Proceedings of the Nutrition Society (2001), **60**, 283–289
DOI: 10.1079/PNS200082

Calcium turnover and nutrition through the life cycle*

Steven A. Abrams
US Department of Agriculture/Agricultural Research Service, Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas 77030, USA

Ca nutritional requirements and bone Ca turnover can be assessed using numerous techniques. Among these techniques are bone mass measurements, tracer kinetic studies, bone histomorphometry and biochemical studies. Stable-isotope-based kinetic studies offer unique advantages in their ability to assess both Ca absorption and turnover. This approach is safe and readily applicable to subjects of all ages. Ca is essential for growth and maintenance of bone mineral throughout life. During pregnancy, increased intestinal absorption of Ca by the mother provides much of the Ca supplied to the fetus. During infancy, Ca supplied in human milk is primarily derived from maternal bone stores, which are quickly replenished during and after weaning. Early childhood is a time of relatively slow bone growth, with a rapid increase occurring during puberty. Recent kinetic studies demonstrate an increase in both Ca absorption and bone Ca deposition associated with early puberty. Bone Ca deposition reaches a maximum in females shortly before menarche. At that time the bone Ca deposition rate is approximately five times that of adulthood. The decline in bone Ca deposition rate is gradual after menarche. Ca absorption from the diet shows a gradual decline in adulthood as well. Ca supplementation, in the presence of adequate vitamin D, is effective in enhancing bone mineral content in childhood and in helping to maintain bone mineral content in adults. Maintaining adequate Ca nutriture throughout life may be necessary to minimize the risk of bone-loss disorders.

Calcium: Bone turnover: Stable isotopes

Identification of calcium nutritional needs and bone calcium turnover

In view of the substantial health and economic impact of osteoporosis on society, it is important to understand developmental changes in bone Ca turnover and the nutritional consequences of these changes during the life cycle. Considerable data regarding Ca absorption were collected in the early part of the century. Bone Ca turnover data in adults were collected via the pioneering radioisotope studies of Bronner, Heaney and other researchers, beginning in the 1950s (Bronner & Harris, 1956; Heaney & Whedon, 1958). A large gap remains, however, in knowledge of bone Ca turnover in children. To understand the recent emphasis on the importance of Ca in childhood and adolescence, it is important to understand the methodologies used to assess Ca nutrition and bone Ca turnover.

The traditional method for determining Ca requirements is mass Ca balance. In this technique, net Ca retention is determined from simultaneous measurements of Ca intake and excretion. The effects of different levels of Ca intake on net Ca retention are calculated and an attempt is made to determine the optimal Ca intake level based on these data (Matkovic, 1991). Mass balances, however, have numerous limitations, especially when applied to paediatric populations. These limitations include potential errors in accurately identifying both intake and total excretion of minerals, and the high cost and substantial difficulty of conducting long-term nutrition balance studies. It is especially difficult to maintain long-term dietary regulation and to perform accurate and complete urine and faecal collections in children. Moreover, mass balances do not provide direct information regarding bone Ca turnover, or readily allow for determination of nutrient interactions (Abrams, 1999).

Radioactive isotopes of Ca (^{45}Ca and ^{47}Ca) have been widely utilized during the past 50 years to measure Ca absorption and bone Ca turnover. Although the radiation exposure from these tracers is relatively small, their use is not considered appropriate in healthy children or during pregnancy and lactation. In addition, a decrease in the use of

Corresponding author: Dr S. A. Abrams, fax +1 713 798 7119, email sabrams@bcm.tmc.edu
*This work is a publication of the US Department of Agriculture (USDA)/Agricultural Research Service (ARS) Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX, USA. Contents of this publication do not necessarily reflect the views or policies of the USDA, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

radioactive mineral tracers in healthy adults may be occurring because of the radiation exposure and the cost of sample disposal.

