Chapter 8. Skeletal Physiology: Fetus and Neonate

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INTRODUCTION

Because of obvious limitations in studying human fetuses and (to a lesser degree) neonates, human regulation of fetal and neonatal mineral homeostasis must be largely inferred from studies in animals. Some observations in animals may not apply to humans. This chapter briefly reviews existing human and animal data, including older studies of surgically manipulated animals and recent studies of mice engineered to lack calciotropic hormones or receptors. Detailed references are available in two comprehensive reviews.[1,2]

FETUS

Much of normal mineral and bone homeostasis in the adult can be explained by the interactions of PTH, 1,25-dihydroxyvitamin D or calcitriol (1,25-D), calcitonin, and the sex steroids. In contrast to the adult, comparatively little has been known about how mineral and bone homeostasis is regulated in the fetus. Fetal mineral metabolism has been uniquely adapted to meet the specific needs of this developmental period, including the requirement to maintain an extracellular level of calcium (and other minerals) that is physiologically appropriate for fetal tissues and to provide sufficient calcium (and other minerals) to fully mineralize the skeleton before birth. Mineralization occurs rapidly in late gestation, such that a human accretes 80% of the required 30 g of calcium in the third trimester, whereas a rat accretes 95% of the required 12.5 mg of calcium in the last 5 days of its 3-week gestation.

Minerals Ions and Calciotropic Hormones

A consistent finding among human and other mammalian fetuses is a total and ionized calcium concentration that is significantly higher than the maternal level during late gestation. Similarly, serum phosphate is significantly elevated, and serum magnesium is minimally elevated above the maternal concentration. The physiological importance of these elevated levels is not known. A calcium level equal to the maternal calcium concentration (and not above it) seems to be sufficient to ensure adequate mineralization of the fetal skeleton, and fetal survival to term is unaffected by extremes of hypocalcemia in several animal models. The increased calcium level is robustly maintained despite chronic, severe maternal hypocalcemia of a variety of causes. For example, adult humans and mice with nonfunctional vitamin D receptors have severe hypocalcemia, but murine fetuses with the same abnormality have normal serum calcium concentrations.[3]

Calciotropic hormone levels are also maintained at levels that differ from the adult. These differences seem to reflect the relatively different roles that these hormones play in the fetus and are not an artifact of altered metabolism or clearance of these hormones. Intact PTH levels are much lower than maternal PTH levels near the end of gestation, but it is unknown whether fetal PTH levels are low throughout gestation after the formation of the parathyroids or only in late gestation. The low level of PTH is critically important, because fetal mice lacking parathyroids and PTH have marked hypocalcemia and undermineralized skeletons.[4] Circulating 1,25-D levels are also lower than the maternal level in late gestation and seem to be largely if not completely derived from fetal sources. The low circulating levels of 1,25-D in the fetus may be a response to high serum phosphate and suppressed PTH levels in late gestation. With respect to 1,25-D, the low levels of this hormone may reflect its relative unimportance for fetal mineral homeostasis, because both vitamin D deficiency and absence of vitamin D receptors do not impair serum mineral concentrations or the mineralization of the fetal skeleton.[3] Fetal calcitonin levels are higher than maternal levels and are thought to reflect increased synthesis of the hormone. Apart from responding appropriately to changes in the serum calcium concentration, there is little evidence of an essential role for calcitonin in fetal mineral homeostasis.[5]

PTH-related protein (PTHrP) is normally not present in the human adult circulation (outside of pregnancy and lactation), but in cord blood, PTHrP levels are up to 15-fold higher than that of PTH. PTHrP is produced in many tissues and plays multiple roles during embryonic and fetal development. The absence of PTHrP (in the *Pthrp*-null fetal mouse) leads to abnormalities of chondrocyte differentiation and skeletal development,[6] modest hypocalcemia,[7] and reduced placental calcium transfer. Such *Pthrp*-null fetuses have increased PTH levels[8] but still remain modestly hypocalcemic, indicating that PTH does not make up for lack of PTHrP in maintaining a normal calcium concentration in the fetal circulation.

The role (if any) of the sex steroids in fetal skeletal development and mineral accretion is unknown, largely because the relevant analyses have not been performed in the relevant mouse models, and corresponding human data are absent. Estrogen receptor α and β knockout mice have been shown to have altered skeletal metabolism that develops postnatally, but the fetal skeleton has not been examined in detail. Similarly, postnatal skeletal roles of RANK, RANKL, and osteoprotegerin have been shown in relevant knockout mice, but the role that this system plays in fetal mineral metabolism is not yet known.

Fetal Parathyroids

Intact parathyroid glands are required for maintenance of normal fetal calcium, magnesium, and phosphate levels; lack of parathyroids and PTH causes a greater fall in the fetal blood calcium than lack of PTHrP. Fetal parathyroids are also required for normal accretion of mineral by the skeleton and may be required for regulation of placental mineral transfer. Studies in fetal lambs have indicated that the fetal parathyroids may contribute to mineral homeostasis by producing both PTH and PTHrP, whereas a detailed study of rats indicates that the fetal parathyroids produce only PTH. Whether human fetal parathyroids produce PTH alone or PTH and PTHrP together is unclear.

Calcium Sensing Receptor

The calcium sensing receptor (CaSR) sets the serum calcium level in adults by regulating PTH, but it does not seem to set the serum calcium level in fetuses. Instead, the fetal serum calcium is driven above the maternal level by the action of PTHrP, while in turn, the CaSR appropriately suppresses PTH in response to this elevated calcium level (Fig. 1A). In the absence of PTHrP (*Pthrp*-null mice), the fetal serum calcium falls to the normal adult level, and the serum PTH is increased,

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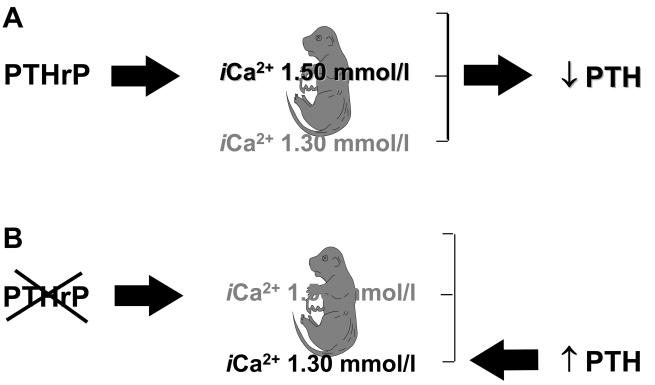

FIG. 1. Fetal blood calcium regulation. (A) Normal high fetal calcium level, which is dependent on PTHrP, activates the parathyroid CaSR, and PTH is suppressed. (B) In the absence of PTHrP, the fetal calcium level falls to a level that is now set by the parathyroid CaSR; PTH is stimulated to maintain the ionized calcium at the normal adult level (maternal). (Reprinted from Pediatric Bone, Glorieux FH, Pettifor JM, Jüppner H, Fetal mineral homeostasis, pp. 271–302, 2003, with permission from Elsevier.)

consistent with the normal function of the CaSR to maintain the calcium concentration at the adult level (Fig. 1B). Inactivating mutations of the CaSR (*Casr*-null fetuses) lead to increases in serum calcium, PTH, 1,25-D, and bone turnover of fetuses, resulting in a lower skeletal calcium content by term. The CaSR is also expressed within placenta as shown in humans and mice, and this may indicate that the CaSR participates in the regulation of placental mineral transfer. *Casr*-null fetuses have a reduced rate of placental calcium transfer, but whether this is a direct consequence of the loss of placental CaSR is not known.[9]

Fetal Kidneys and Amniotic Fluid

Fetal kidneys partly regulate calcium homeostasis by adjusting the relative reabsorption and excretion of calcium, magnesium, and phosphate in response to the filtered load and other factors, such as PTHrP and PTH. The fetal kidneys also synthesize 1,25-D, but because absence of vitamin D receptors in fetal mice does not impair fetal calcium homeostasis or placental calcium transfer, it seems likely that renal production of 1,25-D is relatively unimportant.

Renal calcium handling in fetal life may be less important compared with the adult for the regulation of calcium homeostasis because calcium excreted by the kidneys is not permanently lost. Fetal urine is the major source of fluid and solute in the amniotic fluid, and fetal swallowing of amniotic fluid is a pathway by which excreted calcium can be made available again to the fetus.

Placental Mineral Ion Transport

As noted above, the bulk of placental calcium and other mineral transfer occurs late in gestation at a rapid rate. Active transport of calcium, magnesium, and phosphate across the placenta is necessary for the fetal requirement to be met; only placental calcium transfer has been studied in detail. Analogous to calcium transfer across the intestinal mucosa, it has been theorized that calcium diffuses into calcium-transporting cells through maternal-facing basement membranes, is carried across these cells by calcium-binding proteins, and is actively extruded at the fetal-facing basement membranes by Ca^{2+}-ATPase.

Data from animal models indicates that a normal rate of maternal-to-fetal calcium transfer can usually be maintained despite the presence of maternal hypocalcemia or maternal hormone deficiencies such as aparathyroidism, vitamin D deficiency, and absence of the vitamin D receptor. Whether the same is true for human pregnancies is less certain. A "normal" rate of maternal–fetal calcium transfer does not necessarily imply that the fetus is unaffected by maternal hypocalcemia. Instead, it is an indication of the resilience of the fetal–placental unit to be able to extract the required amount of calcium from a maternal circulation that has a severely lower calcium concentration than normal.

Fetal regulation of placental calcium transfer has been studied in a number of different animal models. Thyroparathyroidectomy in fetal lambs results in a reduced rate of placental calcium transfer, suggesting that the parathyroids are required for this process.[10] In contrast, mice lacking parathyroids as a consequence of ablation of the *Hoxa3* gene have a normal rate of placental calcium transfer.[4] The discrepancy between these findings in lambs and mice may be caused by whether or not the parathyroids are an important source of PTHrP in the circulation. Studies in fetal lambs and in *Pthrp*-null fetal mice are in agreement that PTHrP, and in particular mid-molecular forms of PTHrP, stimulate placental calcium transfer.[7,11,12] PTH does not seem to be involved in this process, and there is little evidence that calcitonin or 1,25-D are required either.[3,5]

Fetal Skeleton

A complete cartilaginous skeleton with digits and intact joints is present by the eighth week of gestation in humans. Primary ossification centers form in the vertebrae and long bones between the 8th and 12th weeks, but it is not until the third trimester that the bulk of mineralization occurs. At the 34th week of gestation, secondary ossification centers form in the femurs, but otherwise most epiphyses are cartilaginous at birth, with secondary ossification centers appearing in other bones in the neonate and child.[13]

The skeleton must undergo substantial growth and be sufficiently mineralized by the end of gestation to support the organism, but as in the adult, the fetal skeleton participates in the regulation of mineral homeostasis. Calcium accreted by the fetal skeleton can be subsequently resorbed to help maintain the concentration of calcium in the blood. Functioning fetal parathyroid glands are needed for normal skeletal mineral accretion, and both hypoparathyroidism (thyroparathyroidectomized fetal lambs and aparathyroid fetal mice) and hyperparathyroidism (including *Casr*-null fetal mice) reduce the net amount of skeletal mineral accreted by term.

Further comparative study of fetal mice lacking parathyroids or PTHrP has clarified the relative and interlocking roles of PTH and PTHrP in the regulation of the development and mineralization of the fetal skeleton. PTHrP produced locally in the growth plate directs the development of the cartilaginous scaffold that is later broken down and transformed into endochondral bone,[14] whereas PTH controls the mineralization of bone through its contribution to maintaining the fetal blood calcium and magnesium.[8] In the absence of PTHrP, a severe chondrodysplasia results,[6] but the fetal skeleton is fully mineralized.[8] In the absence of parathyroids and PTH, endochondral bone forms normally but is significantly undermineralized.[8] The blood calcium and magnesium were also significantly reduced in aparathyroid fetuses, and this may explain why lack of PTH impaired skeletal mineralization. That is, by reducing the amount of mineral presented to the skeletal surface and to osteoblasts, lack of PTH thereby impaired mineral accretion by the skeleton. When both parathyroids and PTHrP are deleted, the typical *Pthrp*-null chondro-

dysplasia results, but the skeleton is smaller and contains less mineral.[8] Therefore, normal mineralization of the fetal skeleton requires intact fetal parathyroid glands and adequate delivery of minerals to the fetal circulation. While both PTH and PTHrP are involved, PTH plays the more critical role in ensuring full mineralization of the skeleton before term.

Fetal Response to Maternal Hyperparathyroidism

In humans, maternal primary hyperparathyroidism has been associated with adverse fetal outcomes, including spontaneous abortion and stillbirth, which are thought to result from suppression of the fetal parathyroid glands. Because PTH cannot cross the placenta, fetal parathyroid suppression may result from increased calcium flux across the placenta to the fetus, facilitated by maternal hypercalcemia. Similar suppression of fetal parathyroids occurs when the mother has hypercalcemia because of familial hypocalciuric hypercalcemia. Chronic elevation of the maternal serum calcium in mice results in suppression of the fetal PTH level,[9] but fetal outcome is not notably affected by this.

Fetal Response to Maternal Hypoparathyroidism

Maternal hypoparathyroidism during human pregnancy can cause fetal hyperparathyroidism. This is characterized by fetal parathyroid gland hyperplasia, generalized skeletal demineralization, subperiosteal bone resorption, bowing of the long bones, osteitis fibrosa cystica, rib and limb fractures, low birth weight, spontaneous abortion, stillbirth, and neonatal death. Similar skeletal findings have been reported in the fetuses and neonates of women with pseudohypoparathyroidism, renal tubular acidosis, and chronic renal failure. These changes in human skeletons differ from what has been found in animal models of maternal hypocalcemia, in which the fetal skeleton and the blood calcium is generally normal.

Integrated Fetal Calcium Homeostasis

The evidence discussed in the preceding sections suggests the following summary models.

Calcium Source. The main flux of calcium and other minerals is across the placenta and into fetal bone, but calcium is also made available to the fetal circulation through several routes (Fig. 2). The kidneys reabsorb calcium; calcium excreted by the kidneys into the urine and amniotic fluid may be swallowed and reabsorbed; calcium is also resorbed from the developing skeleton. Some calcium returns to the maternal circulation (backflux). The maternal skeleton is a potential source of mineral, and it may be compromised in mineral deficiency states to provide to the fetus.

Blood Calcium Regulation. The fetal blood calcium is set at a level higher than the maternal level through the actions of PTHrP and PTH acting in concert (among other potential factors; Fig. 3). The CaSR suppresses PTH in response to the high calcium level, but the low level of PTH is critically required for maintaining the blood calcium and facilitating mineral accretion by the skeleton. 1,25-D synthesis and secretion are, in turn, suppressed by low PTH and high blood calcium and phosphate. The parathyroids may play a central role by producing PTH and PTHrP or may produce PTH alone while PTHrP is produced by the placenta and other fetal tissues.

PTH and PTHrP, both present in the fetal circulation, independently and additively regulate the fetal blood calcium, with PTH having the greater effect. Neither hormone can make up

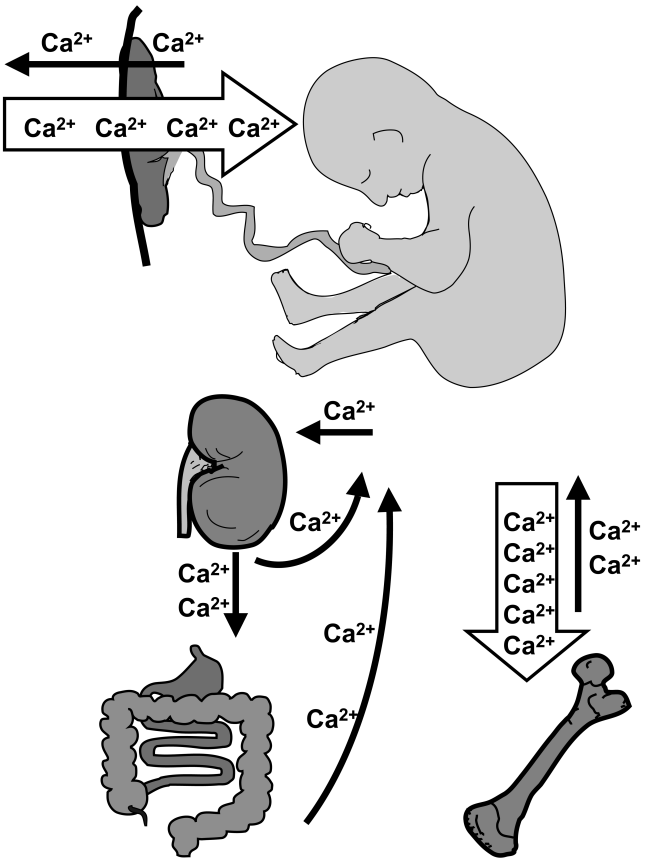

FIG. 2. Calcium sources in fetal life. (Reprinted from Pediatric Bone, Glorieux FH, Pettifor JM, Jüppner H, Fetal mineral homeostasis, pp. 271–302, 2003, with permission from Elsevier.)

for absence of the other: if one is missing the blood calcium is reduced, and if both are missing, the blood calcium is reduced even further. PTH may contribute to the blood calcium through actions on the PTH/PTHrP (PTH1) receptor in classic target tissues (kidney, bone), whereas PTHrP may contribute through placental calcium transfer and actions on the PTH1 receptor and other receptors.

