ELSEVIER

Nutrition 22 (2006) 1057–1066

NUTRITION

www.elsevier.com/locate/nut

Review article

Calcium nutrition and metabolism during infancy

J. Kirk Bass, M.D., and Gary M. Chan, M.D.*

Department of Pediatrics, Division of Neonatology, University of Utah Health Science Center, Salt Lake City, Utah, USA

Manuscript received December 20, 2005; accepted May 23, 2006.

Abstract Calcium is a vital mineral for the developing newborn infant. This review discusses perinatal and neonatal calcium metabolism, with an emphasis on enteral calcium absorption and the nutritional factors affecting calcium bioavailability including the three major endocrine hormones involved in calcium metabolism: parathyroid hormone, vitamin D, and calcitonin. The placenta transports calcium to the fetus throughout pregnancy, with the largest amount of fetal calcium accumulation occurring in the third trimester. At birth, the newborn transitions to intestinal absorption to meet the body's calcium needs. Most calcium is absorbed by paracellular passive diffusion in the small intestine. Calcium intestinal absorption is affected by the type and amount of calcium ingested. It is also affected by the amount of intestinal calcium that is bound to dietary fats and proteins. One major consequence of decreased calcium absorption is metabolic bone disease in which there is a failure of complete mineralization of the bone osteoid.

Keywords: Calcium; Phosphorus; Intestinal absorption; Bone mineral content; Metabolic bone disease

Introduction

Calcium (Ca) is the most abundant mineral in the human body. It is important for intracellular metabolism, bone growth, blood clotting, nerve conduction, muscle contraction, and cardiac functions. Ninety-nine percent of total body Ca is contained in the bones; approximately 1% of this Ca is freely exchangeable with the extracellular fluid. Three hormones—parathyroid hormone, vitamin D, and calcitonin—act in concert to maintain serum Ca concentration at a nearly constant level. These hormones direct intestinal Ca absorption, renal reabsorption, Ca excretion, and use of Ca stores in the bone.

Bone Ca accretion or accumulation is positive during the first 18 y of life, with the highest rate of accumulation occurring in the first year. When growth stops, bone mineral status decreases to a very low level. Peak bone mass is genetically determined [1]. Between 20 and 30 y of age, bone mass accumulation peaks and then slowly declines throughout life.

This review addresses perinatal and neonatal Ca metabolism, emphasizing enteral Ca absorption and the nutritional factors affecting Ca bioavailability.

* Corresponding author. Tel.: +801-585-7508; fax: +801-585-7395.
E-mail address: gchan@hsc.utah.edu (G. M. Chan).

0899-9007/06/$ – see front matter
doi:10.1016/j.nut.2006.05.014

Perinatal calcium metabolism

Calcium needs increase during pregnancy, mainly for fetal skeletal calcification. To meet these needs, maternal Ca absorption increases approximately 33%, with the maximum rate of increase occurring in the final trimester. The placenta actively transports Ca to the fetus and maintains total and ionized Ca at about 1 mg/dL above maternal levels. Thus, the fetus develops in a relatively hypercalcemic state. Placental active transport is driven by a magnesium adenosine triphosphatase-dependent Ca pump. This system enables Ca transfer to the fetus of up to 150 mg of Ca per kilogram of fetal weight/day in the third trimester, during which approximately 80% of fetal Ca accumulation and bone mineralization occurs. From 28 to 40 wk of gestation, fetal weight triples but Ca content quadruples due to increased bone mineral mass [2] (Fig. 1).

Active transport of Ca, and thus Ca homeostasis, is maintained by Ca-sensing receptors (CaSRs) that "sense" extracellular Ca levels and initiate the effectors of Ca homeostasis, i.e., parathyroid hormone (PTH) and vitamin D [3]. These receptors have been found in the placenta and regulate Ca transport from mother to fetus.

Maternal parathyroid activity, 1,25-dihydroxyvitamin D, Ca absorption, and Ca mobilization from bone increase during pregnancy to maintain maternal Ca homeostasis.

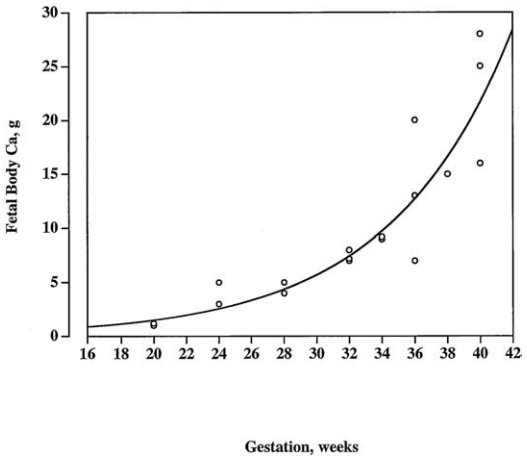

Fig. 1. Fetal body Ca accretion from 20 to 40 wk of gestation. Adapted from Widdowson [88] and Kelly et al. [89]. Ca, calcium.

Despite these changes, when approaching term, Ca transfer to the fetus leads to a modest decrease in maternal Ca levels. Fetal circulation is not affected by maternal PTH, 1,25-dihydroxyvitamin D, or calcitonin levels, because these hormones do not cross the placenta. However, 25-hydroxyvitamin D does cross the placenta, where it is hydroxylated to the physiologically active form, 1,25-dihydroxyvitamin D [4]. The action of maternal 25-hydroxyvitamin D on the fetus is unknown but probably aids in fetal bone mineralization by stimulating bone osteoblastic activity.

At birth, the rich, continuous supply of Ca to the fetus abruptly stops, decreasing fetal serum total and ionized Ca concentrations. In term infants, by 2 h of life there is a 5% decrease in serum total Ca. By 24–36 h serum total Ca concentration reaches its nadir of about 9.0 mg/dL (2.3 mmol/L) and the ionized fraction decreases to 4.9 mg/dL (1.2 mmol/L) [5]. Seventy-five percent of the decrease in total serum Ca occurs in the ionized fraction. Decreased Ca levels stimulate increased PTH release and 1,25-dihydroxyvitamin D formation, thus allowing the Ca concentration to stabilize and then gradually, during the first week of life, increase to levels similar to those found in childhood [5,6].

Hormones affecting calcium metabolism

The three major endocrine hormones involved in Ca homeostasis are PTH, vitamin D, and calcitonin (Table 1).

Parathyroid hormone

Secretion of PTH by the parathyroid glands is regulated by the circulating level of Ca ions sensed by the CaSR in the parathyroids. Elevation of serum Ca inhibits PTH secretion; a decrease in serum Ca stimulates secretion. Functional studies of parathyroid cells have demonstrated the presence of the CaSR, as in the placenta, which is capable of detecting physiologic changes of extracellular Ca and modulating PTH secretion [7,8]. The CaSR also allows regulation of renal tubular Ca reabsorption in response to changes in extracellular Ca concentration. The human CaSR gene is located on chromosome 3q13.3-q21. Loss of function CaSR mutations have been reported in hypercalcemic disorders [3,9,10].