The rate at which bone is formed and resorbed ('turnover') can be assessed by multiple methods. The 'gold standard' method is bone histomorphometry. In this technique, labelling of bone is performed with a tracer such as tetracycline. Subsequently, bone biopsies are obtained for histological section (Clarke *et al.* 1996), and bone volume and bone formation and resorption rates are determined. Although this technique is well suited for evaluation of the effects of therapies such as bisphosphonates on bone kinetics, it has only infrequently been used to evaluate changes associated with normal growth and development for two reasons. First, tetracycline is not safe for administration to small children or pregnant women. Second, bone biopsies are inherently invasive, and thereby limited in their applicability to healthy children or adolescents. Although adults may more readily undergo this procedure, it is relatively expensive, and not well suited to large population studies. One approach that may be useful, especially in children, is to perform bone biopsies on subjects who are undergoing unrelated surgical procedures. Recently, Glorieux *et al.* (2000) used this approach to collect histomorphometric data on a group of children and adolescents. Ultimately, increasing the database of information through this method may further allow for the identification of normal changes in bone histomorphometry related to growth and pubertal development.

In view of the inherent limitations in obtaining large amounts of histomorphometric data, biochemical markers to assess bone turnover have been developed for both clinical and research use. Use of these markers may ultimately allow for important information to be gained relatively non-invasively regarding therapeutic interventions for osteoporosis or other bone diseases. This is an area that is rapidly evolving, as is the understanding of both the utilization and the limitations of various possible markers. For example, the accuracy of bone turnover markers in predicting bone loss during ageing (Dennison *et al*. 1999) has been recently questioned. Furthermore, the use of these markers in children remains investigational, and their use in understanding bone changes related to normal growth may be limited. For example, in one study bone turnover markers were not closely related to rates of bone gain in pubertal girls (Cadogan *et al*. 1998). Nonetheless, the ease of obtaining urine or serum needed for these measurements makes them well suited for further investigation. The continued development of new markers may ultimately lead to their more widespread use in clinical and research studies of paediatric bone mineral metabolism.

Techniques for stable-isotope tracer kinetic studies

The use of stable non-radioactive tracers of Ca allows safe assessment of Ca absorption and bone Ca pool masses and turnover rates in subjects of all ages, including pregnant and lactating women. This technique, described below, is used in studies of young adolescents who participate in our research studies at Baylor College of Medicine, Houston, TX, USA.

Written informed consent is obtained from a parent or legal guardian for each subject. Additionally, written assent is obtained from all study subjects of suitable ages. All protocols are fully reviewed and approved by The Institutional Review Board of Texas Children's Hospital/Baylor College of Medicine. There are no known risks specifically related to the use of stable isotopes in the doses described here for isotope studies. All isotopes are prepared for human use by the pharmacy of the Texas Children's Hospital and are tested for sterility and, where appropriate for pyrogenicity before use. Details of the production, supply and costs related to these isotopes have been reviewed recently (Abrams, 1999). There are six naturally-occurring Ca isotopes. Of these, five are of low abundance (^{42}Ca, ^{43}Ca, ^{44}Ca, ^{46}Ca and ^{48}Ca) and may readily be used for human research studies.

After an overnight fast (except for water), an intravenous line is inserted in the early morning and blood is withdrawn for any desired biochemical or hormonal analysis. Subsequently, each subject is given a breakfast containing approximately 300–350 mg Ca. Towards the end of breakfast, subjects are given one stable isotope of Ca (we typically administer ^{46}Ca at a dose of 0·4 μg/kg) which has been premixed (and allowed to equilibrate in the refrigerator for 12–24 h) with 120 ml milk or Ca-fortified juice. Subsequently, a second Ca isotope (we typically administer ^{42}Ca at a dose of 80 μg/kg) is infused over 2 min via the intravenous catheter or a separately-placed infusion needle. Blood samples (2 ml) are removed for MS analysis of Ca isotope enrichment at 6, 12, 20, 30, 45, 60, 120, 180, 240 and 480 min after completion of the infusion.

Beginning with breakfast, a complete 24 h urine collection is obtained as 8 h samples. For complete studies in which an accurate measurement of secretory Ca losses is to be performed, subjects continue their in-patient stay for at least 6 d, and a complete urine and stool collection is made during this time. However, this procedure is not necessary for most studies in which the end-point goal is a measurement of Ca absorption and bone Ca deposition rate. In this case, subjects may generally be discharged after 24 h and intermittent urine collection (we prefer at least two to three urine samples per d) maintained for a total of at least 5 d (Abrams *et al*. 1996).