The normal elevation of the fetal blood calcium above the maternal calcium concentration was historically taken as proof that placental calcium transfer is an active process. However, the fetal blood calcium level is not simply determined by the rate of placental calcium transfer because placental calcium transfer is normal in aparathyroid mice and increased in mice lacking the PTH1 receptor, but both phenotypes have significantly reduced blood calcium levels.[4,7] Also, *Casr*-null fetuses have reduced placental calcium transfer but markedly increased blood calcium levels.[9]

Placental Calcium Transfer. Placental calcium transfer is regulated by PTHrP but not by PTH (Fig. 4), and the placenta (and possibly the parathyroids) is likely an important source of PTHrP.

Skeletal Mineralization. PTH and PTHrP have separate roles with respect to skeletal development and mineralization (Fig. 5). PTH normally acts systemically to direct the mineralization of the bone matrix by maintaining the blood calcium at the adult level and possibly by direct actions on osteoblasts within the bone matrix. In contrast, PTHrP acts both locally within the growth plate to direct endochondral bone development and

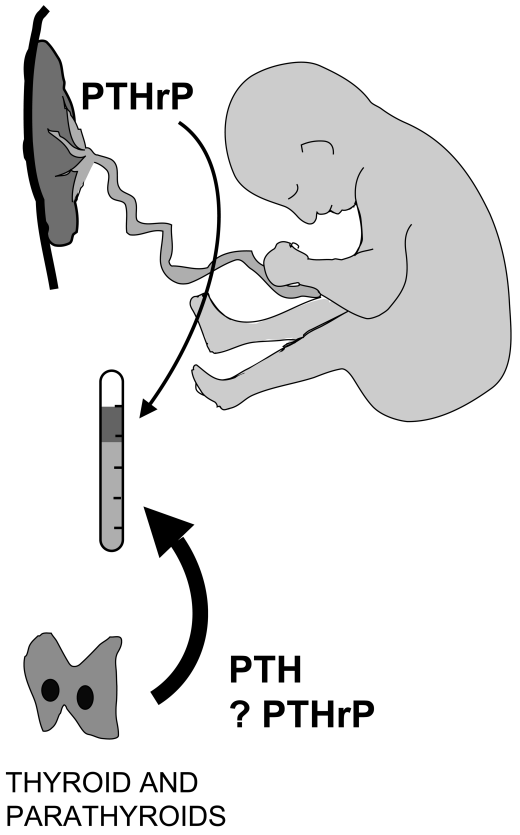

FIG. 3. Fetal blood calcium regulation. PTH has a more dominant effect on fetal blood calcium regulation than PTHrP, with blood calcium represented schematically as a thermometer (light gray, contribution of PTH; dark gray, contribution of PTHrP). In the absence of PTHrP, the blood calcium falls to the maternal level. In the absence of PTH (*Hoxa3*-null that has absent PTH but normal circulating PTHrP levels), the blood calcium falls well below the maternal calcium concentration. In the absence of both PTHrP and PTH (*Hoxa3/Pthrp* double mutant) the blood calcium falls even further than in the absence of PTH alone. (Reprinted from Pediatric Bone, Glorieux FH, Pettifor JM, Jüppner H, Fetal mineral homeostasis, pp. 271–302, 2003, with permission from Elsevier.)

outside of bone to affect skeletal development and mineralization by contributing to the regulation of the blood calcium and placental calcium transfer. PTH has the more critical role in maintaining skeletal mineral accretion.

The rate of placental calcium transfer has been historically considered to be the rate-limiting step for skeletal mineral accretion. However, this is not correct because accretion of mineral was reduced in the presence of both normal and increased placental calcium transfer.[1] The rate-limiting step seems to be the blood calcium level, which in turn is largely determined by PTH. The level of blood calcium achieved in the *Pthrp*-null—that is, the normal adult level—is sufficient to allow normal accretion of mineral, whereas lower levels of blood calcium impair it.

NEONATE

On cutting the umbilical cord and abruptly losing the placental calcium infusion (and placental sources of PTHrP), a rapid adjustment in the regulation of mineral homeostasis occurs over hours to days. The neonate becomes dependent on intestinal calcium intake, skeletal calcium stores, and renal calcium reabsorption to maintain a normal blood calcium at a time of continued skeletal growth. PTH and 1,25-D become more important, whereas PTHrP becomes less involved in neonatal calcium homeostasis.

Mineral Ions and Calciotropic Hormones

Birth marks the onset of a fall in the total and ionized calcium concentration, likely provoked by loss of the placental calcium pump and placental-derived PTHrP and a rise in pH induced by the onset of breathing. Studies in rodents indicate a fall in total and ionized calcium levels to 60% of the fetal value by 6–12 h after birth and a subsequent rise to the normal adult value over the succeeding week. Although data are less complete in humans, the progression in ionized and total calcium values seems to be similar. The ionized calcium in normal neonates falls from the umbilical cord level of 1.45 mM to a mean of 1.20 mM by 24 h after birth.[15] Babies delivered by elective cesarian-section were found to have lower blood calcium and higher PTH levels at birth compared with babies delivered by spontaneous vaginal delivery,[16] indicating that the mode of delivery can affect early neonatal mineral homeostasis.

Phosphate initially rises over the first 24 h of postnatal life in humans and then gradually declines. The intact PTH level has been found to rise briskly to within or near the normal adult range by 24–48 h after birth.[2] The increase in PTH follows the early postnatal drop in the serum ionized calcium and precedes the subsequent rise in ionized calcium and 1,25-D and the fall in phosphate. During the first 48 h, the parathyroid glands have been found to respond sluggishly to more severe falls in the ionized calcium, such as that caused by exchange transfusion with citrated blood. The degree of responsiveness to acute hypocalcemia seems to increase with postnatal age.

In humans, 1,25-D rises to adult levels over the first 48 h of postnatal life, likely in response to the rise in PTH. Serum

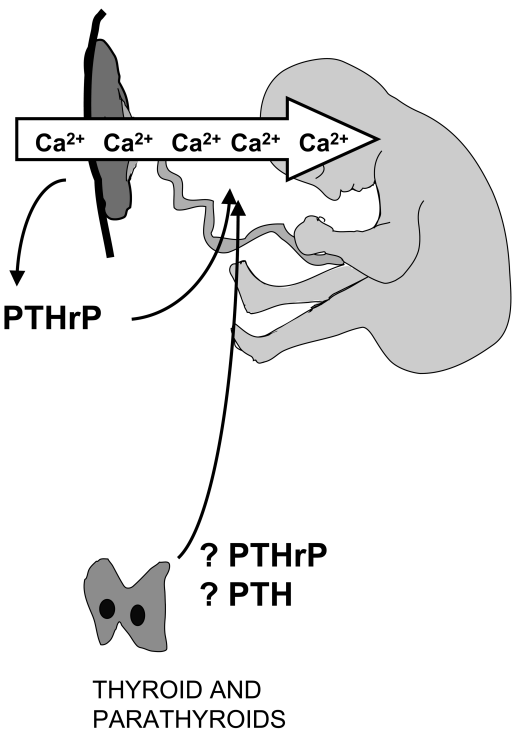

FIG. 4. Placental calcium transfer is regulated by PTHrP but not by PTH; whether the parathyroids produce PTHrP or not is uncertain. (Reprinted from Pediatric Bone, Glorieux FH, Pettifor JM, Jüppner H, Fetal mineral homeostasis, pp. 271–302, 2003, with permission from Elsevier.)

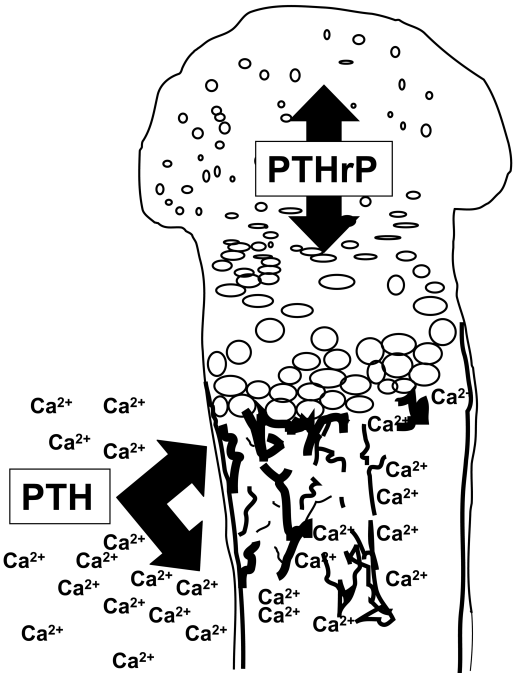

FIG. 5. Schematic model of the relative contribution of PTH and PTHrP to endochondral bone formation and skeletal mineralization. PTHrP is produced within the cartilaginous growth plate and directs the development of this scaffold that will later be broken down and replaced by bone. PTH reaches the skeleton systemically from the parathyroids and directs the accretion of mineral by the developing bone matrix. (Reprinted from Pediatric Bone, Glorieux FH, Pettifor JM, Jüppner H, Fetal mineral homeostasis, pp. 271–302, 2003, with permission from Elsevier.)

calcitonin rises 2- to 10-fold over cord blood levels over the same time interval and then gradually declines. Infants that are premature, asphyxiated, or hypocalcemic have the highest postnatal calcitonin levels; consequently, hypercalcitoninemia has been suggested to cause neonatal hypocalcemia. However, other studies indicate that the postnatal rise in calcitonin levels does not correlate to the fall in serum calcium.

PTHrP secretion from placenta, amnion, and umbilical cord is lost at birth; secretion from the parathyroid glands (if ever present) is also apparently lost sometime after birth, because PTHrP circulates at low to undetectable levels during normal adult life in humans and animals. Animal studies suggest that PTHrP may persist in the neonatal circulation for some time, whether secreted by the parathyroids or caused by absorption of PTHrP from milk (milk contains PTHrP at concentrations 10,000-fold higher than the level in the fetal circulation). Whether PTHrP present in milk contributes to the regulation of neonatal mineral homeostasis is unknown.

Intestinal Absorption of Calcium

In newborns, intestinal calcium absorption is a passive, nonsaturable process that is not dependent on vitamin D and 1,25-D. The high lactose content of milk has been shown to specifically increase the efficiency of intestinal calcium absorption and net bioavailability of dietary calcium through effects on paracellular diffusion in the distal small bowel. With increasing postnatal age, the vitamin D receptor begins to appear in intestinal cells, and mucosal levels of the calcium-binding protein calbindin$_{9K}$-D increase sharply. Around the same time, vitamin D–dependent active transport of calcium becomes noticeable, whereas passive transfer of calcium declines. By the time of weaning in rodents, the intestine is less permeable to passive absorption of calcium, and active transport has become the dominant means by which calcium is transferred into the intestinal mucosa. Data from newborn humans are less complete, but the onset of 1,25-D–dependent active transport of calcium follows a similar postnatal course. The normal postnatal maturation of the neonatal intestine may limit the ability of preterm humans to accrete sufficient calcium for skeletal mineralization and to regulate the blood calcium.

Renal Handling of Calcium

Although data are limited, urinary calcium excretion rises in humans over the first 2 weeks, consistent with a concurrent 2-fold rise in glomerular filtration rate. The neonatal kidney show a response to exogenously administered PTH that increases with postnatal age.

Skeletal Calcium Metabolism

In humans, the neonatal skeleton continues to accrete calcium at a rate of about 150 mg/kg/day, similar to the rate of the late-term fetus. Vitamin D deficiency or loss of the vitamin D receptor (which has no or minimal effect on mineral homeostasis of the fetus) becomes obvious during the neonatal period because of the onset of dependence on intestinal calcium transport for supply of calcium. In human vitamin D deficiency, hypocalcemia appears late in the first or second week, and rickets develops after 2–3 months.

Although parathyroidectomy and vitamin D deficiency in rats and loss of vitamin D receptors in mice will eventually result in hypocalcemia and hyperphosphatemia, by the time of weaning, neonatal rats and mice still have normal serum mineral concentrations and skeletal mineral content. These findings suggest that factors other than PTH and vitamin D (such as lactose and, perhaps, PTHrP in milk) may be required for normal accretion of calcium in the first several weeks when the pup is suckling and intestinal calcium absorption is not yet fully dependent on 1,25-D.

Premature infants are prone to develop metabolic bone disease of prematurity, a form of rickets precipitated by loss of the placental calcium pump at a time when the skeleton is accreting calcium at a peak rate. It is not caused by vitamin D deficiency, but seems to be the consequence of inadequate calcium and phosphate intake to meet the demands of the mineralizing neonatal skeleton. Special oral or parenteral formulas that are high in calcium and phosphorus content will correct the demineralization process and allow normal skeletal accretion of these minerals.

Neonatal Response to Maternal Hyper- or Hypoparathyroidism

Maternal hyperparathyroidism results in suppression of the neonatal parathyroid glands for some time after birth (the suppression can be permanent), and hypocalcemia, tetany, and even death may occur. The mechanism of the prolonged suppression is not known, but may be caused by chronic exposure to increased flux of calcium across the placenta during fetal development. Suppression has been observed in infants of women with familial hypocalciuric hypercalcemia.

Maternal hypoparathyroidism in humans has resulted in neonatal parathyroid gland hyperplasia, as noted above in the fetal section. The serum calcium level of the neonate has usually been reported to be normal while the PTH level (older assays) has been found to be elevated. The skeletal findings generally resolve over the first several months after birth, but acute interventions may be required to raise or lower the blood

calcium in the neonate. In addition, subtotal parathyroidectomy may be required to control more severe, autonomous disease.

Maternal hypocalcemia of any cause may result in parathyroid gland hyperplasia and hyperparathyroidism in the fetus and neonate. In women with pseudohypoparathyroidism, children that do not inherit the genetic disorder are usually normal at birth, although transient neonatal hyperparathyroidism has been reported in some cases. Furthermore, children that did inherit the condition may also be normal at birth and gradually develop the full biochemical features of pseudohypoparathyroidism over the first several years of life.

Neonatal Hypocalcemia

Neonatal hypocalcemia typically presents as seizures that onset between 4 and 28 days of age. The preterm infant is particularly prone to hypocalcemia, having lost the placental calcium infusion at a time when the skeleton is rapidly accreting calcium, and the intestinal calcium absorption is relatively inefficient. In addition to prematurity, other causes of neonatal hypocalcemia include congenital hypoparathyroidism, magnesium deficiency, maternal diabetes, vitamin D deficiency or resistance, and hyperphosphatemia.

Neonatal hypocalcemia can occur as a complication of maternal diabetes in pregnancy in up to 50% of cases, although tight control of the maternal glucose during pregnancy reduces the incidence. The cause of hypocalcemia is likely to be multifactorial but may include neonatal hypomagnesemia as a consequence of maternal glucosuria during pregnancy. Studies in rats have also suggested that maternal diabetes reduces placental mineral transport and skeletal mineral accretion, which in turn predisposes the neonate to develop hypocalcemia and hypomagnesemia.