Low serum Ca levels stimulate PTH production. In bone, PTH increases resorption by stimulating osteoblasts to release factors that increase osteoclast number and activity. Enhanced bone resorption increases serum Ca and phosphate concentrations. In the kidney, PTH increases Ca resorption and decreases phosphate resorption, causing increased phosphaturia. It also upregulates 1-α-hydroxylase activity and thus increases synthesis of 1,25-dihydroxyvitamin D, which causes increased intestinal Ca absorption. The overall result of PTH secretion is increased serum Ca and decreased serum phosphate concentration.

Parathyroid hormone levels are low in cord blood, suppressed by the hypercalcemia caused by active placental Ca transport. In term infants, the endocrine system response to postnatal Ca level changes is sufficient to maintain normal Ca levels. However, in preterm infants, even with hypocalcemia as a stimulus, PTH remains low for the first 2 d of life. During this time, a transient hypocalcemia exists. This “neonatal hypocalcemia” typically resolves within the first week of life and usually requires no treatment [11,12].

Vitamin D

Synthesis of vitamin D occurs in the skin when 7-dehydrocholesterol is exposed to ultraviolet-B light at a wavelength of 295–315 nm and is converted to previtamin D. Vitamin D is also absorbed from dietary sources in the duodenum and jejunum. Each of these forms of vitamin D is hydroxylated in the liver to 25-hydroxyvitamin D. Serum levels of 25-hydroxyvitamin D indicate adequacy of vitamin D stores from intake and sun exposure. A second hydroxylation step occurs in the kidney, forming the active metabolite 1,25-dihydroxyvitamin D.

Newborn stores of vitamin D are usually adequate

Table 1
Hormonal effects on Ca and P concentrations

	Effect on serum Ca	Effect on serum P
Parathyroid hormone	Increase	Decrease
Vitamin D	Increase	Increase
Calcitonin	Decrease	Decrease

Ca, calcium; P, phosphorus

unless there is significant maternal dietary inadequacy. Because skin synthesis of vitamin D requires exposure to ultraviolet-B light and most infants are not exposed to sunlight initially, the majority of vitamin D for infants comes from maternal and dietary sources. Levels of 25-hydroxyvitamin D change with gestational age and thus are lower in premature than in term infants. However, conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D occurs normally in premature infants [13].

In general, synthesis of 1,25-dihydroxyvitamin D in the kidney is increased by hypocalcemia, hypophosphatemia, and PTH [14]. Glucocorticoid use decreases synthesis of 1,25-dihydroxyvitamin D [15]. The concentration of 1,25-dihydroxyvitamin D in small-for-gestational-age infants is lower than in those born at weight appropriate for gestational age [16].

Vitamin D is the major hormone affecting intestinal Ca absorption and is required for effective PTH action on bone and intestines. As a steroid, vitamin D binds to genomic receptors in the intestinal epithelial cells and increases the synthesis of Ca binding proteins (calbindin). Increased levels of calbindin bind a larger portion of intracellular Ca, thus decreasing the intracellular ionized Ca concentration. This causes a change in the Ca concentration gradient between the intestinal lumen and intestinal epithelial cell and mediates increased diffusion of Ca intracellularly, leading to increased Ca absorption. A direct correlation exists between Ca absorption and the amount of Ca binding to calbindin. The use of theophylline has been shown to decrease Ca binding to these proteins, thus decreasing intracellular Ca transport and absorption [17]. Vitamin D also increases intestinal absorption of phosphorus. In bone, 1,25-dihydroxyvitamin D mobilizes Ca and phosphorus by increasing the differentiation of precursor cells (monocytes) into osteoclasts [18,19]. In the kidney, 1,25-dihydroxyvitamin D increases Ca and phosphorus resorption and provides negative feedback upon 1-α-hydroxylase activity. The overall result of this hormone is to increase serum Ca and phosphorus concentrations.

Calcitonin

Calcitonin is secreted by the parafollicular ("C") cells of the thyroid gland. Chronic fetal hypercalcemia leads to high calcitonin levels in the fetus and in the newborn. A positive feedback loop is responsible for further increases of calcitonin. The elevated levels of calcitonin in the newborn period inhibit Ca mobilization from bone by inhibiting osteoclastic bone resorption. Clinically however, it plays a minor role in the regulation of blood Ca levels. Disorders in calcitonin production have not been reported in infants.

Calcium intestinal absorption

Calcium intestinal absorption (percentage of enteral Ca absorbed) is determined by the amount of Ca ingested and the solubility of the Ca salt. In general, the more Ca ingested, the more Ca absorbed. Ca absorption, however, requires that Ca be ionized. If the dissolved Ca is complexed or bound to certain nutrients such as fats or phytates, Ca absorption is diminished.

In infants, Ca absorption rate (percentage of enteral Ca absorbed times intake, measured in milligrams per day) approximates Ca retention or bone accumulation [20,21]. Using mass spectrometric methodology, researchers administered two different isotopes of Ca, one orally and the other intravenously, and then measured true dietary absorption, endogenous fecal excretion, urinary loss, and net Ca retention. Total Ca retention was found to be almost identical to bone accumulation. In 12 preterm infants studied by Abrams et al. [20], net retention on intakes of 189–226 $mg \cdot kg^{-1} \cdot d^{-1}$ averaged 103 ± 38 $mg \cdot kg^{-1} \cdot d^{-1}$ or about 48% Ca retention. Endogenous gastrointestinal excretion was about 7% of the intake; urinary excretion was about 2%. Hillman et al. [21] found greater variability in fecal and urine excretion. These results showed a linear increase in Ca absorption rate with increasing Ca intake from 40 to 120 $mg \cdot kg^{-1} \cdot d^{-1}$, thus supporting the use of formulas containing high Ca content (at least up to 188.5 mg/100 kcal or 1520 mg/L) in an attempt to achieve fetal rates of Ca retention or bone accumulation in preterm infants.

During the first 6 mo of a term infant's life, bone mass accretion is directly related to mineral intake. Infants with low Ca intake during the first 6 mo can catch up by 12 mo if given a formula with high Ca concentration [22].