Samples are analysed for isotopic enrichment using magnetic sector thermal-ionization MS (Finnigan MAT 261; Finnigan, Bremen, Germany). Accuracy and precision of this technique for natural-abundance samples compared with standard data are ≥0·15 %, depending on the ratio being measured.

The compartmental model we use for Ca kinetic determinations is similar to that described by Neer *et al.* (1967). The model is based on three sequential pools before Ca deposition in the 'deep' bone Ca pool. The bone Ca deposition rate is the rate of flow of Ca to the final pool. These models can be used for the indirect determination of the bone Ca removal rate. Compartmental modelling of the data is performed with the aid of the simulation, analysis and modelling program (Berman & Weiss, 1978).

An exciting prospect is the use of a very-long-term tracer, ^{41}Ca, in kinetic studies (Freeman *et al*. 1997). This tracer may be administered at extremely low concentrations with

https://doi.org/10.1079/PNS200082 Published online by Cambridge University Press

very minimal radiation exposure, and can be followed in the subject for many years. This procedure would allow accurate measurements of long-term bone formation and resorption which are not feasible with standard radioactive or stable tracers. Unfortunately, access to ^{41}Ca and analytical methods for its determination is currently limited.

Assessment of calcium turnover and nutrition during the life cycle

Overview

The present review will consider representative data relating to Ca requirements and bone turnover during specific periods of the life cycle. The primary focus will be on data relating to children and pregnant and lactating women, with brief mention of information relating to healthy adults.

Fetal development

At birth the fetus contains approximately 30 g Ca, representing approximately 2·5 % of the typical maternal body Ca stores (Abrams *et al*. 1992). In a young adolescent who has not achieved peak bone mass the percentage may be higher. Evidence suggests that in adult women much of this 30 g comes from increases in dietary Ca absorption during pregnancy (Heaney & Skillman, 1971; Yergey *et al*. 1995; Ritchie *et al*. 1998). Increased body pool masses of Ca and bone Ca turnover during pregnancy are closely related to fetal Ca requirements (Table 1).

The possibility of increasing Ca intake during pregnancy has been considered, weighing potential benefits relating to maternal bone mass and fetal bone development as well as a reduced risk of hypertensive disorders of pregnancy. Remarkably, however, controlled trials have not been able to consistently identify such benefits (Allen, 1998). Thus, current US dietary recommendations do not indicate a need for Ca to be provided at levels greater than the age-appropriate levels (National Academy of Sciences, 1997).

A recent report by Koo *et al*. (1999) found a small but statistically significant ($P < 0·05$) increase in the bone mineral content of infants whose mothers received 2 g Ca/d during pregnancy when the maternal diet was very low in Ca intake. No benefit was seen in other groups, and there is no reason to believe that supplementation above recommended levels of intake is important for fetal bone mineralization.

Bone turnover rates cannot generally be measured directly in the fetus. Bone histomorphometry after tetracycline labelling was performed in two fetuses at approximately 18 weeks gestation (Glorieux *et al*. 1991). In comparing results with those obtained from autopsy specimens from full-term infants they reported that bone and cartilage volume, and trabecular thickness increased while osteoid thickness and indices of resorption decreased in the second half of the gestation period. The authors concluded that fetal bone matrix mineralization is already highly organised at mid-gestation.

An alternate approach to assessing fetal bone turnover is to perform studies in premature infants. We performed Ca stable-isotope kinetic studies in a group of healthy premature infants who were receiving full enteral feedings (Abrams *et al*. 1994). On a body-weight basis, the infants had higher bone Ca deposition rates than at any other time of life (Table 1). Furthermore, when analysed as a function of net Ca absorption, bone Ca deposition increased markedly and significantly as net Ca absorption increased (r 0·70, $P < 0·01$). This finding is in contrast to that with regard to older individuals in whom bone Ca deposition is a relatively constant function of absorption. Our findings indicate that premature infants are readily able to absorb and utilise Ca intake levels which greatly exceed those of older children or adults. Another finding of this study was the relatively smaller response of the bone Ca resorption rate to Ca absorption for the premature infants (r –0·39, $P = 0·18$). This finding also stands in contrast to data in adults, in whom bone Ca resorption constitutes the major regulatory response. This distinction reflects the relatively greater importance of new bone formation in infants compared with the greater importance of bone remodelling in adults.