REFERENCES

1. Kovacs CS 2003 Fetal mineral homeostasis. In: Glorieux FH, Pettifor JM, Jüppner H (eds.) Pediatric Bone: Biology and Diseases. Academic Press, San Diego, CA, USA, pp. 271–302.
2. Kovacs CS, Kronenberg HM 1997 Maternal-fetal calcium and bone metabolism during pregnancy, puerperium and lactation. Endocr.Rev **18:** 832–872.
3. Kovacs CS, Woodland ML, Fudge NJ, Friel JK 2005 The vitamin D receptor is not required for fetal mineral homeostasis or for the regulation of placental calcium transfer. Am J Physiol Endocrinol Metab **289:**E133–E144.
4. Kovacs CS, Manley NR, Moseley JM, Martin TJ, Kronenberg HM 2001 Fetal parathyroids are not required to maintain placental calcium transport. J Clin Invest **107:**1007–1015.
5. McDonald KR, Fudge NJ, Woodrow JP, Friel JK, Hoff AO, Gagel RF, Kovacs CS 2004 Ablation of calcitonin/calcitonin gene related peptide-α impairs fetal magnesium but not calcium homeostasis. Am J Physiol Endocrinol Metab **287:**E218–E226.
6. Karaplis AC, Luz A, Glowacki J, Bronson RT, Tybulewicz VL, Kronenberg HM, Mulligan RC 1994 Lethal skeletal dysplasia from targeted disruption of the parathyroid hormone-related peptide gene. Genes Dev **8:**277–289.
7. Kovacs CS, Lanske B, Hunzelman JL, Guo J, Karaplis AC, Kronenberg HM 1996 Parathyroid hormone-related peptide (PTHrP) regulates fetal-placental calcium transport through a receptor distinct from the PTH/PTHrP receptor. Proc Natl Acad Sci USA **93:**15233–15238.
8. Kovacs CS, Chafe LL, Fudge NJ, Friel JK, Manley NR 2001 PTH regulates fetal blood calcium and skeletal mineralization independently of PTHrP. Endocrinology **142:**4983–4993.
9. Kovacs CS, Ho-Pao CL, Hunzelman JL, Lanske B, Fox J, Seidman JG, Seidman CE, Kronenberg HM 1998 Regulation of murine fetal-placental calcium metabolism by the calcium-sensing receptor. J Clin Invest **101:** 2812–2820.
10. Care AD, Caple IW, Abbas SK, Pickard DW 1986 The effect of fetal thyroparathyroidectomy on the transport of calcium across the ovine placenta to the fetus. Placenta **7:**417–424.
11. Care AD, Abbas SK, Pickard DW, Barri M, Drinkhill M, Findlay JB, White IR, Caple IW 1990 Stimulation of ovine placental transport of calcium and magnesium by mid-molecule fragments of human parathyroid hormone-related protein. Exp Physiol **75:**605–608.
12. Rodda CP, Kubota M, Heath JA, Ebeling PR, Moseley JM, Care AD, Caple IW, Martin TJ 1988 Evidence for a novel parathyroid hormone-related protein in fetal lamb parathyroid glands and sheep placenta: Comparisons with a similar protein implicated in humoral hypercalcaemia of malignancy. J Endocrinol **117:**261–271.
13. Moore KL, Persaud TVN 1998 The Developing Human, 6th ed. Saunders, Philadelphia, PA, USA.
14. Karsenty G 2001 Chondrogenesis just ain't what it used to be. J Clin Invest **107:**405–407.
15. Loughead JL, Mimouni F, Tsang RC 1988 Serum ionized calcium concentrations in normal neonates. Am J Dis Child **142:**516–518.
16. Bagnoli F, Bruchi S, Garosi G, Pecciarini L, Bracci R 1990 Relationship between mode of delivery and neonatal calcium homeostasis. Eur J Pediatr **149:**800–803.

Chapter 9. Childhood and Adolescence

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INTRODUCTION

Childhood and adolescence are characterized by longitudinal growth as well as by changes in skeletal size and shape. Bone mass increases dramatically during growth, and it is becoming increasingly clear that the amount of bone accrued during these life periods may be an important determinant of future resistance to fractures. Thus, considerable interest is placed on defining the determinants that account for the physiological variations in skeletal growth, because they will provide the best means for identification of individuals and populations that are at greatest risk of osteoporosis and other disorders of bone and mineral metabolism.

The development of precise noninvasive methods for measuring BMC and BMD has significantly improved our ability to study the influence of genetic and environmental factors on the attainment of bone mass. Pediatric applications of the most commonly used methods, DXA and QCT will be discussed below. Inherent differences in these measurement methods have sometimes led investigators to different conclusions about the timing and characteristics of bone growth and accumulation of bone mass. Nevertheless, bone densitometry can be an

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effective tool in children both within the clinical and research setting provided that the results are presented in a clinically useful format or the results are interpreted independent of body size and growth.

ACCUMULATION OF BONE MASS AND PEAK BONE MASS

Skeletal mass increases from ~70–95 g at birth to 2400–3300 g in young women and men, respectively.[1] These gains are achieved through longitudinal growth, which results from a combination of bone modeling and remodeling. These processes occur at different rates and at different times at primary and secondary sites of bone formation.

Longitudinal studies of total body BMC measurements show that gains in bone mass are very rapid during adolescence and that up to 25% of peak bone mass (PBM) is acquired during the 2-year period across peak height velocity.[2] At peak height velocity, boys and girls have reached 90% of their adult stature but only 57% of their adult BMC. At least 90% of PBM is acquired by age 18.[2]

The human skeleton contains ~85% cortical bone and 15% cancellous bone, and studies have shown that the patterns of gain during growth (like those of the rate of bone loss with aging) differ considerably between these two skeletal compartments.[3,4] The density of cancellous bone is strongly influenced by hormonal and/or metabolic factors associated with sexual development during late adolescence.[5] On average, cancellous BMD in the spine increases by 13% during puberty in white boys and girls. After controlling for puberty, vertebral BMD fails to correlate significantly with age, sex, weight, height, surface area, or body mass index (BMI).[5] The increase in the density of cancellous bone during the later stages of puberty is likely a reflection of a greater thickness of the trabeculae.

The factors that account for the increase in cancellous vertebral BMD during late puberty remain to be determined. It is reasonable to suspect that many of the physical changes undergone, such as the accelerated growth spurt and the increases in body and bone mass, are, at least in part, mediated by the actions of sex steroids.[6] Some of these effects may be caused by changes in protein and calcium metabolism induced by sex steroids, or, alternatively, they may be secondary to the cascade of events triggered by the increase in growth hormone (GH) and insulin growth factor I (IGF-I) production observed after sex steroid exposure.

The exact age at which values for bone mass reach their peak at various skeletal sites has not yet been determined with certainty. It is likely that the timing of peak values differs between the axial and appendicular skeletons and between men and women. Moreover, differences among studies are, in part, a reflection of the different modalities used for measuring bone mass.

In the axial skeleton, PBM may be achieved by the end of the second decade of life. Studies in women using CT have shown that the density and the size of vertebral bone reach their peak soon after the time of sexual and skeletal maturity,[5,7] which corroborates anatomical data that indicate trabecular bone loss as early as the third decade of life, and no change in the cross-sectional area of the vertebral body from 15 to 90 years of age.[8,9] The data regarding whether vertebral cross-sectional area in men continues to grow after cessation of longitudinal growth are controversial; while some authors find no change in the cross-sectional dimensions after skeletal maturity, others have suggested that vertebral size increases with age throughout adulthood.[9]

In the appendicular skeleton, the range of ages published in cross-sectional studies for the timing of PBM has varied significantly from 17–18 years of age to as late as 35 years of age.[10–13] Longitudinal DXA studies indicate that the rate of increase in skeletal mass slows markedly in late adolescence and that peak values in the femoral neck, such as those in the spine, are achieved near the end of puberty in normal females.[7,14]

According to the "mechanostat" theory, bone mass accrual is tightly controlled by mechanical loads on bone generated from muscle forces.[15,16] Growing muscle and body weight in children exert a load on the skeleton that causes strain (change in dimensions and/or shape of the bone in response to the force load) on bone. Furthermore, strain seems to be an important signaling mechanism in bone to help control bone's structural adaptations to the mechanical use.[15] Miscrostrains (μE) between 800 and 1600 preserve bone, whereas loads on bone that regularly exceeds 1600 μE result in bone becoming stronger. Absorptiometric data from a study in Argentina showed that, in children, both bone and muscle mass increase linearly until puberty, but in girls at 12 years of age, DXA-derived bone mass begins increasing faster than muscle mass. It is hypothesized that an estrogen response may result in the storing of more bone than needed for strictly mechanical reasons in adolescent girls, possibly to provide calcium stores for later use during pregnancy and lactation.[17–19]

GENETIC INFLUENCE ON BONE ACCUMULATION DURING GROWTH

Heredity factors are important determinants of bone mass. Convergent data from mother-daughter pairs, sib pairs, and twin studies have estimated the heritability of bone mass to account for 60–80% of its variance.[20–22] The magnitude of the genetic effect varies with age and between skeletal sites; it is higher in the young than in the elderly and in the spine than in the extremities.[23] Further support for this genetic influence comes from studies showing reduced bone mass in daughters of osteoporotic women compared with controls,[22] in men and women with first-degree relatives who have osteoporosis,[24] and, more recently, in studies reporting a link between several "candidate" genes and bone mass.

In a study of a large group of female subjects, polymorphisms of the *vitamin D receptor* (*VDR*) gene at a *Bsm*I restriction site were associated with BMD in prepubertal and adolescent girls.[25] Girls with the BB genotype had significantly lower spinal BMD SD scores than girls with the Bb and bb genotypes.[25] In contrast, polymorphisms at the start codon site of the *VDR* gene, detected with the *Fok*I restriction enzyme, were not associated with BMD at any skeletal site in prepubertal girls.[26] An association between femoral and spinal BMD and the VDR genotype at the *Apa*I and *Bsm*I restriction sites has been shown using CT in prepubertal American girls of Hispanic descent.[27] In this study, girls with aa and bb genotypes showed significantly higher volumetric BMD values than girls with the other genotypes, both at the spine and the femur. A polymorphism in the Sp1-binding site of the gene encoding for collagen type Ia1 was also found to explain some of the variability in vertebral BMD in this cohort of prepubertal girls.[28] In contrast, no relationship between the VDR genotype at the *Bsm*I site and forearm BMD or rates of gain of BMD was found in Norwegian boys and girls.[29] It would seem that *VDR* polymorphisms are weak determinants of bone mass. This is not surprising because VDR is likely to be confounded by the effect of numerous other genes that influence bone homeostasis and skeletal development in a growing individual. However, the recent emergence of candidate genes (*calcium-sensing re-*

ceptor gene, *α2HS-glycoprotein* gene, *estrogen receptor α* gene, *calcitonin* gene, *PTH* gene, *collagen Iα1* gene, *TGFβ* genes, and others) has opened new concepts in the understanding of the pathophysiology of bone development and loss, but much research is still needed for us to detangle the complexity of gene–gene and gene–environment interactions on bone mass.[30]

Effect of Sex

Observations using CT indicate that, throughout life, females have smaller vertebral cross-sectional area compared with males, even after accounting for differences in body size. On average, the cross-sectional area of the vertebral bodies is 11% smaller in prepubertal girls than in prepubertal boys matched for age, height, and weight.[31] This disparity increases with growth and is greatest at skeletal maturity, when the cross-sectional dimensions of the vertebrae are ~25% smaller in women than in men, even after taking into consideration differences in body size.[32] Thus, the phenotypic basis for the 4- to 8-fold higher incidence of vertebral fractures in women compared with that in men may lie in the smaller size of the female vertebra.

In contrast, the cross-sectional dimensions of the femur do not differ between males and females matched for age, height, and weight.[33] The cross-sectional and cortical bone areas at the midshaft of the femur are primarily related to body weight, regardless of sex, a notion consistent with analytical models proposing that long bone cross-sectional growth is strongly driven by mechanical loads.[33,34]

Recent evidence also indicates that BMD is similar in boys and girls before puberty. Data obtained with DXA in two large samples of healthy subjects clearly indicated that there were no sex differences in BMC and BMD during the prepubertal period.[35,36] In the study by Nguyen et al.,[35] BMD values during puberty in girls were higher in the pelvis and spine, whereas measures in postpubertal boys were higher in the whole skeleton. Peak BMC and BMD was achieved between the ages of 20 and 25 years and occurred much earlier in girls than in boys.

Effect of Ethnicity

Most reports of ethnic differences in bone mass during childhood, based on absorptiometric methods, have indicated a higher bone mass among blacks compared with whites.[36–39] This has led to the generalization that black population groups have greater bone mass and strength compared with white population groups as an attempt to explain the lower fracture rates observed in black population groups. However, not all U.S. studies have observed ethnic differences in BMC and BMD in children.[40,41] Furthermore, black Gambian children have a smaller bone mass than British children,[42] and black children in South Africa have a similar appendicular bone mass to white children.[43] Prepubertal black children in South Africa have greater BMC at the femoral neck than their white peers and similar BMC at the lumbar spine and whole body and lower BMC at the midradius than their white peers. Thus, it is important to recognize that bone mass varies within and among ethnic groups depending on a variety of genetic and environmental factors.

Pediatric studies using CT indicate that, regardless of sex, ethnicity has significant and differential effects on the density and the size of the bones in the axial and appendicular skeletons.[44] In the axial skeleton, the density of cancellous bone in the vertebral bodies is greater in black than in U.S. white adolescents. This difference first becomes apparent during late stages of puberty and persists throughout life.[45] Based on CT data, cancellous BMD is similar in black and white children before puberty, but during puberty it increases in all adolescents. The magnitude of the increase from prepubertal to postpubertal values is, however, substantially greater in black than in white subjects (34% versus 11%, respectively).[44] The cross-sectional areas of the vertebral bodies, however, do not differ between black and white children.[44] Thus, theoretically, the structural basis for the lower vertebral bone strength and the greater incidence of fractures in the axial skeleton of white subjects resides in their lower cancellous BMD. In contrast, in the appendicular skeleton, ethnicity influences the cross-sectional areas of the femora but not the cortical bone area or the material density of cortical bone.[44] Although values for femoral cross-sectional area increase with height, weight, and other anthropometric parameters in all children, this measurement is substantially greater in black children.[44] Because the same amount of cortical bone placed further from the center of the bone results in greater bone strength, the skeletal advantage for blacks in the appendicular skeleton is likely the consequence of the greater cross-sectional size of the bones.[34]

Limited data from Asian and Hispanic youth suggest that their bone mass is similar to that of whites but much lower than that of black children.[41] Differences in bone and body size account for much of the apparent observed ethnic differences in BMD among non-Hispanic, Hispanic, and Asian children.[41]

TRACKING OF BONE MASS

The lack of a meaningful clinical pediatric outcome measure related to BMD examinations is currently a major limitation for their value in children. Unfortunately, studies assessing the relationship of childhood fractures to bone measures have been inconclusive. While several studies have suggested that children with fractures have a deficiency in bone acquisition compared with those who do not fracture, other factors, such as level of activity and the risk associated with youthful behavior, limit the predictive value of bone measures. However, establishing the degree that these determinations can be tracked throughout growth will help us to define the constancy of a child's expected measurements relative to population percentiles. The amount of bone that is gained during adolescence is the main contributor to PBM, which, in turn, is believed to be a major determinant of osteoporosis and fracture risk in the elderly. Available data indicate that the morphological traits that contribute to the strength of the bone track throughout life, from childhood to adulthood.[35,46,47] Longitudinal CT measurements of the cross-sectional areas of the vertebrae and femora and of cancellous BMD in healthy children indicated that measures at early puberty predicted values at sexual maturity.[47] When baseline values were divided into quartiles, a linear relation across pubertal stages was observed for each quartile (Fig. 1). The regression lines differed among quartiles, paralleled each other, and did not overlap. Therefore, individual volumetric BMD and bone size tracked through growth, maintaining the same position in the normal distribution at the end of puberty as was present in the prepubertal period. Establishing whether DXA values also maintain their rank order across time will aid in the identification of those children who are prone to develop low values for PBM and who may be at greater risk for osteoporosis later in life. Available data support the notion of significant tracking for pediatric DXA measures. Indeed, strong correlations between baseline and follow-up DXA values 2 years later have been observed in prepubertal girls.

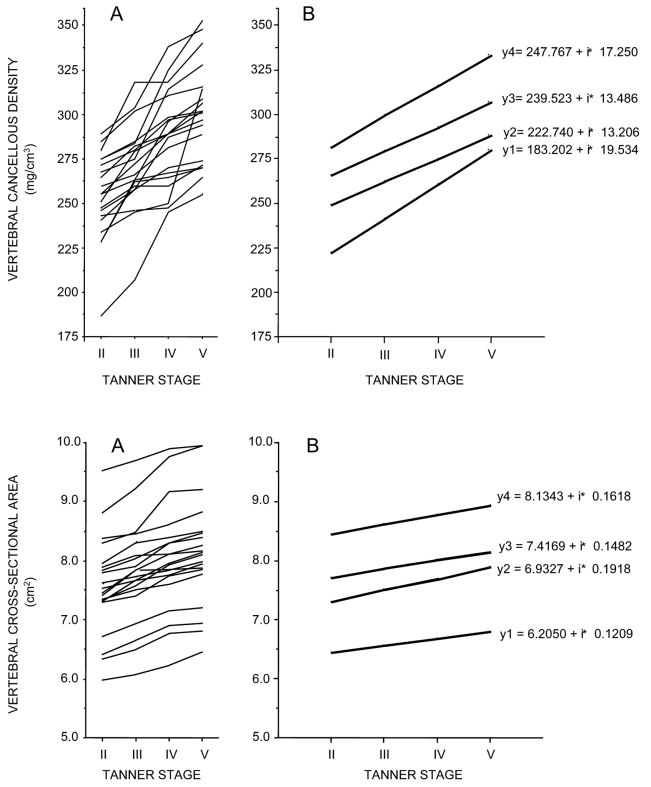

FIG. 1. Longitudinal measurements of vertebral cancellous BMD (top) and vertebral cross-sectional area (bottom) in 20 girls from Tanner stages 2–5 of sexual development. Values are shown (A) for each girl and (B) for each quartile.