Hillman et al. [21] found that, in infants $<$1500 g and gestation $<$33 wk, the mean percentage of Ca retention per kilogram was not different between human milk and preterm formula, with or without Ca fortification, even though absorption, urinary excretion, and endogenous fecal excretion varied greatly. However, Ca-fortified formula (166 mg/100 kcal or 1340 mg/L) yielded a 41% greater average total retention of Ca ($82 mg \cdot kg^{-1} \cdot d^{-1}$) than did formula containing less Ca (117 mg/100 kcal or 940 mg/L; Ca retention of $58 mg \cdot kg^{-1} \cdot d^{-1}$). This study again validates the linear relation for Ca retention and intake up to 200 $mg \cdot kg^{-1} \cdot d^{-1}$ [23].

Several recent studies have reported attempts to improve bioavailability of Ca by adding compounds such as citric acid [24], casein phosphopeptides [25], or highly soluble salts such as Ca gluconate [26] or Ca gluconate-glycerophosphate [27]. Other studies have addressed the effect on Ca bioavailability of rice cereal added to infant formula [28]. None of these studies found a significantly increased Ca bioavailability.

Calcium absorption in the intestine occurs by passive and active mechanisms. In the newborn, the majority of Ca

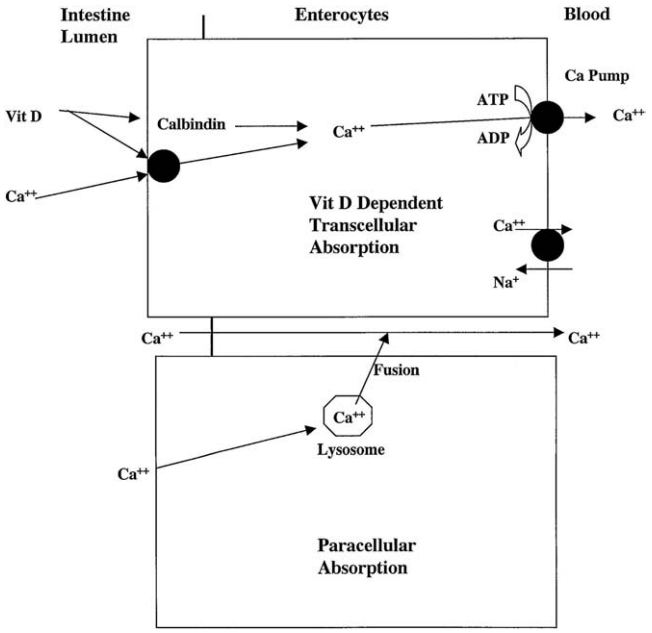

Fig. 2. Ca intestinal absorption showing active vitamin D transcellular absorption and passive paracellular absorption. In active transport, Ca absorption is aided with the vitamin D-induced calbindin protein and excreted out into the blood by an ATP-dependent pump. In passive absorption, Ca is transferred by lysosomes that fuse on the basolateral membrane, where the Ca is extruded out to the blood. Passive absorption is the major path for Ca absorption in the newborn and occurs mainly in the small intestine. ADP, adenosine diphosphate; ATP, adenosine triphosphate; Ca, calcium; Vit, vitamin.

absorption is probably by passive transport occurring mainly in the small intestine (ileum > jejunum). Passive Ca transport is the paracellular diffusion of Ca down a chemical gradient; active transport occurs mainly in the upper small intestine [26] and to a small degree in the colon [29]. Transcellularly, vitamin D-dependent active transport of Ca is not expressed in the preterm infant [23] (Fig. 2).

In infants, Ca absorption is directly related to the degree of Ca deficiency and can vary from 10% to 80% [30]. Having missed the final trimester of Ca accumulation, preterm infants have lower Ca stores than do term infants and so require more Ca than term infants (Fig. 1). Unsupplemented human milk cannot meet the Ca needs of the preterm infant. The recommended intake of Ca is shown in Table 2.

As the preterm infant grows, Ca requirements remain higher than those of a term infant. As presented in Table 3, the Ca content of preterm formulas used in hospitals is higher than that in formulas fed to term infants. When the infant is discharged from the hospital, however, the high Ca requirement continues. The postdischarge preterm infant should receive a Ca-enriched formula instead of a standard term formula [31]. The Ca content of postdischarge formulas is 105–120 mg/100 kcal compared with the term formulas of 78 mg/100 kcal. Preterm infants fed an enriched postdischarge formula containing 97 mg of Ca/100 kcal had 15% greater bone mineralization and faster growth than did infants fed a standard formula [32,33]. The American Academy of Pediatrics Committee on Nutrition has recommended that preterm infants receive a postdischarge formula for 9 mo [34]. However, for the very preterm infant, use of these formulas has been beneficial for up to 12 mo corrected to age, which may be nearly 15 mo postnatal age for many of those born at <25 wk of gestation [35].

High amounts of Ca in human milk fortifiers and formulas, however, can affect Ca retention and fat absorption [26,27]. In a study by Reis et al. [36], infants fed a human milk fortifier containing a Ca gluconate salt grew more slowly than did infants fed a fortifier containing Ca carbonate, an insoluble Ca salt. It was speculated that the Ca was binding with fat in the mother's milk and decreasing Ca and fat absorption. Schanler et al. [37] compared a premature infant formula with fortified human milk and found that efficiency of fat absorption was lower with the Ca gluconate-glycerophosphate product and that fecal Ca loss was increased. Thus, the various mineral salts added to cow milk-based formulas have different coefficients of absorption [37]. It is suggested that, because of their ability to be complexed and bound to fats, the more soluble Ca salts such as Ca chloride and gluconate may cause lower Ca absorption than the insoluble Ca salts such as Ca phosphate and carbonate.

Nutrient aspects of calcium absorption (Table 4)

Carbohydrates

Studies in adults suggest that carbohydrates affect Ca absorption. Researchers found that lactose slightly increases intestinal absorption of Ca. Using a Ca isotope method, the rate of Ca absorption associated with glucose polymers was greater than that associated with lactose in preterm infants [38]. Ca absorption from a lactose-free infant formula is 10% lower than that from lactose-containing formula [39]. Overall, the carbohydrate source does not appear to have significant clinical effects on Ca absorption (Table 4).

In a placebo-controlled trial, Lidestri et al. [40] recently demonstrated that a mixture of fructo- and galactooligosaccharides added to preterm infant formula increased urinary Ca excretion and speculated that oligosaccharides may enhance Ca absorption in preterm infants.

Table 2
Recommended daily enteral intakes*

	Preterm	Term
Calcium	120–230 mg · kg^{-1} · d^{-1}	210–270 mg/d
Phosphorus	60–140 mg · kg^{-1} · d^{-1}	100–275 mg/d
Vitamin D (IU/d)	400	200

* From the American Academy of Pediatrics, [34].