Infancy

There are no data regarding the long-term consequences of different Ca intake levels or feeding sources provided to full-term infants. In a group of healthy breast-fed 5- to 7-month-old infants we calculated a net Ca retention of approximately 70 mg/d (Abrams *et al*. 1997).

Studies evaluating the bone mineral content of full-term infants during the first year of life have generally found a slightly higher value for those fed infant formulas than those fed human milk (Specker *et al*. 1997). However, it is uncertain whether this difference is maintained later in childhood. At present, there is no reason to recommend that high levels of Ca be given to infants, either from infant formulas or weaning foods. Human milk with limited supplementation with weaning foods in the second 6 months of life should remain the standard for infant Ca intake. It is also important to maintain adequate vitamin D status during later infancy to allow for maximal Ca absorption.

Table 1. Bone calcium deposition rates during the life cycle (Abrams *et al*. 1992, 1994, 1996)

Group	Age (years)	Weight (kg)	Bone Ca deposition (mg/kg per d)	Total bone Ca deposition (mg/d)
Premature infants	–	1·34	160	214
Infants	0·8	8	90	720
Prepubertal	8·3	28	52	1456
Pubertal	10·2	35	56	1960
Postpubertal	15·4	59	21	1240
Adults	30–60	60	5–10	300–600

https://doi.org/10.1079/PNS200082 Published online by Cambridge University Press

Data are sparse on Ca turnover and kinetics in healthy infants. Available radiotracer and stable-isotope data suggest values intermediate between those of premature infants and older children (Table 1). It is likely that a rapid drop-off of bone Ca deposition occurs, with the late-childhood–adolescent level of approximately 50 mg/kg per d achieved by the third or fourth year of life (Abrams *et al*. 1992).

Childhood

Few data are available regarding Ca requirements in children before puberty. We recently found an increase in net Ca absorption when the intake of Ca in 3- to 5-year-old children was increased from 500 to 1200 mg/d (Ames *et al*. 1999*b*). The benefit was relatively modest, however, and intermediate intake levels, as might more readily be achieved in preschool children, were not evaluated in this study. The potential benefit to ultimate peak bone mass of increasing Ca intake in this age-group has been studied in groups of prepubertal children. In one controlled Ca supplementation trial an increase in bone mass was found when Ca supplements were given to children as young as 6 years of age (Johnston *et al*. 1992). However, relatively few children this young were studied, and the duration of effect of this supplementation and its impact on peak bone mass are uncertain. Further studies are needed to evaluate different levels of Ca intake in this age-group.

High levels of Ca intake may negatively affect the absorption of other minerals such as Fe and Zn. These minerals may be marginal in toddlers and preschool children, especially in developing countries. Thus, more data regarding the risks and benefits of high Ca intake are needed in children and adolescents. It is likely, however, that adaptation to high Ca intakes occurs such that Fe status and Fe absorption are not harmed over a long period of time (Ilich-Ernst *et al*. 1998; Ames *et al*. 1999*b*). It is reasonable to conclude that greater consumption of Ca-rich foods and beverages can safely be encouraged in small children.

Bone turnover has been measured in healthy prepubertal children using stable-isotope tracer techniques as well as biochemical markers. These data, shown in Table 1, are considered in relation to data in pubertal children.

Puberty

In view of the importance of the pubertal growth spurt in determining ultimate bone mass, this time period has become one of the most studied for evaluation of Ca dietary requirements as well as bone mineral acquisition and turnover. Most, but not all, studies have focused on girls, due to the greater incidence of osteoporosis in females and the shorter duration of pubertal bone growth in girls *v*. boys.

The onset of puberty frequently begins in girls at 9–11 years of age. In a longitudinal multi-ethnic study we found a significant ($P < 0{\cdot}05$) increase in the absorption and bone Ca deposition rate of girls in this age-group, associated with the onset of physical and hormonal signs of puberty (Abrams *et al*. 2000). This finding is consistent with the new lower age of 9 years at which the US National Academy of Sciences (1997) recommended increased Ca intake increase.