PEDIATRIC BONE MASS MEASUREMENT

DXA

The most commonly used quantitative radiologic method to assess bone mass in children, as in adults, is DXA. The standard software from most DXA manufacturers, however, was designed with adult patients in mind, so special software for pediatrics has been developed by some manufacturers. The software may require longer scanning time, which can make cooperation from children difficult, resulting in motion artifacts and other inaccuracies.[48]

The preferred anatomic sites for DXA measurements in adults (lumbar spine, proximal femur, and forearm) are problematic when measured in the growing skeleton where size and shape change with age. There is also tremendous variability in growth and body size at any given chronologic age. Because of these factors that affect regional BMD in children, whole body BMC has been recommended for pediatric studies.[49]

Normative data for DXA values in pediatrics are available in the literature and are included in some DXA software packages. It should, however, be noted that different DXA manufacturers display substantial variation in BMD values of the same bone. Therefore, caution is advised before using published normative data for clinical use, and institutional and device-specific norms are preferable to published references.

Bone mass determinations in children with DXA have been done at all ages, including newborns[50] and infants.[51,52] In general, values measured at different skeletal sites increase from infancy to adulthood (Fig. 2).[14,40,53,54] The relationship between age and BMC in the lumbar spine seems to be represented by a segmented polynomial curve; a rapid increase during childhood is followed by an even greater increase during puberty that ends in the third decade of life.[7,14,55,56] Similar relationships may be seen in the femoral neck[14,53,55] and the entire skeleton.[53,55] However, radial DXA values in children have not been found to be influenced by puberty.[55]

Radiation exposure involved in DXA examinations is extremely low. The subject effective dose has been estimated to be ~0.4 μSv for lumbar spine measurements and ~5.4 μSv for whole skeleton scans.[57] In children, the precision of DXA measurements ranges from 0.8% to 2.5% in most studies.[50,51]

Several limitations of DXA must be stressed with reference to bone measurements during childhood, when major changes in body composition, body size, and skeletal mass occur. DXA is a projectional technique, and its measurements are based on the 2D projection of a 3D structure. Thus, DXA values are a function of three skeletal parameters: the size of the bone being examined, the volume of the bone, and its mineral density.[58] These values are frequently expressed as measurements of the bone content per surface area (g/cm^2), as determined by scan radiographs. However, scan radiographs only provide an approximation of the size of the bone, and any correction based on these radiographs is only a very rough estimate of the "density." Consequently, the interpretation of DXA-derived areal BMD poses major challenges in children because of

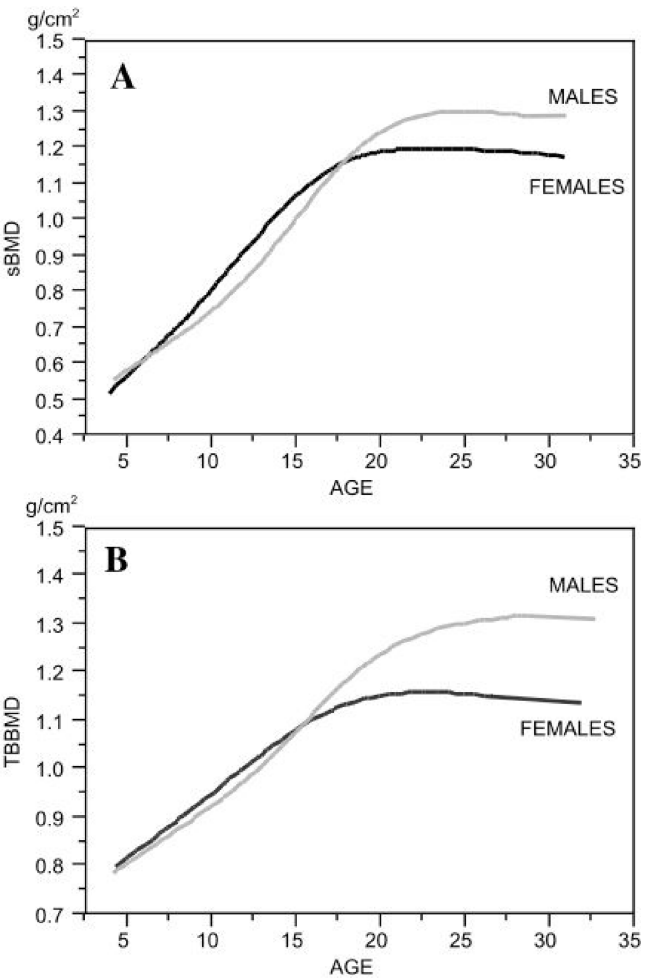

FIG. 2. Changes in BMD values of (A) the lumbar spine and (B) whole body with chronological age. The acceleration during adolescence is followed by a plateau phase in early adulthood. DXA measurements performed in 319 healthy subjects (156 female, 163 male) from 4 to 32 years of age are shown (courtesy of Stefano Mora, MD, Milan, Italy).

changes in bone and body size related to age and pubertal development. A number of approaches have been suggested to overcome this disadvantage with the use of correction factors. A simple correction involves adjusting BMD for the height of the subject. Another method is the determination of bone mineral apparent density (BMAD), which can be calculated in a variety of ways: by dividing BMC by the 3D bone volume calculated from the 2D DXA-derived bone area, assuming the cross-sectional area of the vertebrae is a cube[56,58,59]; or by assuming that the shape approximates a cylinder with a circular base,[54,60] or a cylinder with an elliptic base area.[61] Other size adjustments for BMC include use of lean tissue mass, etc. However, all of these methods are subject to error because there is no closed formula that defines the shape of the vertebrae. Similarly, although correction formulas have been proposed for the femur and the midradius,[54,62] they are also prone to error, because they cannot account for the marked changes in the size and shape of the bone during growth.

Inaccuracies in DXA values can also result from the unknown composition of the soft tissues adjacent to the bone being analyzed. Because corrections for soft tissues are based on a homogenous distribution of fat around the bone, changes in DXA measurements are observed if fat is distributed inhomogeneously around the bone measured. It has been calculated that inhomogeneous fat distribution in soft tissues, resulting in a difference of a 2-cm fat layer between soft tissue area and bone area, will influence DXA measurements by 10%.[63] While this is not of concern when studying subjects whose weight and body size remain constant, longitudinal DXA values in children are subject to considerable error, and measurements may reflect the changes in body size and composition that occur with growth more than true changes in BMD. This disadvantage especially limits the use of DXA in studies of children with eating disorders, such as obesity or anorexia nervosa.

QCT

QCT bone measurements can be obtained at any skeletal site with a standard clinical CT scanner using an external bone mineral reference phantom for calibration and specially developed software. The ability of QCT to assess both the volume and the density of bone in the axial and appendicular skeletons, without influence from body or skeletal size, is the major advantage of this modality when used in children. Unfortunately, CT scanners are expensive, large, nonportable machines that require costly maintenance and considerable technological expertise for proper function. Moreover, this equipment is usually located in the radiology department and is under constant clinical demand, creating a lack of accessibility. These disadvantages have partially been overcome by the recent development of smaller, mobile, less expensive peripheral QCT (pQCT) scanners designed exclusively for bone measurements. These smaller scanners, however, can only assess the bones of the appendicular skeleton; reference data of the distal radius are available for children.[64]

The radiation exposure from QCT measurements is related to the technique used and can be as low as 150 mrem (1.5 mSv) localized to the region of interest in the appendicular or axial skeleton. The total body equivalent dose of radiation is ~4–9 mrems (40–90 μSv), and this figure includes the radiation associated with screening digital radiographs used to localize the site of measurement.[57] This amount of radiation is far lower than that associated with other CT imaging procedures, accounting for the wide range of published figures for the radiation dose associated with CT measurements. It is also less than many other commonly used radiographic diagnostic tests.

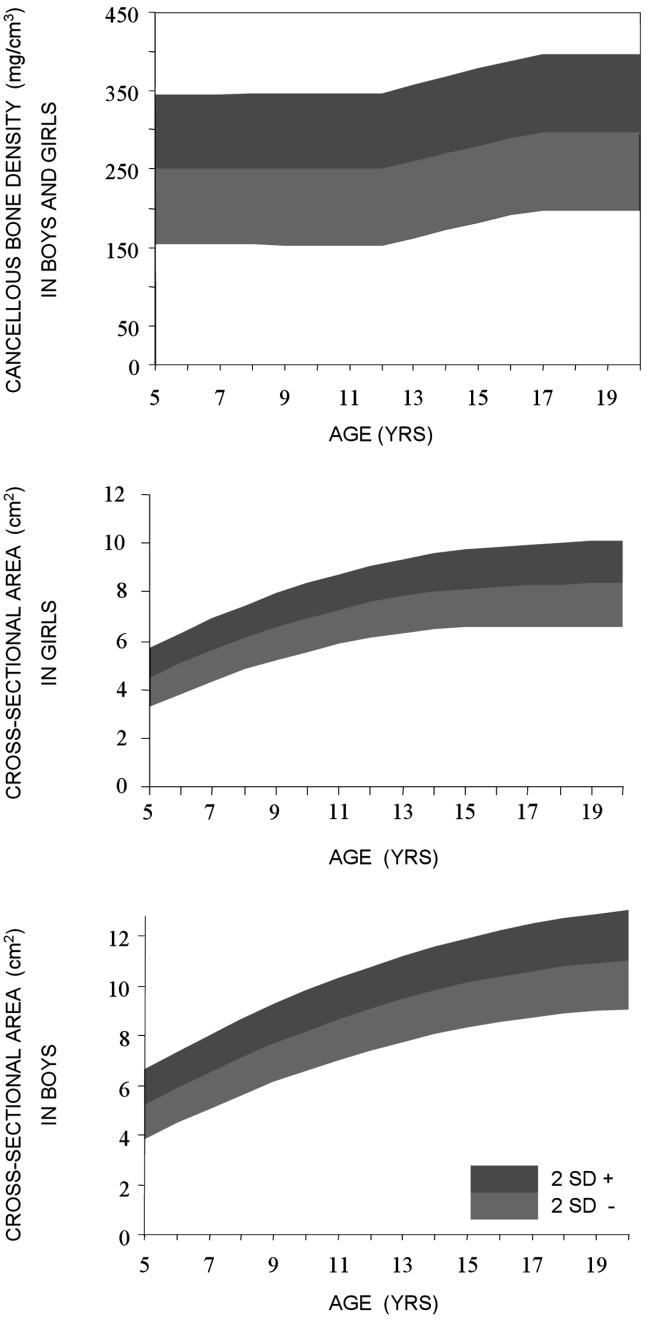

FIG. 3. Normative data for vertebral cancellous BMD and cross-sectional area in children and adolescents. Values for BMD are similar for boys and girls, whereas those for cross-sectional area differ.

In the axial skeleton, QCT has principally been used to determine cancellous bone density (mg/cm^3) in the vertebral bodies, and less frequently, the dimensions of the vertebrae (Fig. 3). It should be noted that because the vertebrae of children contain proportionally more bone and less fat than that of elderly subjects, both the precision and accuracy of QCT cancellous BMD determinations in children are far better than those reported for adults. CVs for determinations of cancellous BMD, vertebral body height, and vertebral cross-sectional area have been calculated as 1.5%, 1.3%, and 0.8%, respectively.[31] Unfortunately, the cortical bone in the vertebral body is not thick enough to avoid inaccuracies associated with bone averaging errors.

In the appendicular skeleton, three bone parameters can be measured by QCT: the cross-sectional area (cm^2) of the bone, the cortical bone area (cm^2), and the cortical bone density.[65,66] To this effect, the outer and inner boundaries of the cortex are identified by specially developed software at the place of the maximum slope of the profile through the bone. The area within the outer cortical shell represents the cross-sectional area, whereas the area between the outer and inner shells represents the cortical bone area. The mean CT numbers of the pixels within the inner and outer cortical shells provide the average density of the bone. The CVs for repeated QCT measurements of cortical BMD, cortical bone area, and cross-sectional area of the femur range between 0.6% and 1.5%.[33,65,66] Unfortunately, the reproducibility of measurements of cancellous BMD is poor because of the large anatomical variability of the metaphysis of the long bone.

ENVIRONMENTAL AND HORMONAL INFLUENCE ON BONE ACCUMULATION DURING GROWTH

Physical Activity

The beneficial effects of exercise on bone mass are well documented through multiple observational and retrospective studies indicating that weight-bearing activities increase bone mass. Studies of prepubertal female gymnasts showed a larger cross-sectional area of the forearm, despite a shorter stature,[67] and that areal BMD values expressed as SD scores were significantly greater than zero (the predicted mean of the controls) in the arms, legs, and spine, all weight-bearing sites.[67] In other studies, children and adolescents who were physically active accrued more bone mineral than their sedentary peers,[2,68] and a more recent study showed that physical activity levels measured by accelerometry and parental report were positively associated with total body BMC and BMD measurements in preschool children.[69]

Studies comparing the effects of different physical exercises on bone indicated that high impact exercises resulted in the greatest increases in bone mass in adolescents.[70] Similarly, gymnasts had higher spine and femur BMD than swimmers or sedentary girls.[71] Amateur athletes involved in weight-bearing sports (rugby, soccer, endurance running, fighting sports, bodybuilding) had higher values for total body and legs BMD than amateur sportsmen involved in active loading activities (swimming, rowing).[72]

Several randomized trials involving weight-bearing activity interventions for bone mass gains have been conducted in children and adolescents.[73–77] Exercise session attendance ranged from 50% to 97%; exercise adherence is therefore a potentially serious threat to the internal validity of the results. However, the most recent studies showed very high rates of adherence. With one exception at 36+ months,[73] the duration of the interventions was 6–12 months. All studies reported significant changes in femoral BMD, and four studies indicated increases in lumbar spine BMD and BMC in the intervention groups.

Whether the beneficial effect of physical activity on the growing skeleton is maintained in adulthood is unknown, because no prospective study has been designed to address this question. However, the results of most, but not all, retrospective analyses indicate that, indeed, the enhancement of bone acquisition during growth because of exercise interventions may be long lasting. Lifetime tennis players, playing at a lower level of intensity then during youth, have remarkably higher forearm BMC than control subjects.[78] Retired soccer players have high BMD during the first 10–20 years after cessation of sport, but their BMD is lower compared with active players.[79] Other studies suggest that BMD values are maintained at ~0.5–1.0 SD above the age-predicted mean in athletes who have been retired for 10–20 years.[67,80,81] Peri- and postmenopausal women who participated in sport activities during adolescence showed BMD measurements at the lumbar spine and femur that were remarkably higher than those of women who did not participate in physical activities during youth.[82] In a recent follow-up study of 27 years, lifetime physical activity was related to adult BMD, indicating the importance of continuing exercise after growth.[83] In contrast, a decrease in spinal BMD has been reported in runners who ceased exercising.[84] Similarly, cessation of exercise led to the return of BMD values to pretraining levels in 12 women who performed unilateral leg press for a year.[85]

Calcium Intake

The earliest data suggesting an influence of dietary calcium on PBM came from a study of two Croatian populations with substantially different calcium intakes.[86] The differences seen in bone mass were present at 30 years of age, suggesting that the effects of dietary calcium probably occurred during growth rather than in adulthood. Moreover, some epidemiological studies have shown an increased prevalence of osteoporosis in regions where dietary calcium intake is extremely low.[87]

The most convincing evidence that calcium consumption influences rates of bone mineral accrual rates comes from controlled supplementation trials in young healthy subjects. These studies showed that subjects given additional calcium for 1–3 years had greater gains than did controls.[88–93] Although bone size increased as a result of added dietary calcium in two studies,[88,92] the response to calcium varied with skeletal site, pretreatment calcium consumption, and pubertal stage. Greater bone mineral gains have been generally reported at cortical skeletal sites in prepubertal subjects and in girls whose habitual dietary intake was <850 mg/day.[92]

Whether short-term increases in bone mineral observed in these trials will translate into a clinically relevant reduction in osteoporosis risk is yet unknown. The magnitude of gains in BMC or BMD in most studies was modest (<5%). Moreover, the beneficial effect of calcium supplementation does not seem to last, and most studies reported that the benefits of intervention disappeared once the treatment was stopped. However, in other studies, the benefits persisted 12 months after discontinuation of supplement.[92]

Hormonal Status

The presence of low bone mass in patients with abnormal pubertal development shows the critical role that pubertal hormone changes have on mineral acquisition. Adult patients with hypogonadotropic hypogonadism commonly have low BMD values, resulting from inadequate bone mineral accrual during puberty.[94] Androgen receptors mediate the effects of testosterone in bone but their function is generally exerted after conversion to estrogen by a specific aromatase present in osteoblastic cells.[95] Thus, the more important sex steroid involved in skeletal maturation is estrogen.[96] Amenorrheic teens have lower lumbar BMD than girls with normal menses.[97] In addition, male patients with aromatase deficiency, or estrogen receptor defects resulting in complete resistance, have a phenotype that includes tall stature, normal secondary sexual characteristics, severe osteoporosis, and skeletal immaturity (delayed physeal closure), despite normal serum levels of testosterone.[98] Idiopathic delayed puberty has also been implicated as a cause of reduced peak bone mass.[99]

The effect that pregnancy and lactation have on bone acquisition in teenagers is yet to be fully defined. Normal pregnancy

places a demand on calcium homeostasis, because the fetus and the placenta draw calcium from the maternal circulation to mineralize the fetal skeleton, and low BMD has been reported during pregnancy. Whether pregnancy during adolescence negatively influences BMD and PBM is the subject of great importance and is yet to be elucidated.