Table 3
Ca content in current formulas

Milk type	Ca (mg/100 kcal)	P (mg/100 kcal)	Ratio	Ca source
Human milk	41	21	1.9	
Full-term formulas				
Similac Advance	78	42	1.9	$CaCO_3$
Enfamil Lipil	78	53	1.5	$CaCl_2$, $Ca(PO_4)_2$
Nestle Nan	75	42	1.8	Ca citrate
Nutricia-Bebelac	64	32	2	$CaCO_3$, $CaCl_2$
Wyeth-SMA Gold	63	36	1.8	$CaCO_3$, $Ca(OH)_2$
Soy-based formulas				
Isomil	105	75	1.4	CaCitrate $Ca_3(PO_4)_2$
Prosobee	105	83	1.3	$Ca_3(PO_4)_2$
Nestle NanSoya	89	63	1.4	Dibasic Ca phosphate ($CaHPO_4$) + Ca pantothenate
Preterm formulas				
Enfamil Premature	165	83	1.9	$CaCO_3$, $CaCl_2$, $Ca(OH)_2$, $Ca_3(PO_4)_2$
Similac Special Care	180	100	1.8	$Ca_3(PO_4)_2$, $CaCO_3$
Wyeth SMA LBW Gold	99	53	1.9	$CaCO_3$, $CaCl_2$

Ca, calcium; P, phosphorus

In several European countries, antiregurgitation milk products are available commercially for infants with evidence of gastroesophageal reflux. Agents such as alginic acid, guar gum, and locust bean gum are used as thickeners. In vitro intestinal simulation studies of formula thickened with locust bean gum (a fibrous, non-digestible carbohydrate) demonstrated decreased availability of Ca for absorption [41,42]. These and other relevant studies [43–48] regarding nutrient absorption have led the European Society for Pediatric Gastroenterology, Hepatology and Nutrition to recommend "that thickening agents should not be used indiscriminately in healthy, thriving infants who spit up" [49].

Protein

The whey or casein portions of cow's milk protein generally have little effect on Ca absorption. Studies in animals and adults suggest that casein may improve passive Ca absorption [25,50]. However, soy protein in infant formulas has been shown to decrease Ca absorption rates. It appears that phytates from soy proteins can bind Ca and inhibit its absorption [51–53]. Soy formulas have been associated with inadequate growth and bone mineralization for term [54,55] and preterm [51,56] infants. To overcome this issue, all soy formulas have 35% more Ca than cow protein-based formulas, 105 versus 78 mg/100 kcal. However, the investigators found that, at 4 mo of age, term infants receiving a soy-based formula with glucose polymers or sucrose as the carbohydrate source had lower bone mineral content than did breast-fed infants [57]. Because of the issue of bone mineralization, the American Academy of Pediatrics advises against the use of soy formulas for the preterm infant [34].

Calcium-to-phosphorus ratio

The Ca-to-phosphorus (Ca-to-P) ratio in the formula may be an important determinant of Ca absorption and retention. In human milk, the Ca-to-P ratio is approximately 2.0 in terms of mass. The ratios in currently available preterm infant formulas are similar to those in human milk, although the absolute amounts are larger than in human milk and term formulas. The ratios for term infant formulas vary from 1.5 to 2.0. Whether this difference in ratio causes any Ca absorption problems has not been tested in term infants (Table 3).

Investigators studied 30 preterm infants until they reached 1900 g and six similar control infants who received their own mothers' milk [58]. The experimental group was fed one of three diets: a commercial premature formula containing 948 mg of Ca per liter and 474 mg of P per L, the same formula with a higher P (664 mg/L), or the same formula with higher concentrations of Ca (1134 mg/L) and P (664 mg/L). The Ca-to-P mass ratios of these diets were 2.0, 1.4, and 1.7, respectively. For the first and third formulations, bone mineralization rates were above the fifth percentile of the reference intrauterine rate. Thus, a Ca-to-P ratio of 1.7 in the formula was adequate at 948 mg of Ca per liter, whereas a ratio of 1.4 at the same Ca concentration

Table 4
Nutrition factors affecting Ca absorption

Nutrients	Effect on Ca absorption
Lactose	Increase
Casein	Increase
Soy protein	Decrease
High Ca/P ratio	Increase
Medium-chain triacylglycerols	Increase
Palm olein fatty acid	Decrease

Ca, calcium; P, phosphorus

was not. The American Academy of Pediatrics Committee on Nutrition (2003) recommended that premature infants receive 175 mg of Ca/100 kcal. That is equivalent to 1418 mg of Ca per liter of formula at a caloric concentration of 810 kcal/L or 24 kcal/oz. The recommendation for P was 91.5 mg/100 kcal. Hence, the recommended Ca-to-P ratio would be 1.9 [34]. The Committee on Nutrition of the Preterm Infant of the European Society of Paediatric Gastroenterology and Nutrition (1987) recommended the Ca-to-P mass ratio be 1.4–2.0 [59].

Effects of fats on calcium absorption

Dietary fats and fat-soluble vitamins are emulsified by bile for absorption. Specifically, adequate bile secretion is necessary for vitamin D absorption. Deficient bile secretion in infants with chronic liver or biliary disease leads to diminished vitamin D absorption and subsequent decreased Ca absorption. Bile salts also affect the vitamin D-dependent active transport of Ca [60]. Bile itself has a direct positive effect on Ca absorption, probably by solubilizing the Ca and inhibiting the formation of Ca and fat complexes [30].

Calcium absorption rates increase when medium-chain triacylglycerols are used as the fat source [61]. However with longer fat chains, the fat combines with Ca to form Ca soaps that decrease Ca and fat absorption. Triacylglycerol provides half of dietary energy for infants fed human milk and infant formula. About one-fourth of the fatty acids in human milk triacylglycerol are composed of the saturated fatty acid palmitic acid. Human milk palmitic acid is well absorbed. Lucas et al. [62] found that, when synthetic triacylglycerols similar to human milk were added to a formula diet in preterm infants, palmitate and Ca absorption improved significantly (Ca from 42% to 57%) and the percentage of milk fat excreted as fatty acid Ca soaps was reduced by about 50% to 3.3%. These findings were validated in another study with term infants in which infant formulas containing the palmitate similar to human milk resulted in higher whole-body bone mineral content and reduced stool Ca excretion at 12 wk of age [63]. However, when a vegetable palm oil structurally different from the human milk palmitic acid was used, fat and Ca absorption decreased. Neville et al. [64] and Koo et al. [65] demonstrated that, during the first 6 mo of life, term infants ingesting infant formula containing palm oils with palmitate in the Sn-1 and Sn-3 or end positions developed reduced total body bone mineral content compared with infants ingesting an infant formula without any palm oils. The more soluble Ca salt such as Ca gluconate-glycerophosphate can complex with the palmitate more readily than an insoluble Ca salt such as Ca carbonate or phosphate. Thus, to overcome the loss of calories and Ca, greater intake is required for adequate weight gains [27]. It is recommended that infants should not ingest vegetable palm oils with palmitate in the Sn-1 and Sn-3 or end positions. Instead, infants should ingest formulas with palm oil in the Sn-2 position, which is the structure found in human milk. In Europe, there are formulas available with synthetic lipids (palm oil) in the Sn-2 position [66]. However, in the United States, the palm oils in infant formulas are in the Sn-1 and Sn-3 or end positions.