In understanding the rapid mineralization related to puberty, it is important to consider the effects of pubertal hormones. We found that changes in Ca metabolism and bone Ca deposition were significantly ($P < 0{\cdot}05$) associated with maturation of the hypothalamic–pituitary axis, as measured by the level of luteinizing hormone, and occurred after initial increases in oestradiol in most girls.

Our studies further demonstrated a sharp decrease in the rate of absorption and skeletal gain of Ca beginning within 12–24 months of menarche (Abrams & Stuff, 1994; Abrams *et al*. 1996). This finding is consistent with data from Weaver *et al*. (1997) who also reported decreased rates of bone Ca deposition within a few years of menarche.

Identification of the optimal intake level of Ca for adolescents has been primarily based on achieving a maximum level of Ca retention. These data were used to establish 1300 mg/d as the adequate intake level in the USA (National Academy of Sciences, 1997). Most of the data from which this intake level was derived came from studies of Caucasians of European descent. Similarly, most studies of Ca supplementation in adolescents have been performed in those populations.

To evaluate the possible effect of ethnicity on Ca metabolism, we measured Ca absorption and kinetics in groups of Caucasian, African-American and Mexican-American girls. Among subjects receiving diets with low Ca levels we found increased Ca absorption in African-American girls relative to Caucasian girls post-menarche (Abrams *et al*. 1995). Smaller ethnic differences were seen before menarche. In contrast, we did not identify any difference in Ca absorption or kinetics in prepubertal 7- and 8-year-old Mexican-American girls relative to Caucasians (Abrams *et al*. 1999). Further studies are needed, however, to identify the adaptation by children of different ethnic groups to very low Ca intakes, and to assess ethnic differences in the role of other factors, such as exercise, in Ca metabolism. At the present time, however, no data specifically indicate that dietary requirement levels should be adjusted based on ethnicity.

During the last 5 years there has been considerable interest in the role of genetic markers in determining bone mass. We recently evaluated the relationship between Ca absorption and polymorphisms of the vitamin D receptor gene in seventy-two healthy children age 7–12 years (Ames *et al*. 1999*a*). We found that the *Fok* 1 polymorphism at the vitamin D receptor translation initiation site was significantly positively associated with Ca absorption ($P = 0{\cdot}04$). Children who were FF homozygotes had a mean Ca absorption that was 41·5 % greater than those who were ff homozygotes, and 17 % greater absorption than Ff heterozygotes.

Eventually, if these data are confirmed, and further genetic links to bone mass, Ca absorption and kinetics are identified, it may be possible to target groups of children who, based on their genetic background, have a higher (or lower) risk of osteoporosis than other groups. If such identification can be made, it will then be necessary to conduct controlled trials to evaluate the effects of long-term levels of Ca and vitamin D intake on these risks. However, this level of knowledge remains quite distant, and, at present, the data do not support altering the Ca dietary

requirements based on available genetic factors. It seems reasonable to assume that the genetic component of osteoporosis is related to multiple genetic sites, and that these sites are only beginning to be understood.

Adults

Data regarding the dietary requirements for Ca in adults are based both on balance studies, such as those described for children and adolescents, and numerous Ca supplementation trials. Consideration of these data in detail is beyond the scope of the present review. In general, there appears to be a benefit to providing intakes of Ca and vitamin D above the levels frequently found in American and European populations (Chapuy *et al*. 1992). Although most studies have used Ca supplements in pill form, it is also feasible and perhaps optimal to provide some or all the additional Ca via dietary sources (Heaney *et al*. 1999).

Bone mineralization studies indicate that although peak bone mass is approached by the age of 15–16 years in most girls, there is a small increment in bone mass at some sites during the 18–30-year age range. This finding is consistent with our Ca kinetic studies in older adolescents, in whom we found the persistence of a higher bone Ca deposition rate 4–5 years post-menarche compared with later in adulthood (Abrams *et al*. 1996).