Reduced BMD is commonly seen in GH–deficient children who fail to acquire bone mineral at the expected rate.[100] Part of the bone mass deficit in these patients is caused by reduced bone size. Much of the GH action on bone is mediated through IGF-I, which functions as a bone trophic hormone that positively affects osteoblasts and stimulates collagen synthesis. In humans, IGF-I serum levels have been found to be positively correlated to bone size measured at the midshaft of the femur.[101]

CONCLUSION

Skeletal mass is accrued throughout childhood and adolescence and is largely determined by genetic and/or familial factors. A child's sex affects bone mass after puberty, while many ethnic differences seem to be present throughout growth. The influence of gonadal steroids is of major importance during puberty, while many factors such as other hormonal influences, physical activity type and intensity, and dietary calcium intake affect bone acquisition throughout growth. Bone mass measurements such as DXA and CT, while limited in some respects for pediatric applications, can be very important in assessing a child's skeletal status and growth.

REFERENCES

1. Trotter M, Hixon BB 1974 Sequential changes in weight, density, and percentage ash weight of human skeletons from an early fetal period through old age. Anat Rec **179:**1–18.
2. Bailey DA, McKay HA, Mirwald RL, Crocker PR, Faulkner RA 1999 A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: The University of Saskatchewan bone mineral accrual study. J Bone Miner Res **14:**1672–1679.
3. Riggs BL, Wahner HW, Dunn WL, Mazess RB, Offord KP, Mellon LJ 1981 Differential changes in bone mineral density of the appendicular skeleton with aging. J Clin Invest **67:**328–335.
4. Mora S, Goodman WG, Loro ML, Roe TF, Sayre J, Gilsanz V 1994 Age-related changes in cortical and cancellous vertebral bone density in girls: Assessment with quantitative CT. AJR Am J Roentgenol **162:**405–409.
5. Gilsanz V, Gibbens DT, Carlson M, Boechat MI, Cann CE, Schulz EE 1988 Peak trabecular vertebral density: A comparison of adolescent and adult females. Calcif Tissue Int **43:**260–262.
6. Mauras N, Haymond MW, Darmaun D, Vieira NE, Abrams SA, Yergey A 1994 Calcium and protein kinetics in prepubertal boys—positive effects of testosterone. J Clin Invest **93:**1014–1019.
7. Theintz G, Buchs B, Rizzoli R, Slosman D, Clavien H, Sizonenko PC, Bonjour JP 1992 Longitudinal monitoring of bone mass accumulation in healthy adolescents: Evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. J Clin Endocrinol Metab **75:**1060–1065.
8. Marcus R, Kosen J, Pfefferbaum A, Horning A 1983 Age-related loss of trabecular bone in premenopausal women: A biopsy study. Calcif Tiss Int **35:**406–409.
9. Mosekilde L 1989 Sex differences in age-related loss of vertebral trabecular bone mass and structure—biomechanical consequences. Bone **10:**425–432.
10. Halioua L, Anderson JJB 1990 Age and anthropometric determinants of radial bone mass in premenopausal Caucasian women: A cross-sectional study. Osteoporos Int **1:**50–55.
11. Gordon CL, Halton JM, Atkinson S, Webber CE 1991 The contributions of growth and puberty to peak bone mass. Growth Dev Aging **55:**257–262.
12. Recker RR, Davies KM, Hinders SM, Heaney RP, Stegman MR, Kimmel DB 1992 Bone gain in young adult women. J Am Med Assoc **268:**2403–2408.
13. Matkovic V, Jelic T, Wardlaw GM, Ilich JZ, Goel PK, Wright JK, Andon MB, Smith KT, Heaney RP 1994 Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. J Clin Invest **93:**799–808.
14. Bonjour JP, Theintz G, Buchs B, Slosman B, Rizzoli R 1991 Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. J Clin Endocrinol Metab **73:**555–563.
15. Frost HM 1996 Bone development during childhood: Insights from a new paradigm. In: Schonau E (ed.) New Developments in Diagnostics and Therapy. Elsevier Science, Amsterdam, The Netherlands, pp. 3–39.
16. Frost HM 1997 Perspectives: Why do long distance runners not have more bone? A vital biomechanical explanation and an estrogen effect. J Bone Miner Metab **15:**9–16.
17. Zanchetta JR, Plotkin H, Alvarez-Figueira ML 1995 ztiBone mass in children: Normative values for the 2–20 year-old population. Bone **16**(Suppl)**:**393–399.
18. Neu CM, Manz F, Rauch F, Merkel A, Schoenau E 2001 Bone densities and bone size at the distal radius in healthy children and adolescents: A study using peripheral quantitative computed tomography. Bone **28:**227–232.
19. Schoenau E, Neu MC, Manz F 2004 Muscle mass during childhood—relationship to skeletal development. J Musculoskelet Neuron Interact **4:**105–108.
20. Christian JC, Yu PL, Slemenda CW, Johnston CC 1989 Heritability of bone mass: A longitudinal study in ageing male twins. Am J Hum Genet **44:**429–433.
21. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Ebert S 1991 Genetic determinants of bone mass in adults: A twin study. J Clin Invest **80:**706–710.
22. Seeman E, Hopper JL, Bach LA, Cooper ME, Parkinson E, McKay J, Jerums G 1989 Reduced bone mass in daughters of women with osteoporosis. N Engl J Med **320:**554–558.
23. Slemenda CW, Christian JC, Williams CJ, Norton JA, Johnston CC Jr 1991 Genetic determinants of bone mass in adult women: A reevaluation of the twin model and the potential importance of gene interaction on heritability estimates. J Bone Miner Res **6:**561–567.
24. Evans RA, Marel GH, Lancaster EK, Kos S, Evans M, Wong YP 1988 Bone mass is low in relatives of osteoporotic patients. Ann Intern Med **109:**870–873.
25. Ferrari SL, Rizzoli R, Slosman DO, Bonjour JP 1998 Do dietary calcium and age explain the controversy surrounding the relationship between bone mineral density and vitamin D receptor gene polymorphism? J Bone Miner Res **13:**363–370.
26. Ferrari SL, Rizzoli R, Manen D, Slosman DA, Bonjour JP 1998 Vitamin D receptor gene start codon polymorphisms (*Fok*I) and bone mineral density: Interaction with age, dietary calcium, and 3′-end region polymorphisms. J Bone Miner Res **13:**925–930.
27. Sainz J, Van Tornout JM, Loro ML, Sayre J, Roe TF, Gilsanz V 1997 Vitamin D receptor gene polymorphisms and bone density in prepubertal girls. N Engl J Med **337:**77–82.
28. Sainz J, Van Tornout JM, Sayre J, Kaufman F, Gilsanz V 1999 Association of collagen type 1 a1 gene polymorphism with bone density in early childhood. J Clin Endocrinol Metab **84:**853–855.
29. Gunnes M, Berg JP, Hasle J, Lehmann EH 1997 Lack of relationship between vitamin D receptor genotype and forearm bone gain in healthy children, adolescents, and young adults. J Clin Endocrinol Metab **82:**851–855.
30. Rizzoli R, Bonjour J-P, Ferrari SL 2001 Osteoporosis, genetics and hormones. J Molecul Endocrinol **26:**79–94.
31. Gilsanz V, Boechat MI, Roe TF, Loro ML, Sayre JW, Goodman WG 1994 Gender differences in vertebral body sizes in children and adolescents. Radiology **190:**673–677.
32. Gilsanz V, Boechat MI, Gilsanz R, Loro ML, Roe TF, Goodman WG 1994 Gender differences in vertebral sizes in adults: Biomechanical implications. Radiology **190:**678–682.
33. Gilsanz V, Kovanlikaya A, Costin G, Roe TF, Sayre J, Kaufman F 1997 Differential effect of gender on the size of the bones in the axial and appendicular skeletons. J Clin Endocrinol Metab **82:**1603–1607.
34. Van der Meulen MCH, Beaupre GS, Carter DR 1993 Mechanobiologic influences in long bone cross-sectional growth. Bone **14:**635–642.
35. Nguyen TV, Maynard LM, Towne B, Roche AF, Wisemandle W, Li J, Guo SS, Chumlea WC, Siervogel RM 2001 Sex differences in bone mass acquisition during growth. J Clin Densitom **4:**147–157.
36. Nelson DA, Simpson PM, Johnson CC, Barondness DA, Kleerekoper M 1997 The accumulation of whole body skeletal mass in third- and fourth-grade children: Effects of age, gender, ethnicity and body composition. Bone Miner **20:**73–78.
37. Bell NH, Shary J, Stevens J, Garza M, Gordon L, Edwards J 1991

Demonstration that bone mass is greater in black than in white children. J Bone Miner Res **6:**719–723.
38. Li JY, Specker BL, Ho ML, Tsang RC 1989 Bone mineral content in black and white children 1 to 6 years of age. Early appearance of race and sex differences. Am J Dis Child **143:**1346–1349.
39. Thomas KA, Cook SD, Bennett JT, Whitecloud TSHI, Rice JC 1991 Femoral neck and lumbar spine mineral densities in a normal population 3–20 years of age. J Pediatric Orthopad **11:**48–58.
40. Southard RN, Morris JD, Mahan JD, Hayes JR, Torch MA, Sommer A, Zipf WB 1991 Bone mass in healthy children: Measurements with quantitative DXA. Radiology **179:**735–738.
41. Bachrach LK, Hastie T, Wang MC, Balasubramanian N, Marcus R 1999 Bone mineral acquisition in healthy asian, hispanic, black, and caucasian youth: A longitudinal study. J Clin Endocrinol Metab **84:**4702–4712.
42. Prentice A, Laskey MA, Shaw J, Cole TJ, Fraser DR 1990 Bone mineral content of Gambian and British children aged 0–36 months. Bone Miner **10:**211–224.
43. Patel DN, Pettifor JM, Becker PJ, Grieve C, Leschner K 1992 The effect of ethnic group on appendicular bone mass in children. J Bone Miner Res **7:**263–272.
44. Gilsanz V, Skaggs DL, Kovanlikaya A, Sayre J, Loro ML, Kaufman F, Korenman SG 1998 Differential effect of race on the axial and appendicular skeletons of children. J Clin Endocrinol Metab **83:**1420–1427.
45. Kleerekoper M, Nelson DA, Flynn MJ, Pawluszka AS, Jacobsen G, Peterson EL 1994 Comparison of radiographic absorptiometry with dual energy x-ray absorptiometry and quantitative computed tomography in normal older white and black women. J Bone Miner Res **9:**1745–1750.
46. Ferrari S, Rizzoli R, Slosman D, Bonjour JP 1998 Familial resemblance for bone mineral mass is expressed before puberty. J Clin Endocrinol Metab **83:**358–361.
47. Loro ML, Sayre J, Roe TF, Goran MI, Kaufman FR, Gilsanz V 2000 Early identification of children predisposed to low peak bone mass and osteoporosis later in life. J Clin Endocrinol Metab **85:**3908–3918.
48. Koo WWK, Massom LR, Walters J 1995 Validation of accuracy and precision of dual energy x-ray absorptiometry for infants. J Bone Miner Res **10:**1111–1115.
49. Nelson DA, Koo WK 1995 Interpretation of bone mass measurements in the growing skeleton. Calcif Tissue Int **65:**1–3.
50. Braillon PM, Salle BL, Brunet J, Glorieux FH, Delmas PD, Meunier PJ 1992 Dual energy x-ray absorptiometry measurement of bone mineral content in newborns: Validation of the technique. Pediatr Res **32:**77–80.
51. Koo WW, Walters J, Bush AJ 1995 Technical considerations of dual-energy x-ray absorptiometry-based bone mineral measurements for pediatric subjects. J Bone Miner Res **10:**1998–2004.
52. Rupich RC, Specker BL, Lieuw-A-Fa M, Ho M 1996 Gender and race differences in bone mass during infancy. Calcif Tissue Int **58:**395–397.
53. Faulkner RA, Bailey DA, Drinkwater DT, McKay HA, Arnold C, Wilkinson AA 1996 Bone densitometry in canadian children. Calcif Tissue Int **59:**344–351.
54. Kröger H, Kotaniemi A, Vainio P, Alhava E 1992 Bone densitometry of the spine and femur in children by dual-energy x-ray absorptiometry. Bone Miner **17:**75–85.
55. Zanchetta JR, Plotkin H, Alvarez-Filgueira ML 1995 Bone mass in children: Normative values for the 2–20-year-old population. Bone **14:** 3S–399S.
56. Katzman DK, Bachrach LK, Carter DR, Marcus R 1991 Clinical and anthropometric correlates of bone mineral acquisition in healthy adolescent girls. J Clin Endocrinol Metab **73:**1332–1339.
57. Kalender WA 1992 Effective dose values in bone mineral measurements by photon absorptiometry and computed tomography. Osteoporos Int **2:**82–87.
58. Carter DR, Bouxsein ML, Marcus R 1992 New approaches for interpreting projected bone densitometry data. J Bone Miner Res **7:**137–145.
59. Jergas M, Breitenseher M, Gluer CC, Genant HK 1995 Estimates of volumetric bone density from projectional measurements improve the discriminatory capability of dual x-ray absorptiometry. J Bone Miner Res **10:**1101–1110.
60. Salle BL, Braillon P, Glorieux FG, Brunet J, Cavero E, Meunier PJ 1992 Lumbar bone mineral content measured by dual energy X-ray absorptiometry in newborns and infants. Acta Paediatr **81:**953–958.
61. Peel NFA, Eastell R 1994 Diagnostic value of estimated volumetric bone mineral density of the lummbar spine in osteoporosis. J Bone Miner Res **9:**317–320.
62. Lu PW, Cowell CT, Lloyd-Jones SA, Briody JN, Howman-Giles R 1996 Volumetric bone mineral density in normal subjects, aged 5–27 years. J Clin Endocrinol Metab **81:**1586–1590.
63. Hangartner TN 1990 Influence of fat on bone measurements with dual-energy absorptiometry. Bone Miner **9:**71–78.
64. Rauch F, Schoenau E 2005 Peripheral quantitative computed tomography of the distal radius in young subjects—new reference data and interpretation of results. J Musculoskelet Neuronal Interact **5:**119–126.
65. Hangartner TN, Gilsanz V 1996 Evaluation of cortical bone by computed tomography. J Bone Miner Res **11:**1518–1525.
66. Kovanlikaya A, Loro ML, Hangartner TN, Reynolds RA, Roe TF, Gilsanz V 1996 Osteopenia in children: CT assessment. Radiology **198:**781–784.
67. Bass S, Pearce G, Bradney M, Hendrich E, Delmas PD, Harding A, Seeman E 1998 Exercise before puberty may confer residual benefits in bone density in adulthood: Studies in active prepubertal and retired female gymnasts. J Bone Miner Res **13:**500–507.
68. Cooper C, Cawley M, Bhalla A, Egger P, Ring F, Morton L, Barker D 1995 Childhood growth, physical activity, and peak bone mass in women. J Bone Miner Res **10:**940–947.
69. Janz KF, Burns TL, Torner JC, Levy SM, Paulos R, Willing MC, Warren JJ 2001 Physical activity and bone measures in young children: The Iowa bone development study. Pediatrics **107:**1387–1393.
70. Lima F, DeFalco V, Baima J, Carazzato JG, Pereira RM 2001 Effect of impact load and active load on bone metabolism and body composition of adolescent athletes. Med Sci Sports Exerc **33:**1318–1323.
71. Courteix D, Lespessailles E, Peres SL, Obert P, Germain P, Benhamou CL 1998 Effect of physical training on bone mineral density in prepubertal girls: A comparative study between impact-loading and non-impact-loading sports. Osteoporos Int **8:**152–158.
72. Morel J, Combe B, Francisco J, Bernard J 2001 Bone mineral density of 704 amateur sportsmen involved in different physical activities. Osteoporos Int **12:**152–157.
73. Sundberg M, Gardsell P, Johnell O, Karlsson MK, Ornstein E, Sandstedt B, Sernbo I 2001 Peripubertal moderate exercise increases bone mass in boys but not in girls: A population-based intervention study. Osteoporos Int **12:**230–238.
74. Petit MA, McKay HA, MacKelvie KJ, Heinonen A, Khan KM, Beck TJ 2002 A randomized school-based jumping intervention confers site and maturity specific benefits on bone structural properties in girls: A hip structural analysis study. J Bone Miner Res **17:**363–372.
75. Fuchs RK, Buauer JJ, Snow CM 2001 Jumping improves hip and lumbar spine bone mass in prepubescent children: A randomized controlled trial. J Bone Miner Res **16:**148–156.
76. Bradney M, Pearce G, Naughton G, Sullivan C, Bass S, Beck T, Carlson J, Seeman E 1998 Moderate exercise during growth in prepubertal boys: Changes in bone mass, size, volumetric density, and bone strength: A controlled prospective study. J Bone Miner Res **13:**1814–1821.
77. Binkley T, Specker B 2004 Increased periosteal circumference remains present 12 months after as exercise intervention in preschool children. Bone **35:**1383–1388.
78. Huddleston AL, Rockwell D, Kulund DN, Harrison RB 1980 Bone mass in lifetime tennis athletes. JAMA **244:**1107–1109.
79. Karlsson MK, Linden C, Karlsson C, Johnell O, Obrant K, Seeman E 2000 Exercise during growth and bone mineral density and fractures in old age. Lancet **355:**469–470.
80. Khan KM, Grren RM, Saul A, Bennell KL, Crichton KJ, Hopper JL 1996 Retired elite female ballet dancers and nonathletic controls have similar bone mineral density at weight bearing sites. J Bon Miner Res **11:**1566–1574.
81. Etherington J, Harris PA, Nandra D, Hart DJ, Wolman RL, Doyle DV, Spector TD 1996 The effect of weight-bearing exercise on bone mineral density: A study of female ex-elite athletes and the general population. J Bone Miner Res **11:**1333–1338.
82. Puntila E, Kroger H, Lakke T, Honkanen R, Tupperainen M 1997 1997 Physical activity in adolescence and bone density in peri- and postmenopausal women: A population based study. Bone **21:**363–367.
83. Delvaux K, Lefevre J, Philippaerts R, Dequeker J, Thomis M, Vanreusel B, Claessens A, Eynde BV, Beunen G, Lysens R 2001 Bone mass and lifetime physical activity in Flemish males: A 27 year follow-up study. Med Sci Sports Exerc **33:**1868–1875.
84. Michel BA, Lane NE, Bjorkengren A, Bloch DA, Fries JF 1992 Impact of running on lumbar bone density: A 5-year longitudinal study. J Rheumatol **19:**1759–1763.
85. Vuori I, Heinonen A, Sievanan H, Kannus P, Pasanen M, Oja P 1994 Effect of unilateral strength training and detraining on bone mineral density and content in young women: A study of mechanical loading and deloading on human bones. Calcif Tissue Int **55:**59–67.
86. Matkovic V, Kostial K, Simonovic I, Buzinz R, Brodarec A, Nordin BE 1979 Bone status and fracture rates in two regions of Yugoslavia. Am J Clin Nutr **32:**540–549.
87. Heaney RP 1992 Calcium in the prevention and treatment of osteoporosis. J Intern Med **231:**169–180.