Researchers have reported that formula feedings with or without palm olein oil during the first 4 mo of life had no effect on subsequent bone mineralization at 4 y of age [67]. Unfortunately, this study was not randomized and formula selection may have been biased. The investigators reported their findings in 65 infants who received a palm oil formula versus 56 infants without palm oils. This sample was too small to detect any differences. To detect a minimum of a 5% difference in bone mineral content, a sample with >100 children in each group should have been evaluated. The dietary history of the feeding groups between 4 mo and 4 y was not obtained and the Ca and vitamin D intakes during that period could have affected their conclusions.

Calcium content in human milk and formulas

Compared with commercial formulas, human milk has the lowest Ca content; this remains fairly constant during the first year of lactation, ranging from 4 mmol/l (38 mg/100 kcal) to 4.9 mmol/l (41 mg/100 kcal) [64,68]. The Ca content of milk from mothers giving birth prematurely is not significantly different from that of term milk during the first month of nursing. However, preterm milk has a higher concentration of ionized or soluble Ca than does term milk [69]. The Ca content of preterm infant formulas that are currently available in the United States ranges from 165 to 180 mg/100 kcal (almost five times that of human milk). Term infant formulas have 60–78 mg of Ca/100 kcal. Despite the differences in concentration of Ca, Ca absorption from human milk and cow-based formula is similar at 58–61% [28,70]. Table 4 summarizes the nutrition factors affecting Ca absorption.

Metabolic bone disease

One important result of low Ca absorption by the preterm infant is metabolic bone disease (MBD). This condition is characterized by a failure of complete mineralization of osteoid and encompasses disturbances ranging from mild undermineralization (osteopenia) to severe bone disease with fractures (rickets). Rickets is characterized by the accumulation of unmineralized osteoid that interrupts the mineralization of the growth plate. MBD is common (50–60%) in infants <28 wk of gestation and in those with birth weights <1000 g. In these infants, the cause is usually inadequate Ca and phosphate intake [71]. Major risk factors for this bone disease are prematurity, prolonged feeding intolerance and parenteral nutrition, ingestion of unsupple-

mented breast milk, chronic lung disease, and prolonged immobility.

Diagnosis of MBD of prematurity is a challenge clinically due to a lack of accurate diagnostic criteria. Infants who develop this disease are typically asymptomatic. Physical examination is normal except in cases of severe, advanced disease. In this situation, clinical features appear between the 6th and 12th postnatal weeks with fractures (ribs and extremities are most common); slow linear growth with sustained head growth; respiratory distress or failure to wean from mechanical ventilation due to a relatively compliant chest wall and/or rib fractures; dolichocephaly from softened cranial bones; craniotabes (flattening of the posterior skull); enlargement of wrists, knees, and ankles; frontal bossing, enlarged anterior fontanel, and widely split cranial sutures; rachitic rosary (swelling of the costochondral junctions); or hypotonia.

Biochemical measurements of serum Ca, inorganic phosphate (iP), and alkaline phosphatase (AP) activity are used routinely to identify infants with MBD. However, in this disease, serum Ca can be normal, high, or low and serum iP and AP alone are not adequately sensitive for diagnostic purposes. Backstrom et al. [72] reported that a combination of an AP level >900 IU/L and a serum iP concentration <1.8 mmol/l in a group of 43 preterm infants yielded a sensitivity of 100% and a specificity of 70% in detecting low bone mineral density at 3 mo corrected age. Lucas et al. [73] evaluated 857 infants weighing <1850 g and found that a peak AP level >1200 IU/L was associated with significant linear growth reduction that persisted through the neonatal period to 18 mo of age. However, Faerk et al. [74], using dual X-ray absorptiometry in 108 low–birth-weight infants, found no correlation between AP or serum iP level and whole body mineral content at term. Studies to date are difficult to compare because of inconsistencies in the measurement of serum biochemical markers and bone mineral content leading to inconsistent results.

In phosphate depletion states, renal tubular reabsorption of iP can reach 98% of the filtered load [75–77]. Urine Ca excretion is increased in phosphate deficiency, presumably because of decreased Ca accretion as apatite secondary to an inadequate Ca-to-P ratio. Investigators have proposed that following urinary Ca and iP levels may be helpful in early detection of mineral deficiency in low–birth-weight infants [77–79].

Hormone evaluation also aids in the diagnosis of MBD and in evaluating the effectiveness of therapy. PTH is typically increased with Ca deficiency. Vitamin D deficiency is present if levels of 25-hydroxyvitamin D are low. The level of 1,25-dihydroxyvitamin D is inversely related to a patient's Ca and iP needs and thus will be increased with Ca and/or iP deficiency. Each of these levels begins to revert to normal after 1–2 wk of adequate replacement therapy. Vitamin D-deficient rickets has been reported in term infants who were breast fed, received no vitamin supplementation, and had minimal sunlight exposure [80,81].

Diagnosis of MBD can be made with X-ray evaluation of long bone metaphyses when using the grading system of disease severity developed by Koo et al. [82]. However, bone mineral content must be reduced by 20–50% before radiographic changes become evident. Therefore, radiographic evaluation underestimates the frequency of disease and should not be the sole method of diagnosis. Currently, dual X-ray absorptiometry, although primarily a research tool, is considered the best method for diagnosis of bone mineral content and bone mineral density in infants.

Screening for MBD should begin at about 6 wk of age in infants with the risk factors listed above. Serial measurements every 1–2 wk allow early identification of the biochemical changes of MBD. The major nutritional goal in treating MBD is to provide sufficient Ca and phosphate and adequate vitamin D [83]. The goal is to approach intrauterine rates of bone mineralization. This requires 200 $mg \cdot kg^{-1} \cdot d^{-1}$ of Ca and 90 $mg \cdot kg^{-1} \cdot d^{-1}$ of P enterally assuming 60% absorption of Ca and 80% absorption of P. Early trophic enteral feeding significantly enhances establishment of full-volume enteral intake and thus increases Ca accumulation and decreases osteopenia. The American Academy of Pediatrics recommends that very-low–birth-weight infants receiving oral feedings should not be given >400 IU/d of vitamin D [84]. Along with the use of preterm formulas and human milk fortifiers, consider supplementing additional Ca and P after discharge in extremely preterm infants until they reach 3.5–4 kg. For infants with osteopenia or rickets and fractures, additional Ca and P should also be provided until AP level has normalized or until the infant is 4–6 mo of age.