Data regarding bone Ca turnover changes in healthy adults are derived from several sources. Considerable bone histomorphometric data are available for healthy adults as well as those with osteoporosis. These data generally show the expected age-related decline in bone volume and mineral apposition rate in men and women (Recker *et al*. 1988; Clarke *et al*. 1996). Radioisotope kinetic studies show a similar pattern of changes (Bronner *et al*. 1963). Detailed description of these findings in adults is beyond the scope of the present review.

Substantial bone loss occurs in the early post-menopausal period in women. Using bone density measurements and biochemical bone-turnover markers, Mazzuoli *et al*. (2000) found that bone loss begins in the first year post-menopause and that this rapid bone loss phase is completed within 6 years (peaking during years 2–4) of menopause. It results in an approximately 15 % overall bone loss at the lumbar spine. In the initial post-menopausal period bone turnover is increased, with bone resorption appearing to predominate over bone formation. After this time it appears that there is a relative favouring of bone formation again, which slows down the rate of bone loss.

Lactation

During lactation, a period of 6 months of exclusive breast-feeding would lead to the secretion by the mother of an additional 45 g Ca. Although some of this Ca is provided by decreased urinary Ca excretion during lactation, there is extensive evidence demonstrating a loss of maternal bone Ca during lactation (Cross *et al*. 1995; Kalkwarf *et al*. 1997). However, in adult women bone remineralization occurs post-weaning, and neither pregnancy nor lactation is associated with persistent bone loss (Hopkinson *et al*. 2000). Recent data indicated that Ca supplementation is not effective in preventing lactation-associated bone loss or enhancing post-weaning bone mass recovery (Kalkwarf *et al*. 1997). Thus, US dietary recommendations do not suggest increases in Ca intake during lactation above the 1000 mg/d recommended for non-lactating adult women (National Academy of Sciences, 1997). Similarly, these recommendations do not suggest increased intake during lactation for adolescents above the age-appropriate levels.

Lactation-associated increases in bone formation and resorption with low- and normal-Ca diets have been found in stable-isotope studies of Ca kinetics, as well as in studies using biochemical markers of bone turnover (Yergey *et al*. 1995; Prentice *et al*. 1998). Although the stable-isotope studies involved few subjects, they support the basic pattern of increased bone resorption relative to bone formation during lactation which has been suggested by studies using biochemical markers.

Consideration of some unanswered questions

There have been tremendous advances in understanding bone mineral nutrition during the last 20 years. Widespread use of bone mass measurements and improvements in evaluating Ca turnover have led to increased knowledge of normal bone development. Among the most important objectives in upcoming years is achieving a greater appreciation of the nature of variations in bone development and their genetic and biological basis. Fundamental to this goal is an increased knowledge of the molecular and cellular biology of bone development, as well as the consequences of lifestyle choices such as diet and exercise on bone development. This process will require an integration of basic and clinical science. Isotope-based studies and biochemical bone-turnover studies are among the techniques that will be needed to evaluate future genetic findings.

Acknowledgements

This project has been funded in part with federal funds from the USDA/ARS under Cooperative Agreement number 58-6250-6-001, the NIH, NCRR General Clinical Research for Children Grant number RR00188 and NIH AR43740.

References

Abrams SA (1999) Using stable isotopes to assess mineral requirements in children. *American Journal of Clinical Nutrition* **70**, 955–964.

Abrams SA, Copeland KC, Gunn SK, Gundberg CM, Klein KO & Ellis KJ (2000) Calcium absorption, bone mass accretion and kinetics increase during early pubertal development in girls. *Journal of Clinical Endocrinology and Metabolism* **85**, 1805–1809.

Abrams SA, Copeland KC, Gunn SK, Stuff JE, Clarke LL & Ellis KJ (1999) Calcium absorption and kinetics are similar in 7- and