88. Lee WTK, Leung SSF, Leung DM, Cheng JC 1996 A follow-up study on the effects of calcium-supplement withdrawal and puberty on bone acquisition in children. Am J Clin Nutr **64:**71–77.
89. Lloyd T, Andon MB, Rollings N, Martel JK, Landis JR, Demers LM, Eggli DF, Kieselhorst K, Kulin HE 1993 Calcium supplementation and bone mineral density in adolescent girls. JAMA **270:**841–844.
90. 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. N Engl J Med **327:**82–87.
91. Chan GM, Hoffman K, McMurry M 1995 Effects of dairy products on bone and body composition in pubertal girls. J Pediatr **126:**551–556.
92. Bonjour JP, Carrie AL, Ferrari S, Clavien H, Slosman D, Theintz G, Rizzoli 1997 Calcium-enriched foods and bone mass growth in prepubertal girls: A randomized double blind, placebo-controlled trial. J Clin Invest **99:**1287–1294.
93. Nowson CA, Green RM, Hopper JL, Sherwin AJ, Young D, Kaymakci B, Guest CS, Smid M, Larkins RG, Wark J 1997 A co-twin study of the effect of calcium supplementation on bone density during adolescence. Osetoporos Int **7:**219–225.
94. Finkelstein JS, Klibanski A, Neer RM, Greenspan SL, Rosenthal DI, Crowley WF Jr 1987 Osteoporosis in men with idiopathic hypogonadotropic hypogonadism. Ann Intern Med **106:**354–361.
95. Abu EO, Horner A, Kusec V, Triffitt JT, Compston JE 1997 The localization of androgen receptors in human bone. J Clin Endocrinol Metab **82:**3493–3497.
96. Frank GR 1995 The role of estrogen in pubertal skeletal physiology: Epiphyseal maturation and mineralization of the skeleton. Acta Paediatr **84:**627–630.
97. Hergenroeder AC 1995 Bone mineralization, hypothalamic amenorrhea, and sex steroid therapy in female adolescents and young adults. J Pediatr **126:**683–689.
98. Carani C, Qin K, Simoni M, Faustini-Fustini M, Serpente S, Boyd J, Korach KS, Simpson ER 1997 Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med **337:**91–95.
99. Finkelstein JS, Klibanski A, Neer RM 1996 A longitudinal evaluation of bone mineral density in adult men with histories of delayed puberty. J Clin Endocrinol Metab **81:**1152–1155.
100. Baroncelli GI, Bertelloni S, Ceccarelli C, Saggese G 1998 Measurement of volumetric bone mineral density accurately determines degree of lumbar undermineralization in children with growth hormone deficiency. J Clin Endocrinol Metab **83:**3150–3154.
101. Mora S, Pitukcheewanont P, Nelson JC, Gilsanz V 1999 Serum levels of insulin-like growth factor-I and the density, volume and cross-sectional area of bone in children. J Clin Endocrinol Metab **84:**2780–2783.

Chapter 10. Skeletal Physiology: Pregnancy and Lactation

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INTRODUCTION

Normal pregnancy places a demand on the calcium homeostatic mechanisms of the human female, because the fetus and placenta draw calcium from the maternal circulation to mineralize the fetal skeleton. Similar demands are placed on the lactating woman, to supply sufficient calcium to the breast milk and enable continued skeletal growth in a nursing infant. Despite a similar magnitude of calcium demand presented to pregnant and lactating women, the adjustments made in each of these reproductive periods differ significantly (Fig. 1). These hormone-mediated adjustments normally satisfy the daily calcium needs of the fetus and infant without long-term consequences to the maternal skeleton. Detailed references on this subject are available in two comprehensive reviews.[1,2]

PREGNANCY

In total, the developing fetal skeleton gains up to 33 g of calcium, and ~80% of the accretion occurs during the third trimester when the fetal skeleton is rapidly mineralizing. This calcium demand seems to be largely met by a doubling of maternal intestinal calcium absorption, mediated by 1,25-dihydroxyvitamin D and other factors.

Mineral Ions and Calciotropic Hormones

Normal pregnancy results in altered levels of calcium and the calciotropic hormones as schematically depicted in Fig. 2.[1] The total serum calcium falls early in pregnancy because of a fall in the serum albumin. This decrease should not be mistaken for true hypocalcemia, because the ionized calcium (the physiologically important fraction) remains constant. Serum phosphate levels are also normal during pregnancy.

The serum PTH level, when measured with a two-site immunoradiometric assay, falls to the low-normal range (i.e., 10–30% of the mean nonpregnant value) during the first trimester, but increases steadily to the midnormal range by term. Total 1,25-dihydroxyvitamin D levels double early in pregnancy and maintain this increase until term; free 1,25-dihydroxyvitamin D levels are increased from the third trimester and possibly earlier. The rise in 1,25-dihydroxyvitamin D may be largely independent of changes in PTH, because PTH levels are typically decreasing at the time of the increase in 1,25-dihydroxyvitamin D. The maternal kidneys likely account for most, if not all, of the rise in 1,25-dihydroxyvitamin D during pregnancy, although the decidua, placenta, and fetal kidneys may contribute a small amount. The relative contribution of the maternal kidneys is based on several lines of evidence,[1] including the report of an anephric woman on hemodialysis who had low 1,25-dihydroxyvitamin D levels before and during a pregnancy. The renal 1α-hydroxylase is upregulated in response to factors such as PTH-related protein (PTHrP), estradiol, prolactin, and placental lactogen. Serum calcitonin levels are also increased during pregnancy.

PTHrP levels are increased during pregnancy, as determined by assays that detect PTHrP fragments encompassing amino acids 1–86. Because PTHrP is produced by many tissues in the fetus and mother (including the placenta, amnion, decidua, umbilical cord, fetal parathyroids, and breast), it is not clear which source(s) contribute to the rise detected in the maternal circulation. PTHrP may contribute to the elevations in 1,25-dihydroxyvitamin D and suppression of PTH that are noted during pregnancy. PTHrP may have other roles during pregnancy, such as regulating placental calcium transport in the fetus.[1,3] Also, PTHrP may have a role in protecting the maternal skeleton during pregnancy, because the carboxy-terminal

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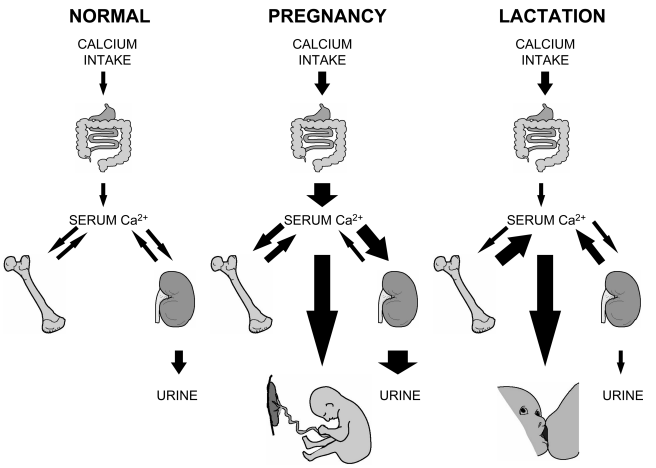

FIG. 1. Schematic illustration contrasting calcium homeostasis in human pregnancy and lactation compared with normal. The thickness of arrows indicates a relative increase or decrease with respect to the normal and nonpregnant state. (Modified with permission of The Endocrine Society from Kovacs CS, Kronenberg HM 1997 Maternal-fetal calcium and bone metabolism during pregnancy, puerperium and lactation. Endocr Rev **18:** 832–872. Copyright 1997, The Endocrine Society.)

portion of PTHrP (osteostatin) has been shown to inhibit osteoclastic bone resorption.[4]

Pregnancy induces significant changes in the levels of other hormones, including the sex steroids, prolactin, placental lactogen, and IGF-1. Each of these may have direct or indirect effects on calcium and bone metabolism during pregnancy, but these issues have been largely unexplored.

Intestinal Absorption of Calcium

Intestinal absorption of calcium is doubled during pregnancy from as early as 12 weeks of gestation (the earliest time-point studied); this seems to be a major maternal adaptation to meet the fetal need for calcium. This increase may be largely the result of a 1,25-dihydroxyvitamin D–mediated increase in intestinal calbindin$_{9K}$-D and other proteins; prolactin and placental lactogen (and possibly other factors) may also mediate part of the increase in intestinal calcium absorption. The increased absorption of calcium early in pregnancy may allow the maternal skeleton to store calcium in advance of the peak fetal demands that occur later in pregnancy.

Renal Handling of Calcium

The 24-h urine calcium excretion is increased as early as the 12th week of gestation (the earliest time-point studied), and the amounted excreted may exceed the normal range. The elevated calcitonin levels of pregnancy might also promote renal calcium excretion. Because fasting urine calcium values are normal or low, the increase in 24-h urine calcium likely reflects the increased intestinal absorption of calcium (absorptive hypercalciuria).

Skeletal Calcium Metabolism

Animal models indicate that histomorphometric parameters of bone turnover are increased during pregnancy and that BMC may increase; however, comparable histomorphometric data are not available for human pregnancy. In one study,[5] 15 women who electively terminated a pregnancy in the first trimester (8–10 weeks) had bone biopsy evidence of increased bone resorption, including increased resorption surface and increased numbers of resorption cavities. These findings were

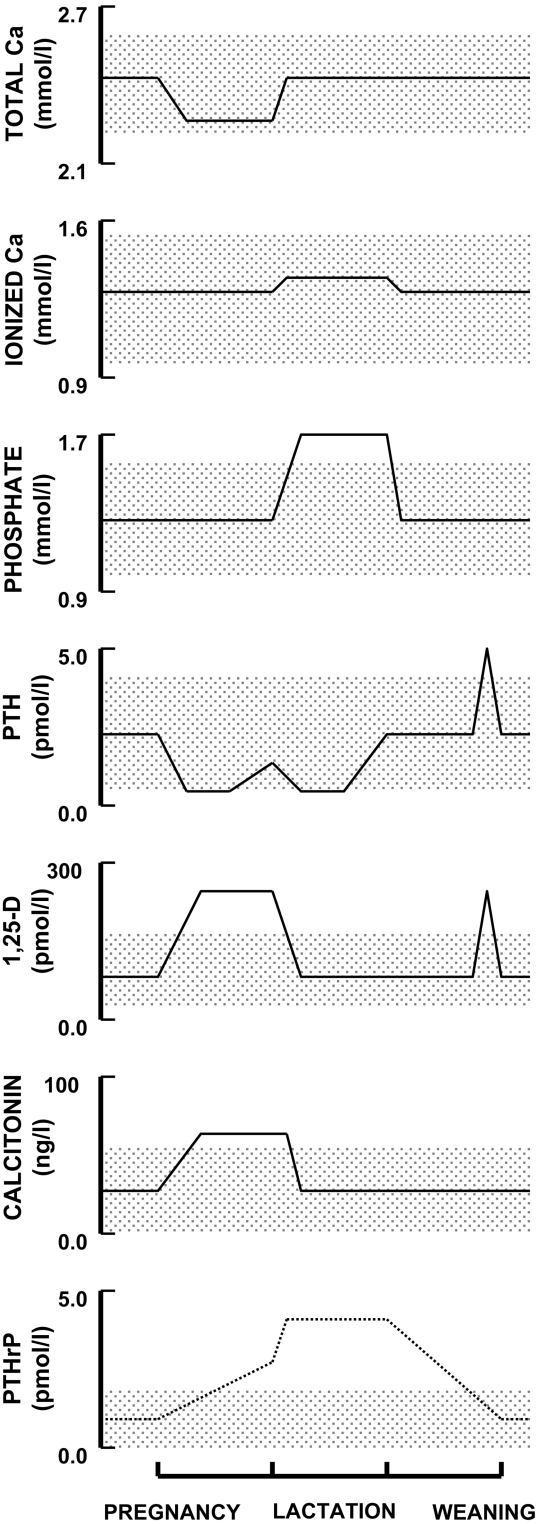

FIG. 2. Schematic illustration of the longitudinal changes in calcium, phosphate, and calciotropic hormone levels that occur during pregnancy and lactation. Normal adult ranges are indicated by the shaded areas. The progression in PTHrP levels has been depicted by a dashed line to reflect that the data are less complete; the implied comparison of PTHrP levels in late pregnancy and lactation are uncertain extrapolations because no reports followed patients serially. In both situations, PTHrP levels are elevated. (Adapted with permission of The Endocrine Society from Kovacs CS, Kronenberg HM 1997 Maternal-fetal calcium and bone metabolism during pregnancy, puerperium, and lactation. Endocr Rev **18:**832–872. Copyright 1997, The Endocrine Society.)

not present in biopsy specimens obtained from nonpregnant controls or in biopsy specimens obtained at term from 13 women who had elective cesarian-sections.

Most human studies of skeletal calcium metabolism in pregnancy have examined changes in serum markers of bone formation and urine markers of bone resorption. These studies are fraught with a number of confounding variables, including lack of prepregnancy baseline values; effects of hemodilution in pregnancy on serum markers; increased glomular filtration rate (GFR); altered creatinine excretion; placental, uterine, and fetal contribution to the levels of markers in blood; degradation and clearance by the placenta; and lack of diurnally timed or fasted specimens. Given these limitations, many studies have reported that urinary markers of bone resorption (24-h collection) are increased from early to mid-pregnancy (including deoxypyridinoline, pyridinoline, and hydroxyproline). Conversely, serum markers of bone formation (generally not corrected for hemodilution or increased GFR) are often decreased from prepregnancy or nonpregnant values in early or mid-pregnancy, rising to normal or above before term (including osteocalcin, procollagen I carboxypeptides, and bone-specific alkaline phosphatase). It is conceivable that the bone formation markers are artifactually lowered by normal hemodilution and increased renal clearance of pregnancy, obscuring any real increase in the level of the markers. Total alkaline phosphatase rises early in pregnancy due largely to contributions from the placental fraction; it is not a useful marker of bone formation in pregnancy.