Importance of infant bone mineralization

Calcium accretion or bone mineralization is greatest during the first year of life. Many nutritionists believe that early bone mass accumulation is important for preventing poor childhood growth and later adult osteoporosis. Follow-up studies of preterm infants fed human milk or formula showed that these children were smaller and had lower total body bone mineral content compared with term children at 8–12 y of age [85,86]. Ca intake and bone mineralization are indeed important during the first year of life.

Complications of calcium-enriched formulas

Can too much Ca intake result in hypercalcemia and potentially nephrocalcinosis? In general, the infant is able to decrease Ca absorption and increase Ca fecal excretion to avoid hypercalcemia. When using a dual-tracer method, no significant relation between dietary and urinary Ca was identified [87]. In this study, bone resorption was the source of urinary Ca rather than Ca-enriched feedings. Thus, if an infant develops nephrocalcinosis, urinary Ca is from the

bone and cessation of high Ca feedings is not indicated. However, the clinician must keep in mind that hypercalciuria occurs in hypophosphatemic states in which serum Ca can not be used for bone formation due to lack of sufficient P intake.

Summary

Calcium is an essential mineral for the fetus and the infant. The fetus actively accumulates Ca during gestation. At birth, the infant must rely on intestinal absorption of Ca to meet its Ca needs. Ca absorption is affected by the amount of Ca ingested and the type of Ca salt. The majority of Ca absorption occurs by paracellular passive absorption in the intestines and is influenced by how Ca is complexed or bound to fats or proteins.

References

[1] Chan G. Calcium and bone mineral status in infant's and children's nutrition. 2nd ed. New York: Raven Press; 1989.

[2] Steichen JJ, Gratton TL, Tsang RC. Osteopenia of prematurity: the cause and possible treatment. J Pediatr 1980;96(pt 2):528–34.

[3] Felderbauer P, Hoffmann P, Klein W, Bulut K, Ansorge N, Epplen JT, et al. Identification of a novel calcium-sensing receptor gene mutation causing familial hypocalciuric hypercalcemia by single-strand conformation polymorphism analysis. Exp Clin Endocrinol Diabetes 2005;113:31–4.

[4] Evans KN, Bulmer JN, Kilby MD, Hewison M. Vitamin D and placental-decidual function. J Soc Gynecol Investig 2004;11:263–71.

[5] Loughead JL, Mimouni F, Tsang RC. Serum ionized calcium concentrations in normal neonates. Am J Dis Child 1988;142:516–8.

[6] Wandrup J, Kroner J, Pryds O, Kastrup KW. Age-related reference values for ionized calcium in the first week of life in premature and full-term neonates. Scand J Clin Lab Invest 1988;48:255–60.

[7] Nemeth EF. Regulation of cytosolic calcium by extracellular divalent cations in C-cells and parathyroid cells. Cell Calcium 1990;11:323–7.

[8] Brown EM. Extracellular Ca2+ sensing, regulation of parathyroid cell function, and role of Ca2+ and other ions as extracellular (first) messengers. Physiol Rev 1991;71:371–411.

[9] Speer G, Toth M, Niller HH, Salamon D, Takacs I, Miheller P, et al. Calcium metabolism and endocrine functions in a family with familial hypocalciuric hypercalcemia. Exp Clin Endocrinol Diabetes 2003; 111:486–90.

[10] Lam CW, Lee KF, Chan AO, Poon PM, Law TY, Tong SF. Novel missense mutation in the CASR gene in a Chinese family with familial hypocalciuric hypercalcemia. Clin Chim Acta 2005;360: 167–72.

[11] Venkataraman PS, Blick KE, Fry HD, Rao RK. Postnatal changes in calcium-regulating hormones in very-low-birth-weight infants. Effect of early neonatal hypocalcemia and intravenous calcium infusion on serum parathyroid hormone and calcitonin homeostasis. Am J Dis Child 1985;139:913–6.

[12] Venkataraman PS, Tsang RC, Steichen JJ, Grey I, Neylan M, Fleischman AR. Early neonatal hypocalcemia in extremely preterm infants. High incidence, early onset, and refractoriness to supraphysiologic doses of calcitriol. Am J Dis Child 1986;140:1004–8.

[13] Chan GM, Tsang RC, Chen IW, DeLuca HF, Steichen JJ. The effect of 1,25(OH)2 vitamin D3 supplementation in premature infants. J Pediatr 1978;93:91–6.

[14] Fraser DR. Regulation of the metabolism of vitamin D. Physiol Rev 1980;60:551–613.

[15] Colette C, Monnier L, Pares Herbute N, Blotman F, Mirouze J. Calcium absorption in corticoid treated subjects effects of a single oral dose of calcitriol. Horm Metab Res 1987;19:335–8.

[16] Namgung R, Tsang RC, Specker BL, Sierra RI, Ho ML. Reduced serum osteocalcin and 1,25-dihydroxyvitamin D concentrations and low bone mineral content in small for gestational age infants: evidence of decreased bone formation rates. J Pediatr 1993;122:269–75.

[17] Favus MJ, Tembe V. The use of pharmacologic agents to study mechanisms of intestinal calcium transport. J Nutr 1992;122(suppl): 683–6.

[18] Brommage R, Neuman WF. Mechanism of mobilization of bone mineral by 1,25-dihydroxyvitamin D3. Am J Physiol 1979;237: E113–20.

[19] Raisz LG, Trummel CL, Holick MF, DeLuca HF. 1,25-dihydroxycholecalciferol: a potent stimulator of bone resorption in tissue culture. Science 1972;175:768–9.

[20] Abrams SA, Esteban NV, Vieira NE, Yergey AL. Dual tracer stable isotopic assessment of calcium absorption and endogenous fecal excretion in low birth weight infants. Pediatr Res 1991;29:615–8.

[21] Hillman LS, Johnson LS, Lee DZ, Vieira NE, Yergey AL. Measurement of true absorption, endogenous fecal excretion, urinary excretion, and retention of calcium in term infants by using a dual-tracer, stable-isotope method. J Pediatr 1993;123:444–56.

[22] Specker BL, Beck A, Kalkwarf H, Ho M. Randomized trial of varying mineral intake on total body bone mineral accretion during the first year of life. Pediatrics 1997;99:E12.

[23] Bronner F. Current concepts of calcium absorption: an overview. J Nutr 1992;122(suppl):641–3.

[24] Lacour B, Tardivel S, Drueke T. Stimulation by citric acid of calcium and phosphorus bioavailability in rats fed a calcium-rich diet. Miner Electrolyte Metab 1997;23:79–87.

