, boron, zinc, magnesium and long-chain poly-unsaturated fatty acids (47;48). Several studies have further looked in combination at increasing absorbed calcium while also increasing exercise so as to increase both BMM and bone volume. This may lead to greater mineral density as well as BMC and thus decreased fracture risk (49).

Strategies to promote increased calcium intake among adolescents have been largely ineffective, and supplementation studies have had minimal effect on BMM over a long period of time (7;11;14). The current “adequate intake” for calcium of 1300 mg

per day was derived by the National Academy of Sciences based on data indicating that this value approximated an asymptotic intake amount above which further calcium intake would not lead to increased calcium absorption or total body calcium retention (5;6).

The accuracy of both this calcium intake value and of the concept of a fixed asymptotic value for maximal calcium retention are uncertain. Rather, it is likely that there is no absolute value above which no or negligible further calcium can be absorbed or if there is such an intake, it is much higher than 1300 mg/d. On the other hand, the long-term catch-up of BMM suggests that reaching a "near asymptote" may be unnecessary. This has led to suggestions that excessive public health emphasis on achieving calcium intakes of 1300 mg/d, a level far beyond usual adolescent intakes and not achieved by > 80% of adolescent girls, may be somewhat misplaced. Rather, emphasis on overall healthy diets and lifestyle to support bone growth and mineralization should be provided.

This does not mean that adequate calcium intake should not be encouraged, however. Very low calcium intakes, especially those < 600-800 mg/d, are unlikely to be associated with maximal or near maximal total calcium absorption or BMM accretion (5;11;20;50;51). Catch-up mineralization with such a severely deficient intake may not be possible, although the exact level that is "too low" is unknown and may be affected by gender and ethnicity. Furthermore, it may be preferable to achieve higher levels of calcium absorption during *early puberty* both to enhance the likelihood of reaching peak BMM and to avoid an increased risk of fractures. It should be noted that it is unlikely that an intake of 1300 mg/d or more of calcium is harmful (up to an unknown maximum, probably at least 2500 mg/d for most adolescents), but additional strategies are needed to increase the total *absorbed* calcium (20).

**Vitamin D and Calcium Absorption in Adolescents**

One potential approach to increasing calcium absorption at lower calcium intakes is to increase vitamin D status and thus increase transcellular calcium absorption. In studies in adults, a combination of calcium and vitamin D supplementation leads to important increases in BMD and fewer fractures (5;52). However, similar research data are not available for adolescents. Few data are available specific to vitamin D supplementation, and these data sets are not convincing, especially for American populations (53).

Of physiological importance is that there appears to be a difference in the relationship between vitamin D status and calcium absorption in children and adolescents compared to adults. The available data in adults indicate a relatively linear increase in calcium absorption with serum 25-OHD concentration up to about 35 ng/mL (54). These data, as well as results from supplementation trials, have led to a strong push to increase vitamin D status in adults using a variety of methods (55).

In children and adolescents, however, the data are less clear regarding this relationship. Low maternal vitamin D status has been linked to low BMM in infants and children, although confirmatory and safety data, especially in an American cohort, are needed before recommendations can be made regarding maternal high dose supplementation for this purpose (56;57). Nutritional vitamin D deficiency rickets in older infants and small children is well recognized and has increased in recent years, although as rickets is not a reportable disease, the true incidence and secular trends are unknown in the United States (58;59). Although some cases of adolescent rickets have been reported globally, this is not a common concern among most populations in the United States (60).

The relationship between serum 25-OHD concentrations and calcium absorption in children remains somewhat puzzling. The inverse relationship between 25-OHD levels

and parathyroid hormone levels seen in adults is also seen in adolescents in multiple studies (24;61). In adolescents, no specific consequences of moderately low 25-OHD concentrations or elevated PTH concentrations have been clearly demonstrated. Cheng et al. (62) found some evidence for lower bone mineralization in early adolescent (10-12-year-old) girls in Finland with low serum 25-OHD or high PTH. Effects were seen primarily in girls with serum 25-OHD < 10 ng/mL, a level well below that commonly seen in most adolescents in the United States.