8-year-old Mexican-American and Caucasian girls despite hormonal differences. *Journal of Nutrition* **129**, 666–671.
Abrams SA, Esteban NV, Vieira NE, Sidbury JB, Specker BL & Yergey AL (1992) Developmental changes in calcium kinetics in children assessed using stable isotopes. *Journal of Bone and Mineral Research* **7**, 287–293.
Abrams SA, O'Brien KO, Liang LK & Stuff JE (1995) Differences in calcium absorption and kinetics between black and white girls age 5–16 years. *Journal of Bone and Mineral Research* **10**, 829–833.
Abrams SA, O'Brien KO & Stuff JE (1996) Changes in calcium kinetics associated with menarche. *Journal of Clinical Endocrinology and Metabolism* **81**, 2017–2020.
Abrams SA, Schanler RJ, Yergey AL, Vieira NE & Bronner F (1994) Compartmental analysis of calcium metabolism in very low birth weight infants. *Pediatric Research* **36**, 424–428.
Abrams SA & Stuff JE (1994) Calcium metabolism in girls: current dietary intakes lead to low rates of calcium absorption and retention during puberty. *American Journal of Clinical Nutrition* **60**, 739–743.
Abrams SA, Wen J & Stuff JE (1997) Absorption of calcium, zinc and iron from breast milk by 5- to 7-month-old infants. *Pediatric Research* **41**, 384–390.
Allen LH (1998) Women's dietary calcium requirements are not increased by pregnancy or lactation. *American Journal of Clinical Nutrition* **67**, 591–592.
Ames SK, Ellis KJ, Gunn SK, Copeland KC & Abrams SA (1999*a*) Vitamin D receptor gene *Fok*1 polymorphism predicts calcium absorption and bone mineral density in children. *Journal of Bone and Mineral Research* **14**, 740–746.
Ames SK, Gorham BM & Abrams SA (1999*b*) Effects of high vs low calcium intake on calcium absorption and red blood cell iron incorporation by small children. *American Journal of Clinical Nutrition* **70**, 44–48.
Berman M & Weiss M (1978) *SAAM Manual. USDHEW Publication* no. (NIH) 78–180. Washington, DC: US Government Printer.
Bronner F & Harris RS (1956) Absorption and metabolism of calcium in human beings, studied with calcium^{45}. *Annals of the New York Academy of Sciences* **64**, 314–325.
Bronner F, Richelle LJ, Saville PD, Nicholas JA & Cobb JR (1963) Quantitation of calcium metabolism in postmenopausal osteoporosis and in scoliosis. *Journal of Clinical Investigation* **42**, 898–904.
Cadogan J, Blumsohn A, Barker ME & Eastell R (1998) A longitudinal study of bone gain in pubertal girls: anthropometric and biochemical correlates. *Journal of Bone and Mineral Research* **13**, 1602–1612.
Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD & Meunier PJ (1992) Vitamin D_3 and calcium to prevent hip fractures in elderly women. *New England Journal of Medicine* **327**, 1637–1642.
Clarke BL, Ebeling PR, Jones JD, Wahner HW, O'Fallon WM, Riggs BL & Fitzpatrick LA (1996) Changes in quantitative bone histomorphometry in aging healthy men. *Journal of Clinical Endocrinology and Metabolism* **81**, 2264–2270.
Cross NA, Hillman LS, Allen SH & Krause GF (1995) Changes in bone mineral density and markers of bone remodeling during lactation and postweaning in women consuming high amounts of calcium. *Journal of Bone and Mineral Research* **10**, 1312–1320.
Dennison E, Eastell R, Fall CH, Kellingray S, Wood PJ & Cooper C (1999) Determinants of bone loss in elderly men and women: a prospective population-based study. *Osteoporosis International* **10**, 384–391.
Freeman S, King JC, Vieira NE, Woodhouse LR & Yergey AL (1997) Human calcium metabolism including bone resorption measured with ^{41}Ca tracer. *Nuclear Instruments and Methods in Physics Research* **123**B, 266–270.
Glorieux FH, Salle BL, Travers R & Audra PH (1991) Dynamic histomorphometric evaluation of human fetal bone formation. *Bone* **12**, 377–381.
Glorieux FH, Travers R, Taylor A, Bowen JR, Rauch F, Norman M & Parfitt AM (2000) Normative data for iliac bone histomorphometry in growing children. *Bone* **26**, 103–109.