[25] Hansen M, Sandstrom B, Jensen M, Sorensen SS. Casein phosphopeptides improve zinc and calcium absorption from rice-based but not from whole-grain infant cereal. J Pediatr Gastroenterol Nutr 1997;24:56–62.

[26] Pansu D, Duflos C, Bellaton C, Bronner F. Solubility and intestinal transit time limit calcium absorption in rats. J Nutr 1993;123:1396–404.

[27] Schanler RJ, Abrams SA. Postnatal attainment of intrauterine macromineral accretion rates in low birth weight infants fed fortified human milk. J Pediatr 1995;126:441–7.

[28] Lifschitz CH, Abrams SA. Addition of rice cereal to formula does not impair mineral bioavailability. J Pediatr Gastroenterol Nutr 1998;26: 175–8.

[29] Sandstrom B, Cederblad A, Kivisto B, Stenquist B, Andersson H. Retention of zinc and calcium from the human colon. Am J Clin Nutr 1986;44:501–4.

[30] Halbert KETR. Neonatal calcium, phosphorus, and magnesium homeostasis. Philadelphia: WB Saunders; 1992.

[31] Chan GM. Growth and bone mineral status of discharged very low birth weight infants fed different formulas or human milk. J Pediatr 1993;123:439–43.

[32] Bishop NJ, King FJ, Lucas A. Increased bone mineral content of preterm infants fed with a nutrient enriched formula after discharge from hospital. Arch Dis Child 1993;68(5 Spec No):573–8.

[33] Lucas A, Bishop NJ, King FJ, Cole TJ. Randomised trial of nutrition for preterm infants after discharge. Arch Dis Child 1992;67:324–7.

[34] Kleinman RE, 2003–2004 CoN, editors. Pediatric nutrition handbook. 5th ed. Elk Grove, IL: American Academy of Pediatrics; 2004.

[35] Carver JD, Wu PY, Hall RT, Ziegler EE, Sosa R, Jacobs J, et al. Growth of preterm infants fed nutrient-enriched or term formula after hospital discharge. Pediatrics 2001;107:683–9.

[36] Reis BB, Hall RT, Schanler RJ, Berseth CL, Chan G, Ernst JA, et al. Enhanced growth of preterm infants fed a new powdered human milk fortifier: a randomized, controlled trial. Pediatrics 2000;106:581–8.