Based on the scant bone biopsy data and the measurements of bone markers (with aforementioned confounding factors), one may cautiously conclude that bone resorption is increased in pregnancy from as early as the 10th week of gestation. There is comparatively little maternal–fetal calcium transfer occurring at this stage of pregnancy compared with the peak rate of calcium transfer in the third trimester. One might have anticipated that markers of bone resorption would increase particularly in the third trimester, but in fact, no marked increase is seen at that time.

Changes in skeletal calcium content have been assessed through the use of sequential BMD studies during pregnancy. Because of concerns about fetal radiation exposure, few such studies have been done. Such studies are confounded by the changes in body composition and weight during normal pregnancy that can lead to artifactual changes in the BMD reading obtained. Using single and/or dual-photon absorptiometry (SPA/DPA), several prospective studies did not find a significant change in cortical or trabecular BMD during pregnancy.[1] Several recent studies have used DXA before conception (range, 1–8 months prior, but not always stated) and after delivery (range, 1–6 weeks postpartum).[2] Most studies involved 16 or fewer subjects. One study found no change in lumbar spine BMD measurements obtained preconception and within 1–2 weeks after delivery, whereas the other studies reported decreases of 4–5% in lumbar spine BMD, with the postpartum measurement taken between 1 and 6 weeks after delivery. Because the puerperium is associated with BMD losses of 1–3% per month, it is possible that obtaining the second measurement 2–6 weeks after delivery contributed to the bone loss documented in many of the studies. Other longitudinal studies have found a progressive decrease during pregnancy in indices thought to correlate with BMD, as determined by ultrasonographic measurements at another peripheral site, the os calcis. None of all the aforementioned studies can address the question as to whether skeletal calcium content is increased early in pregnancy in advance of the third trimester. Further studies, with larger numbers of patients, will be needed to clarify the extent of bone loss during pregnancy.

It seems certain that any acute changes in bone metabolism during pregnancy do not cause long-term changes in skeletal calcium content or strength. Numerous studies of osteoporotic or osteopenic women have failed to find a significant association of parity with BMD or fracture risk.[1,6] Although many of these studies could not separate out the effects of parity from those of lactation, it may be reasonable to conclude that if parity has any effect on BMD or fracture risk later in life, it must be only a very modest effect.

Osteoporosis in Pregnancy

Occasionally, a woman may present with fragility fractures and low BMD during or shortly after pregnancy; the possibility that the woman had low BMD before pregnancy cannot be excluded. Some women may experience excessive resorption of calcium from the skeleton because of changes in mineral metabolism induced by pregnancy and other factors such as low dietary calcium intake and vitamin D insufficiency. The apparently increased rate of bone resorption in pregnancy may contribute to fracture risk, because a high rate of bone turnover is an independent risk factor for fragility fractures outside of pregnancy. Therefore, fragility fractures in pregnancy or the puerperium may be a consequence of preexisting low BMD and increased bone resorption, among other possible factors. During lactation, additional changes in mineral metabolism occur that may further increase fracture risk in some women.

Focal, transient osteoporosis of the hip is a rare, self-limited form of pregnancy-associated osteoporosis. It is probably not a manifestation of altered calciotropic hormone levels or mineral balance during pregnancy, but rather might be a consequence of local factors. The theories proposed to explain the condition include femoral venous stasis caused by the gravid uterus, reflex sympathetic dystrophy, ischemia, trauma, viral infections, marrow hypertrophy, immobilization, and fetal pressure on the obturator nerve. These patients present with unilateral or bilateral hip pain, limp, and/or hip fracture in the third trimester. There is objective evidence of reduced BMD of the symptomatic femoral head and neck, which has been shown by MRI to be the consequence of increased water content of the femoral head and the marrow cavity; a joint effusion may also be present. The symptoms and the radiological appearance usually resolve within 2–6 months postpartum.

Primary Hyperparathyroidism

Although probably a rare condition (there are no data available on its prevalence), primary hyperparathyroidism in pregnancy has been associated in the literature with an alarming rate of adverse outcomes in the fetus and neonate, including a 30% rate of spontaneous abortion or stillbirth. The adverse postnatal outcomes are thought to result from suppression of the fetal and neonatal parathyroid glands; this suppression may occasionally be prolonged after birth for months. To prevent these adverse outcomes, surgical correction of primary hyperparathyroidism during the second trimester has been almost universally recommended. Several case series have found elective surgery to be well tolerated and to dramatically reduce the rate of adverse events compared with the earlier cases reported in the literature. Many of the women in those early cases had a relatively severe form of primary hyperparathyroidism that is not often seen today (symptomatic with nephrocalcinosis and renal insufficiency). While mild, asymptomatic primary hyperparathyroidism during pregnancy has been followed conservatively with successful outcomes, complications continue to occur; therefore, in the absence of definitive data, surgery during the second trimester remains the most common recommendation.[7]

Familial Hypocalciuric Hypercalcemia

Although familial hypocalciuric hypercalcemia (FHH) has not been reported to adversely affect the mother during pregnancy, maternal hypercalcemia can cause fetal and neonatal parathyroid suppression with subsequent tetany.

Hypoparathyroidism and Pseudohypoparathyroidism

Early in pregnancy, hypoparathyroid women may have fewer hypocalcemic symptoms and require less supplemental calcium. This is consistent with a limited role for PTH in the pregnant woman and suggests that an increase in 1,25-dihydroxyvitamin D and/or increased intestinal calcium absorption will occur in the absence of PTH. However, it is clear from other case reports that some pregnant hypoparathyroid women may require increased calcitriol replacement to avoid worsening hypocalcemia. It is important to maintain a normal ionized calcium level in pregnant women because maternal hypocalcemia has been associated with the development of intrauterine fetal hyperparathyroidism and fetal death. Levels of ionized calcium rather than that of total calcium should be followed, because of the fall of serum albumin during pregnancy. Late in pregnancy, hypercalcemia may occur in hypoparathyroid women unless the calcitriol dosage is substantially reduced or discontinued. This effect may be mediated by the increasing levels of PTHrP in the maternal circulation in late pregnancy.

In limited case reports of pseudohypoparathyroidism, pregnancy has been noted to normalize the serum calcium level, reduce the PTH level by one-half, and increase the 1,25-dihydroxyvitamin D level 2- to 3-fold.[8] The mechanism by which these changes occur despite pseudohypoparathyroidism remains unclear.

LACTATION

The typical daily loss of calcium in breast milk has been estimated to range from 280 to 400 mg, although daily losses as great as 1000 mg calcium have been reported. A temporary demineralization of the skeleton seems to be the main mechanism by which lactating humans meet these calcium requirements. This demineralization does not seem to be mediated by PTH or 1,25-dihydroxyvitamin D, but may be mediated by PTHrP in the setting of a fall in estrogen levels.

Mineral Ions and Calciotropic Hormones

The normal lactational changes in maternal calcium, phosphate, and calciotropic hormone levels are schematically depicted in Fig. 2.[1] The mean ionized calcium level of exclusively lactating women is increased, although it remains within the normal range. Serum phosphate levels are also higher during lactation, and the level may exceed the normal range. Because reabsorption of phosphate by the kidneys seems to be increased, the increased serum phosphate levels may, therefore, reflect the combined effects of increased flux of phosphate into the blood from diet and from skeletal resorption in the setting of decreased renal phosphate excretion.

Intact PTH, as determined by a two-site immunoradiometric assay (IRMA) assay, has been found to be reduced 50% or more during the first several months of lactation. It rises to normal at weaning, but may rise above normal after weaning. In contrast to the high 1,25-dihydroxyvitamin D levels of pregnancy, maternal free and bound 1,25-dihydroxyvitamin D levels fall to normal within days of parturition and remain there throughout lactation. Calcitonin levels fall to normal after the first 6 weeks postpartum. Mice lacking the calcitonin gene lose twice the normal amount of BMC during lactation, which indicates that physiological levels of calcitonin may protect the maternal skeleton from excessive resorption during this time period.[9,10] Whether calcitonin plays a similar role in human physiology is unknown.

PTHrP levels, as measured by two-site IRMA assays, are significantly higher in lactating women than in nonpregnant controls. The source of PTHrP may be the breast, because PTHrP has been detected in breast milk at concentrations exceeding 10,000 times the level found in the blood of patients with hypercalcemia of malignancy or normal human controls. Furthermore, lactating mice with the *PTHrP* gene ablated only from mammary tissue have lower blood levels of PTHrP than control lactating mice.[11] Studies in animals suggest that PTHrP may regulate mammary development and blood flow and the calcium content of milk. In addition, PTHrP reaching the maternal circulation from the lactating breast may cause resorption of calcium from the maternal skeleton, renal tubular reabsorption of calcium, and (indirectly) suppression of PTH. In support of this hypothesis, deletion of the *PTHrP* gene from mammary tissue at the onset of lactation resulted in more modest losses of BMC during lactation in mice.[11] In humans, PTHrP levels correlate with the amount of BMD lost, negatively with PTH levels, and positively with the ionized calcium levels of lactating women.[12–14] Furthermore, observations in aparathyroid women provide evidence of the impact of PTHrP in calcium homeostasis during lactation (see below).

Intestinal Absorption of Calcium

Intestinal calcium absorption decreases to the nonpregnant rate from the increased rate of pregnancy. This corresponds to the fall in 1,25-dihydroxyvitamin D levels to normal.

Renal Handling of Calcium

In humans, the glomerular filtration rate falls during lactation, and the renal excretion of calcium is typically reduced to levels as low as 50 mg/24 h. This suggests that the tubular reabsorption of calcium must be increased to account for reduced calcium excretion in the setting of increased serum calcium.

Skeletal Calcium Metabolism

Histomorphometric data from animals consistently show increased bone turnover during lactation, and losses of 35% or more of bone mineral are achieved during 2–3 weeks of normal lactation in the rat.[1] Comparative histomorphometric data are lacking for humans, and in place of that, serum markers of bone formation and urinary markers of bone resorption have been assessed in numerous cross-sectional and prospective studies of lactation. Some of the confounding factors discussed with respect to pregnancy apply to the use of these markers in lactating women. In this instance, the GFR is reduced, and the intravascular volume is contracted. Urinary markers of bone resorption (24-h collection) have been reported to be elevated 2- to 3-fold during lactation and are higher than the levels attained in the third trimester. Serum markers of bone formation (not adjusted for hemoconcentration or reduced GFR) are generally high during lactation and increased over the levels attained during the third trimester. Total alkaline phosphatase falls immediately postpartum because of loss of the placental fraction, but may still remain above normal because of the elevation in the bone-specific fraction. Despite the confounding variables, these findings suggest that bone turnover is significantly increased during lactation.

Serial measurements of BMD during lactation (by SPA, DPA, or DXA) have shown a fall of 3–10.0% in BMC after 2–6 months of lactation at trabecular sites (lumbar spine, hip, femur, and distal radius), with smaller losses at cortical sites.[1,6] The loss occurs at a peak rate of 1–3% per month, far

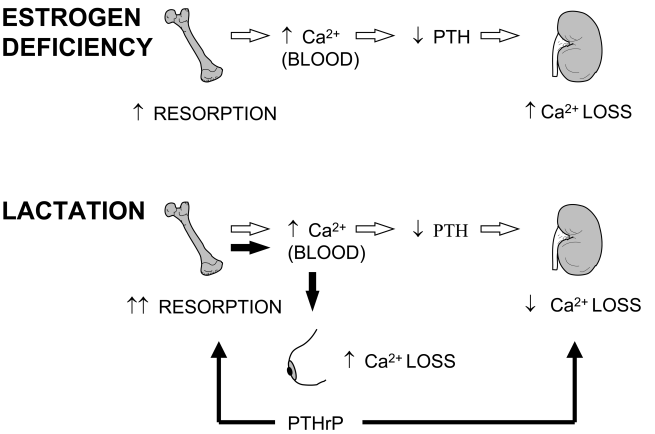

FIG. 3. Acute estrogen deficiency (e.g., GnRH analog therapy) increases skeletal resorption and raises the blood calcium; in turn, PTH is suppressed and renal calcium losses are increased. During lactation, the combined effects of PTHrP (secreted by the breast) and estrogen deficiency increase skeletal resorption, reduce renal calcium losses, and raise the blood calcium, but calcium is directed into breast milk. (Reprinted with permission of The Endocrine Society from Kovacs CS, Kronenberg HM 1997 Maternal-fetal calcium and bone metabolism during pregnancy, puerperium and lactation. Endocr Rev **18:**832–872. Copyright 1997, The Endocrine Society.)

exceeding the rate of 1–3% per year that can occur in women with postmenopausal osteoporosis who are considered to be losing bone rapidly. Loss of bone mineral from the maternal skeleton seems to be a normal consequence of lactation and may not be preventable by raising the calcium intake above the recommended dietary allowance. Several studies have shown that calcium supplementation does not significantly reduce the amount of bone lost during lactation.[15–18] Not surprisingly, the lactational decrease in BMD correlates with the amount of calcium lost in the breast milk.[19]

The mechanisms controlling the rapid loss of skeletal calcium content are not well understood. The reduced estrogen levels of lactation are clearly important but are unlikely to be the sole explanation. To estimate the effects of estrogen deficiency during lactation, it is worth noting the alterations in calcium and bone metabolism that occur in reproductive age women who have estrogen deficiency induced by gonadotropin-releasing hormone (GnRH) agonist therapy for endometriosis and other conditions. Six months of acute estrogen deficiency induced by GnRH agonist therapy leads to 1–4% losses in trabecular (but not cortical) BMD, increased urinary calcium excretion, and suppression of 1,25-dihydroxyvitamin D and PTH levels.[1] In lactation, women are not as estrogen deficient but lose more BMD (at both trabecular and cortical sites), have normal (as opposed to low) 1,25-dihydroxyvitamin D levels, and have reduced (as opposed to increased) urinary calcium excretion. The difference between isolated estrogen deficiency and lactation may be caused by the effects of other factors (such as PTHrP) that add to the effects of estrogen withdrawal in lactation (Fig. 3).

The BMD losses of lactation seem to be substantially reversed during weaning.[1,6,16] This corresponds to a gain in BMD of 0.5–2% per month in the woman who has weaned her infant. The mechanism for this restoration of BMD is uncertain and largely unexplored. In the long term, the consequences of lactation-induced depletion of bone mineral seem clinically unimportant. The vast majority of epidemiologic studies of pre- and postmenopausal women have found no adverse effect of a history of lactation on peak bone mass, BMD, or hip fracture risk.

Osteoporosis of Lactation

Rarely, a woman will suffer a fragility fracture during lactation, and osteoporotic readings will be confirmed by DXA. Like osteoporosis in pregnancy, this may represent a coincidental, unrelated disease; the woman may have had low BMD before conception. Alternatively, some cases might represent an exacerbation of the normal degree of skeletal demineralization that occurs during lactation and a continuum from changes in BMD and bone turnover that may have occurred during pregnancy. For example, excessive PTHrP release from the lactating breast into the maternal circulation could cause excessive bone resorption, osteoporosis, and fractures in some of these cases. PTHrP levels were high in one case of lactational osteoporosis and were found to remain elevated for months after weaning.[20]

Hypoparathyroidism and Pseudohypoparathyroidism

Levels of calcitriol/calcium supplementation required for treatment of hypoparathyroid women fall early in the postpartum period, especially if the woman breastfeeds, and hypercalcemia may occur if the calcitriol dosage is not substantially reduced.[21] As observed in one recent case, this is consistent with PTHrP reaching the maternal circulation in amounts sufficient to allow stimulation of 1,25-dihydroxyvitamin D synthesis and maintenance of normal (or slightly increased) maternal serum calcium.[22]

The management of pseudohypoparathyroidism has been less well documented. Because these patients are likely resistant to the renal actions of PTHrP and the placental sources of 1,25-dihydroxyvitamin D are lost at parturition, the calcitriol requirements might well increase and may require further adjustments during lactation.

IMPLICATIONS

In both pregnancy and lactation, novel regulatory systems specific to these settings complement the usual regulators of calcium homeostasis. The studies of pregnant women suggest that the fetal calcium demand is met in large part by intestinal calcium absorption, which more than doubles from early in pregnancy, correlating with the increase in 1,25-dihydroxyvitamin D. The studies of biochemical markers of bone turnover, DXA, and ultrasound are not conclusive, but are compatible with the possibility that the maternal skeleton does contribute calcium to the developing fetus. In comparison, the studies in lactating women suggest that skeletal calcium resorption is a dominant mechanism by which calcium is supplied to the breast milk, whereas renal calcium conservation is also apparent. These changes seem to be driven by PTHrP in association with estrogen deficiency. These observations indicate that the maternal adaptations to pregnancy and lactation have evolved differently over time, such that dietary calcium absorption dominates in pregnancy, whereas the temporary borrowing of calcium from the skeleton seems to dominate during lactation. Lactation seems to program an obligatory skeletal calcium loss irrespective of maternal calcium intake, but the calcium is completely restored to the skeleton after weaning. The rapidity of calcium loss and regain by the skeleton of the lactating woman are through mechanisms that are, at best, only partly understood. A full elucidation of the mechanisms of bone loss and restoration in the lactating woman might lead to the development of novel approaches to the treatment of osteoporosis and other metabolic bone diseases. Finally, while it is apparent that some women will experience fragility fractures as a consequence of pregnancy or lactation, the vast majority of women can be assured that the changes in calcium and bone metabolism during pregnancy and lactation are normal, healthy, and without adverse consequences in the long term.