Furthermore, it appears that adolescents adapt to lower 25-OHD concentrations and increased serum PTH by increasing 1,25 dihydroxyvitamin D concentrations and maintaining calcium absorption (24). Our studies and smaller additional trials have all been cross-sectional, however. There are no controlled trials of vitamin D supplementation in adolescents in which calcium absorption was used as an endpoint, and there are negligible data in the U.S. relating specifically to vitamin D supplementation in adolescents and any outcomes. Pending such data, the cross-sectional data do not support a role for recommended vitamin D supplements in most adolescents substantially beyond those contained in the current dietary recommendations as a means of enhancing peak BMM (5). Clearly, avoidance of very low vitamin D intakes and very low serum 25-OHD concentrations is necessary, especially when solar exposure is limited, but high dose supplementation or a specific target serum 25-OHD concentration in the population above 30 ng/mL, as has been suggested for adults, is not supported by currently published data.

The current dietary recommendation for vitamin D, as with calcium, is an “adequate intake” (200 IU/d). The use of an adequate intake, a single, poorly-defined reference value, for both calcium and vitamin D intakes is highly inappropriate as current data are more than adequate to develop both an estimated adequate requirement (EAR) and a recommended dietary requirement (RDA). Many other nutrients about which far less scientific data exist have these guidelines in place. These values would greatly enhance dietary planning for both individuals and populations and should be developed as soon as possible as a matter of highest priority.

It is possible that a higher value than the current adequate vitamin D intake of 200 IU/d for adolescents would be determined to be an appropriate estimated average requirement or RDA. However, dietary recommendations well above this level, e.g., intakes above 400-500 IU/d, must be tested in controlled trials in adolescents before being advocated. Outcomes that must be tested would include calcium absorption, BMM accumulation and safety parameters including serum and urinary calcium concentrations. Such trials should be conducted in both northern and southern parts of the United States. They should be multi-ethnic and be conducted across multiple seasons. Long-term outcomes, including overall fracture rates during adolescence and BMM in adulthood after adolescent supplementation, would be ideal outcomes, although more difficult to assess within a reasonable time period.

**Other Dietary Factors**

Other dietary factors may also enhance calcium absorption and BMM accumulation (63-66). We have shown that the addition of a prebiotic inulin-type fructan to the diet of young adolescents increases calcium absorption and increases total body BMC and BMD (46). The effect is relatively small, but may be of importance given the increased use of such products and the persistence of low calcium intakes.

Other dietary factors are increasingly being considered in enhancing peak BMM. These include a possible role for long-chain polyunsaturated fatty acid supplementation. Current data are limited, however, and principally derived from animal models (66). A role for supplementation for other nutrients, especially magnesium and zinc, has been considered (63;64). No trials exist for these nutrients related to BMM in adolescents. A deficiency of other nutrients

may also be a factor in peak bone mass. These may include boron and vitamin K (63;65). However, no data exist in adolescents, nor any suggestion that interventions with these nutrients may be of benefit.

## Summary and Conclusions

In summary, a programmed large increase in fractional absorption of calcium occurs during early adolescence. This increase appears to be mediated by hormonal changes of puberty and does not appear to be associated with serum 25-OHD concentrations as long as overt vitamin D deficiency is avoided. From an evolutionary perspective, these conclusions are consistent with the speculation that adolescent bone growth, like pregnancy and lactation, has evolved to achieve success with minimal reliance on calcium and vitamin D intake. Interventions to enhance the total absorption of calcium using dietary strategies or supplements to increase calcium intake have had minimal long-term success in the United States and have not been widely accepted by adolescents.

Ultimately, it is necessary to further define the factors needed to approximate the maximal (peak) BMM. Increasing the bioavailability of calcium in the diet, enhanced exercise and increased vitamin D intake are all possibilities that have been explored minimally in adolescents compared to adults. Long-term efficacy data are especially weak. It is likely in the future that further genetic studies will increase the confidence in any approach as being likely to benefit an individual or subset of the population. Pending such data, avoidance of very low calcium intakes (< 600-800 mg/d), provision of adequate dietary vitamin D, and appropriate lifestyle habits including exercise may be recommended in adolescents as well as children. Urgent revision of dietary requirements for calcium and vitamin D to provide both an estimated average requirement and an RDA is needed.

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