[37] Schanler RJ, Abrams SA, Garza C. Bioavailability of calcium and phosphorus in human milk fortifiers and formula for very low birth weight infants. J Pediatr 1988;113(pt 1):95–100.
[38] Stathos TH, Shulman RJ, Schanler RJ, Abrams SA. Effect of carbohydrates on calcium absorption in premature infants. Pediatr Res 1996;39(pt 1):666–70.
[39] Abrams SA, Griffin IJ, Davila PM. Calcium and zinc absorption from lactose-containing and lactose-free infant formulas. Am J Clin Nutr 2002;76:442–6.
[40] Lidestri M, Agosti M, Marini A, Boehm G. Oligosaccharides might stimulate calcium absorption in formula-fed preterm infants. Acta Paediatr Suppl 2003;91(441):91–2.
[41] Bosscher D, Van Caillie-Bertrand M, Van Dyck K, Robberecht H, Van Cauwenbergh R, Deelstra H. Thickening infant formula with digestible and indigestible carbohydrate: availability of calcium, iron, and zinc in vitro. J Pediatr Gastroenterol Nutr 2000;30:373–8.
[42] Bosscher D, Van Caillie-Bertrand M, Van Cauwenbergh R, Deelstra H. Availabilities of calcium, iron, and zinc from dairy infant formulas is affected by soluble dietary fibers and modified starch fractions. Nutrition 2003;19:641–5.
[43] Gee JM, Lee-Finglas WE, Wortley GM, Pell JD, Johnson IT. Influence of non-starch polysaccharides on gastrointestinal endocrine mechanisms. Eur J Clin Nutr 1995;49(suppl 3):S170–2.
[44] Southon S, Gee JM, Johnson IT. The effect of dietary protein source and guar gum on gastrointestinal growth and enteroglucagon secretion in the rat. Br J Nutr 1987;58:65–72.
[45] Fuessl HS, Williams G, Adrian TE, Bloom SR. Guar sprinkled on food: effect on glycaemic control, plasma lipids and gut hormones in non–insulin dependent diabetic patients. Diabet Med 1987;4:463–8.
[46] Johnson IT, Gee JM. Inhibitory effect of guar gum on the intestinal absorption of glucose in vitro. Proc Nutr Soc 1980;39:52A.
[47] Johnson IT, Gee JM. Gastrointestinal adaptation in response to soluble non-available polysaccharides in the rat. Br J Nutr 1986;55:497–505.
[48] Harmuth-Hoene AE, Meier-Ploeger A, Leitzmann C. [Effect of carob bean flour on the resorption of minerals and trace elements in man]. Z Ernahrungswiss 1982;21:202–13.
[49] Aggett PJ, Agostoni C, Goulet O, Hernell O, Koletzko B, Lafeber HL, et al. Antireflux or antiregurgitation milk products for infants and young children: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 2002;34:496–8.
[50] Erba D, Ciappellano S, Testolin G. Effect of the ratio of casein phosphopeptides to calcium (w/w) on passive calcium transport in the distal small intestine of rats. Nutrition 2002;18:743–6.
[51] Naude SP, Prinsloo JG, Haupt CE. Comparison between a humanized cow's milk and a soy product for premature infants. S Afr Med J 1979;55:982–6.
[52] Heaney RP, Weaver CM, Fitzsimmons ML. Soybean phytate content: effect on calcium absorption. Am J Clin Nutr 1991;53:745–7.
[53] Allen LH. Calcium bioavailability and absorption: a review. Am J Clin Nutr 1982;35:783–808.
[54] Kohler L, Meeuwisse G, Mortensson W. Food intake and growth of infants between six and twenty-six weeks of age on breast milk, cow's milk formula, or soy formula. Acta Paediatr Scand 1984;73:40–8.
[55] Cherry FF, Cooper MD, Stewart RA, Platou RV. Cow versus soy formulas. Comparative evaluation in normal infants. Am J Dis Child 1968;115:677–92.
[56] Kulkarni PB, Dorand RD, Bridger WM, Payne JH III, Montiel DC, Hill JG. Rickets in premature infants fed different formulas. South Med J 1984;77:13–6, 20.
[57] Chan GM, Leeper L, Book LS. Effects of soy formulas on mineral metabolism in term infants. Am J Dis Child 1987;141:527–30.
[58] Chan GM, Mileur L, Hansen JW. Calcium and phosphorus requirements in bone mineralization of preterm infants. J Pediatr 1988;113(pt 2):225–9.
[59] Committee on Nutrition of the Preterm Infant, European Society of Paediatric Gastroenterology and Nutrition (ESPAN). Nutrition and feeding of preterm infants. Oxford, England: Blackwell Scientific Publications; 1987.
[60] Lee DB, Hu MS, Kayne LH, Nakhoul F, Jamgotchian N. The importance of non-vitamin D–mediated calcium absorption. Contrib Nephrol 1991;91:14–20.
[61] Sulkers EJ, Lafeber HN, Degenhart HJ, Lindemans J, Sauer PJ. Comparison of two preterm formulas with or without addition of medium-chain triglycerides (MCTs). II: Effects on mineral balance. J Pediatr Gastroenterol Nutr 1992;15:42–7.
[62] Lucas A, Quinlan P, Abrams S, Ryan S, Meah S, Lucas PJ. Randomised controlled trial of a synthetic triglyceride milk formula for preterm infants. Arch Dis Child Fetal Neonatal Ed 1997;77:F178–84.
[63] Kennedy K, Fewtrell MS, Morley R, Abbott R, Quinlan PT, Wells JC, et al. Double-blind, randomized trial of a synthetic triacylglycerol in formula-fed term infants: effects on stool biochemistry, stool characteristics, and bone mineralization. Am J Clin Nutr 1999;70:920–7.
[64] Neville MC, Allen JC, Archer PC, Casey CE, Seacat J, Keller RP, et al. Studies in human lactation: milk volume and nutrient composition during weaning and lactogenesis. Am J Clin Nutr 1991;54:81–92.
[65] Koo WW, Hammami M, Margeson DP, Nwaesei C, Montalto MB, Lasekan JB. Reduced bone mineralization in infants fed palm olein-containing formula: a randomized, double-blinded, prospective trial. Pediatrics 2003;111(pt 1):1017–23.
[66] Carnielli VP, Luijendijk IH, van Goudoever JB, Sulkers EJ, Boerlage AA, Degenhart HJ, et al. Feeding premature newborn infants palmitic acid in amounts and stereoisomeric position similar to that of human milk: effects on fat and mineral balance. Am J Clin Nutr 1995;61:1037–42.
[67] Antonson DYR, Heires P, Murray N, Merkel K, Ferguson P, Moore T. Effect of neonatal and infant feeding on bone density at 4 years. In: Section on perinatal pediatrics; October 10, 2004. San Francisco: Journal of Perinatology; 2004, pp. 571–609.
[68] Dewey KG, Finley DA, Lonnerdal B. Breast milk volume and composition during late lactation (7–20 months). J Pediatr Gastroenterol Nutr 1984;3:713–20.
[69] Chan GM. Human milk calcium and phosphate levels of mothers delivering term and preterm infants. J Pediatr Gastroenterol Nutr 1982;1:201–5.
[70] Abrams SA, Grusak MA, Stuff J, O'Brien KO. Calcium and magnesium balance in 9–14-y-old children. Am J Clin Nutr 1997;66:1172–7.
[71] Masel JP, Tudehope D, Cartwright D, Cleghorn G. Osteopenia and rickets in the extremely low birth weight infant–a survey of the incidence and a radiological classification. Australas Radiol 1982;26:83–96.
[72] Backstrom MC, Kouri T, Kuusela AL, Sievanen H, Koivisto AM, Ikonen RS, et al. Bone isoenzyme of serum alkaline phosphatase and serum inorganic phosphate in metabolic bone disease of prematurity. Acta Paediatr 2000;89:867–73.
[73] Lucas A, Brooke OG, Baker BA, Bishop N, Morley R. High alkaline phosphatase activity and growth in preterm neonates. Arch Dis Child 1989;64(7 Spec No):902–9.
[74] Faerk J, Peitersen B, Petersen S, Michaelsen KF. Bone mineralisation in premature infants cannot be predicted from serum alkaline phosphatase or serum phosphate. Arch Dis Child Fetal Neonatal Ed 2002;87:F133–6.
[75] Bishop N. Bone disease in preterm infants. Arch Dis Child 1989;64(10 Spec No):1403–9.
[76] Senterre J, Salle B. Renal aspects of calcium and phosphorus metabolism in preterm infants. Biol Neonate 1988;53:220–9.
[77] Karlen J, Aperia A, Zetterstrom R. Renal excretion of calcium and phosphate in preterm and term infants. J Pediatr 1985;106:814–9.
[78] Catache M, Leone CR. Role of plasma and urinary calcium and phosphorus measurements in early detection of phosphorus deficiency in very low birthweight infants. Acta Paediatr 2003;92:76–80.

[79] Pohlandt F. Prevention of postnatal bone demineralization in very low-birth-weight infants by individually monitored supplementation with calcium and phosphorus. Pediatr Res 1994;35:125–9.
[80] Kruger DM, Lyne ED, Kleerekoper M. Vitamin D deficiency rickets. A report on three cases. Clin Orthop Relat Res 1987(224): 277–83.
[81] Greer FR. Issues in establishing vitamin D recommendations for infants and children. Am J Clin Nutr 2004;80(suppl):1759S–62S.
[82] Koo WW, Gupta JM, Nayanar VV, Wilkinson M, Posen S. Skeletal changes in preterm infants. Arch Dis Child 1982;57:447–52.
[83] Johnson CB. Neonatal rickets: metabolic bone disease of prematurity. Neonatal Netw 1991;9:13–7.
[84] Gartner LM, Greer FR. Prevention of rickets and vitamin D deficiency: new guidelines for vitamin D intake. Pediatrics 2003;111(pt 1):908–10.
[85] Fewtrell MS, Prentice A, Jones SC, Bishop NJ, Stirling D, Buffenstein R, et al. Bone mineralization and turnover in preterm infants at 8–12 years of age: the effect of early diet. J Bone Miner Res 1999;14:810–20.
[86] Fewtrell MS, Cole TJ, Bishop NJ, Lucas A. Neonatal factors predicting childhood height in preterm infants: evidence for a persisting effect of early metabolic bone disease? J Pediatr 2000;137:668–73.
[87] Abrams SA, Schanler RJ, Yergey AL, Vieira NE, Bronner F. Compartmental analysis of calcium metabolism in very-low-birth-weight infants. Pediatr Res 1994;36:424–8.
[88] Widdowson EMDJ. Chemical composition of the body. Orlando: Academic Press; 1964.
[89] Kelly HJ, Sloan RE, Hoffman W, Saunders C. Accumulation of nitrogen and six minerals in the human fetus during gestation. Hum Biol 1951;23:61–74.