Heaney RP, McCarron DA, Dawson-Hughes B, Oparil S, Berga SL, Stern JS, Barr SI & Rosen CJ (1999) Dietary changes favorably affect bone remodeling in older adults. *Journal of the American Dietetic Association* **99**, 1228–1233.
Heaney RP & Skillman TG (1971) Calcium metabolism in normal human pregnancy. *Journal of Clinical Endocrinology and Metabolism* **33**, 661–670.
Heaney RP & Whedon GD (1958) Radiocalcium studies of bone formation rate in human metabolic bone disease. *Journal of Clinical Endocrinology and Metabolism* **35**, 1246–1267.
Hopkinson JM, Butte NF, Ellis K & Smith EO (2000) Lactation delays postpartum bone mineral accretion and temporarily alters its regional distribution in women. *Journal of Nutrition* **30**, 777–783.
Ilich-Ernst JZ, McKenna AA, Badenhop NE, Clairmont AC, Andon MB, Nahhas RW, Goel P & Matkovic V (1998) Iron status, menarche, and calcium supplementation in adolescent girls. *American Journal of Clinical Nutrition* **68**, 880–887.
Johnston CC Jr, Miller JZ, Slemenda CW, Reister TK, Hui S, Christian JC & Peacock M (1992) Calcium supplementation and increases in bone mineral density in children. *New England Journal of Medicine* **327**, 82–87.
Kalkwarf HJ, Specker BL, Bianchi DC, Ranz J & Ho M (1997) The effect of calcium supplements on bone density during lactation and after weaning. *New England Journal of Medicine* **337**, 523–528.
Koo WWK, Walters JC, Esterlitz J, Levine RJ, Bush AJ & Sibai B (1999) Maternal calcium supplementation and fetal bone mineralization. *Obstetrics and Gynecology* **94**, 577–582.
Matkovic V (1991) Calcium metabolism and calcium requirements during skeletal modeling and consolidation of bone mass. *American Journal of Clinical Nutrition* **54**, 245S–260S.
Mazzuoli G, Acca M, Pisani D, Dianinti D, Scarda A, Scarnecchia L, Pacitti MT, D'Erasmo E, Minisola S, Bianchi G & Manfredi G (2000) Annual skeletal balance and metabolic bone marker changes in healthy early postmenopausal women: Results of a prospective study. *Bone* **26**, 381–386.
National Academy of Sciences (1997) *Dietary Reference Intakes*. Washington, DC: National Academy Press.
Neer R, Berman M, Fisher F & Rosenberg LE (1967) Multicompartmental analysis of calcium kinetics in normal adult males. *Journal of Clinical Investigation* **46**, 1364–1378.
Prentice A, Jarjou LMA, Stirling DM, Buffenstein R & Fairweather-Tait S (1998) Biochemical markers of calcium and bone metabolism during eighteen months of lactation in Gambian women accustomed to a low calcium intake and in those consuming a calcium supplement. *Journal of Clinical Endocrinology and Metabolism* **83**, 1059–1066.
Recker RR, Kimmell DB, Parfitt AM, Davies KM, Deshawarz N & Hinders S (1988) Static and tetracycline-based bone histomorphometric data from 34 normal postmenopausal females. *Journal of Bone and Mineral Research* **3**, 133–144.
Ritchie LD, Fung EB, Halloran BP, Turnlund JR, Van Loan MD, Cann CE & King JC (1998) A longitudinal study of calcium

homeostasis during human pregnancy and lactation and after resumption of menses. *American Journal of Clinical Nutrition* **67**, 93–701.

Specker BL, Beck A, Kalkwarf H & Ho M (1997) Randomized trial of varying mineral intake on total body bone mineral accretion during the first year of life. *Pediatrics* **99**, E12.

Weaver CM, Peacock M, Martin BR, McCabe GP, Zhao J, Smith DL & Wastney ME (1997) Quantification of biochemical markers of bone turnover by kinetic measures of bone formation and resorption in young healthy females. *Journal of Bone and Mineral Research* **12**, 1714–1720.

Yergey AL, Abrams SA, Vieira NE, Esteban NV & Bronner F (1995) Calcium debit to the maternal skeleton during pregnancy and lactation. In *Kinetic Models of Trace Element and Mineral Metabolism*, pp. 29–35 [ME Wastney and KN Siva Subramaniam, editors]. Boca Raton, FL: CRC Press.