REFERENCES

1. Kovacs CS, Kronenberg HM 1997 Maternal-fetal calcium and bone metabolism during pregnancy, puerperium and lactation. Endocr Rev **18:**832–872.
2. Kovacs CS, El-Hajj Fuleihan G 2005 Calcium and bone disorders during pregnancy and lactation. Endocrinol Metab Clin North Am **35:**21–51.
3. Kovacs CS, Lanske B, Hunzelman JL, Guo J, Karaplis AC, Kronenberg HM 1996 Parathyroid hormone-related peptide (PTHrP) regulates fetal-placental calcium transport through a receptor distinct from the PTH/PTHrP receptor. Proc Natl Acad Sci USA **93:**15233–15238.
4. Cornish J, Callon KE, Nicholson GC, Reid IR 1997 Parathyroid hormone-related protein-(107–139) inhibits bone resorption in vivo. Endocrinology **138:**1299–1304.
5. Purdie DW, Aaron JE, Selby PL 1988 Bone histology and mineral homeostasis in human pregnancy. Br J Obstet Gynaecol **95:**849–854.
6. Sowers M 1996 Pregnancy and lactation as risk factors for subsequent bone loss and osteoporosis. J Bone Miner Res **11:**1052–1060.
7. Schnatz PF, Curry SL 2002 Primary hyperparathyroidism in pregnancy: Evidence-based management. Obstet Gynecol Surv **57:**365–76.
8. Breslau NA, Zerwekh JE 1986 Relationship of estrogen and pregnancy to calcium homeostasis in pseudohypoparathyroidism. J Clin Endocrinol Metab **62:**45–51.
9. Woodrow JP, Noseworthy CS, Fudge NJ, Hoff AO, Gagel RF, Kovacs CS 2003 Calcitonin/calcitonin gene-related peptide protect the maternal skeleton from excessive resorption during lactation. J Bone Miner Res **18:**S2; S37.
10. Woodrow JP, Hoff AO, Gagel RF, Kovacs CS 2004 Calcitonin treatment rescues calcitonin-null mice from excessive bone resorption during lactation. J Bone Miner Res **19:**S1;SA518.
11. VanHouten JN, Dann P, Stewart AF, Watson CJ, Pollak M, Karaplis AC, Wysolmerski JJ 2003 Mammary-specific deletion of parathyroid hormone-related protein preserves bone mass during lactation. J Clin Invest **112:** 1429–1436.
12. Kovacs CS, Chik CL 1995 Hyperprolactinemia caused by lactation and pituitary adenomas is associated with altered serum calcium, phosphate, parathyroid hormone (PTH), and PTH-related peptide levels. J Clin Endocrinol Metab **80:**3036–3042.
13. Dobnig H, Kainer F, Stepan V, Winter R, Lipp R, Schaffer M, Kahr A, Nocnik S, Patterer G, Leb G 1995 Elevated parathyroid hormone-related peptide levels after human gestation: Relationship to changes in bone and mineral metabolism. J Clin Endocrinol Metab **80:**3699–3707.
14. Sowers MF, Hollis BW, Shapiro B, Randolph J, Janney CA, Zhang D, Schork A, Crutchfield M, Stanczyk F, Russell-Aulet M 1996 Elevated parathyroid hormone-related peptide associated with lactation and bone density loss. J Am Med Assoc **276:**549–554.
15. Kolthoff N, Eiken P, Kristensen B, Nielsen SP 1998 Bone mineral changes during pregnancy and lactation: A longitudinal cohort study. Clin Sci (Colch) **94:**405–412.
16. Polatti F, Capuzzo E, Viazzo F, Colleoni R, Klersy C 1999 Bone mineral changes during and after lactation. Obstet Gynecol **94:**52–56.
17. Kalkwarf HJ, Specker BL, Bianchi DC, Ranz J, Ho M 1997 The effect of calcium supplementation on bone density during lactation and after weaning. N Engl J Med **337:**523–528.
18. 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. J Bone Miner Res **10:**1312–1320.
19. Laskey MA, Prentice A, Hanratty LA, Jarjou LM, Dibba B, Beavan SR, Cole TJ 1998 Bone changes after 3 mo of lactation: Influence of calcium intake, breast-milk output, and vitamin D-receptor genotype. Am J Clin Nutr **67:**685–692.
20. Reid IR, Wattie DJ, Evans MC, Budayr AA 1992 Post-pregnancy osteoporosis associated with hypercalcaemia. Clin Endocrinol (Oxf) **37:**298–303.
21. Caplan RH, Beguin EA 1990 Hypercalcemia in a calcitriol-treated hypoparathyroid woman during lactation. Obstet Gynecol **76:**485–489.
22. Mather KJ, Chik CL, Corenblum B 1999 Maintenance of serum calcium by parathyroid hormone-related peptide during lactation in a hypoparathyroid patient. J Clin Endocrinol Metab **84:**424–427.

Chapter 11. Menopause

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INTRODUCTION

Menopause refers to the cessation of menstruation, which occurs at ~48–50 years of age in healthy women. The decline in ovarian hormone production is gradual and starts several years before the last period. Changes in bone mass and calcium metabolism are evident during this perimenopausal transition. Estrogen is the ovarian product that has the greatest impact on mineral metabolism, although both progesterone and ovarian androgens may have some influence. Menopause ushers in a period of bone loss that extends until the end of life and that is the central contributor to the development of osteoporotic fractures in older women.

EFFECTS ON BONE

Before menopause, there is virtually no bone loss in most regions of the skeleton, and fracture rates are stable. The most obvious effect of menopause on bone is an increase in the incidence of fractures; in the forearms and vertebrae, this is clearly apparent within the first postmenopausal decade. It is attributable to the rapid decline in bone mass that occurs in the perimenopausal years. Bone loss is more marked in trabecular than in cortical bone because the former has a far greater surface area over which bone resorption can take place. Thus, the fractures that occur early in menopause are in trabecular-rich regions of the skeleton such as the distal forearm and vertebrae. The loss of bone and increase in fracture rates are preventable with estrogen replacement.

The perimenopausal increase in bone loss is driven by increased bone resorption.[1] Bone biopsy specimens in normal postmenopausal women show an increase in the proportion of bone surfaces at which resorption is taking place and an increase in the depth of resorption pits. These changes follow from an increase in the activation frequency of remodeling units and a prolongation of their resorptive phase. Indices of bone resorption are twice the levels found in premenopausal women, whereas markers of bone formation are only ~50% above premenopausal levels,[2] leading to negative bone balance. The resulting loss of bone leads to the perforation and loss of trabeculae and increased porosity in cortical bone. The changes in histomorphometric indices and biochemical markers can be returned to premenopausal levels with estrogen replacement therapy.

The changes in bone turnover that accompany menopause are in part accounted for by the direct actions of estrogen on bone cells. Estrogen receptors are present in both osteoblasts and osteoclasts. Estrogen promotes the development of osteoblasts in preference to adipocytes from their common precursor cell,[3] increases osteoblast proliferation,[4] and increases pro-

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duction of a number of osteoblast proteins (e.g., IGF-1, type I procollagen, TGF-β, and BMP-6). Thus, estrogen tends to have an anabolic effect on the isolated osteoblast, which is complemented by its inhibition of apoptosis in osteocytes[5] and osteoblasts.[6] In vivo, however, the initiation of estrogen replacement therapy is usually associated with a reduction in osteoblast numbers and activity.[7] This is accounted for by the tight coupling of osteoblast activity to that of osteoclasts and the overriding effect of estrogen to reduce osteoclastic bone resorption. However, there is now evidence that high concentrations of estrogen increase some histomorphometric indices of osteoblast activity (e.g., mean wall thickness) in humans, possibly by increasing osteoblast synthesis of growth factors.[8]

Estrogen's suppression of osteoclast activity is contributed to by increased osteoclast apoptosis,[9] by reduced osteoblast/stromal cell production of RANKL, and by increased production of osteoprotegerin.[10] These direct effects are buttressed by estrogen action on bone marrow stromal and mononuclear cells and on T cells. The former produce cytokines, such as IL-1, IL-6, and TNF-α, which are potent stimulators of osteoclast recruitment and/or activity.[11] Estrogen decreases production of each of these cytokines[12] and modulates levels of IL-1 receptors.[13] Bone loss after ovariectomy is reduced by blockers of these cytokines. IL-1 and TNF-α may act in part by regulating stromal cell production of IL-6 and macrophage colony-stimulating factor.[14] Estrogen's reduction in bone resorption is also contributed to by its increasing levels of NO and TGF-β, both of which are potent inhibitors of osteoclast differentiation and bone resorption.[15] TGF-β acts through regulation of T-cell production of TNF-α,[16] and estrogen also influences T-cell proliferation through interferon-γ.[17] These effects on osteoclasts and bone marrow cytokines are supported by estrogen's regulation of the release of systemic factors, such as growth hormone.[18]

EFFECTS ON CALCIUM METABOLISM

The bone loss that follows menopause is accompanied by negative changes in external calcium balance, which are approximately equally contributed to by decreases in intestinal calcium absorption and by increases in urinary calcium loss.[19] Menopause is associated with reduced circulating concentrations of total, but not free, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], implying that its main effect is on vitamin D binding protein. However, intestinal mucosal cells contain estrogen receptors and respond directly to 17β-estradiol with enhanced calcium transport,[20] probably through regulation of the epithelial calcium channel CaT1,[21] suggesting estrogen's effects are independent of vitamin D.

In the kidney, it is clear that tubular reabsorption of calcium is higher in the presence of estrogen[22,23]. One study[23] found higher PTH concentrations in the presence of estrogen and inferred that this was the mechanism of the renal calcium conservation. However, higher PTH levels have not been the finding in a number of other studies. Thus, it is likely that estrogen directly modulates renal tubular calcium absorption through its own receptor in the kidney, as suggested by in vitro studies of renal tubule cells that show a stimulatory effect of 17β-estradiol on calcium membrane transport.[24]

The changes in the handling of calcium by the gut and kidney could each be a cause of postmenopausal bone loss or they could represent homeostatic responses to it. If the former was the case, PTH concentrations would be elevated in postmenopausal women to maintain plasma calcium concentrations in the face of intestinal and renal losses. This, in turn, would cause bone loss. If, on the other hand, bone loss were the primary event, suppression of PTH would be expected, leading to secondary declines in intestinal and renal calcium absorption. The effect of menopause on PTH concentrations has been addressed many times without any consistent pattern emerging. This suggests that estrogen has direct effects on bone, kidney and gut, and the opposing effects of these actions on PTH secretion leads to inconsistent changes in PTH concentrations. Furthermore, estrogen may directly modulate PTH secretion.[25,26]

There are small but consistently demonstrable effects of menopause on circulating concentrations of calcium. Total calcium is 0.05 mM higher after menopause.[22,27] This is partly attributable to a contraction of the plasma volume and resulting increase in albumin concentrations that occurs in the absence of estrogen,[28,29] and partly to an increase in plasma bicarbonate that leads to an increase in the complexed fraction of plasma calcium. The higher bicarbonate levels of postmenopausal women are attributable to a respiratory acidosis that results from the loss of the respiratory stimulatory effects of progesterone on the central nervous system, an action that is potentiated by estrogen.[30,31] Despite changes in protein-bound and complexed calcium fractions, ionized calcium concentrations are usually found to be the same in pre- and postmenopausal women.

SUMMARY

The effects of menopause on skeletal physiology are summarized in Fig. 1. The major effect is an increase in bone turnover, which is dominantly an increase in bone resorption. This results in bone loss that may be contributed to by reductions in both intestinal and renal tubular absorption of calcium. Bone loss persists throughout the entire postmenopausal period and results in a high risk of fractures in those women whose peak bone mass was in the lower part of the normal range.

REFERENCES

1. Heaney RP, Recker RR, Saville PD 1978 Menopausal changes in bone remodeling. J Lab Clin Med **92:**964–970.
2. Garnero P, Sornayrendu E, Chapuy MC, Delmas PD 1996 Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis. J Bone Miner Res **11:**337–349.
3. Okazaki R, Inoue D, Shibata M, Saika M, Kido S, Ooka H, Tomiyama H, Sakamoto Y, Matsumoto T 2002 Estrogen promotes early osteoblast differentiation and inhibits adipocyte differentiation in mouse bone marrow stromal cell lines that express estrogen receptor (ER) alpha or beta. Endocrinology **143:**2349–2356.
4. Fujita M, Urano T, Horie K, Ikeda K, Tsukui T, Fukuoka H, Tsutsumi O, Ouchi Y, Inoue S 2002 Estrogen activates cyclin-dependent kinases 4 and 6 through induction of cyclin D in rat primary osteoblasts. Biochem Biophys Res Commun **299:**222–228.
5. Tomkinson A, Reeve J, Shaw RW, Noble BS 1997 The death of osteocytes via apoptosis accompanies estrogen withdrawal in human bone. J Clin Endocrinol Metab **82:**3128–3135.
6. Gohel A, McCarthy MB, Gronowicz G 1999 Estrogen prevents glucocorticoid-induced apoptosis in osteoblasts in vivo and in vitro. Endocrinology **140:**5339–5347.
7. Vedi S, Compston JE 1996 The effects of long-term hormone replacement therapy on bone remodeling in postmenopausal women. Bone **19:**535–539.
8. Bord S, Beavan S, Ireland D, Horner A, Compston JE 2001 Mechanisms by which high-dose estrogen therapy produces anabolic skeletal effects in postmenopausal women: Role of locally produced growth factors. Bone **29:**216–222.
9. Kameda T, Mano H, Yuasa T, Mori Y, Miyazawa K, Shiokawa M, Nakamaru Y, Hiroi E, Hiura K, Kameda A, Yang NN, Hakeda Y, Kumegawa M 1997 Estrogen inhibits bone resorption by directly inducing apoptosis of the bone-resorbing osteoclasts. J Exp Med **186:**489–495.
10. Syed F, Khosla S 2005 Mechanisms of sex steroid effects on bone. Biochem Biophys Res Comm **328:**688–696.
11. Manolagas SC, Jilka RL 1995 Mechanisms of disease: Bone marrow,

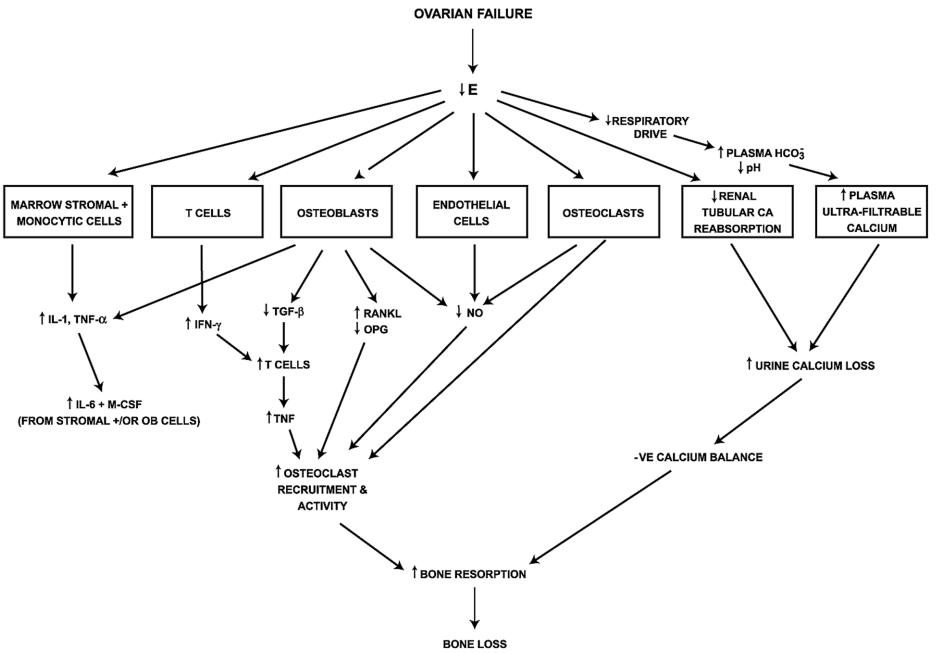

FIG. 1. The potential pathways by which menopause leads to bone loss. For simplicity, a contribution from loss of any anabolic effect of estrogen on the osteoblast is not shown. The fall in ovarian production of androgens and progesterone also contributes to some of these changes.

cytokines, and bone remodeling—emerging insights into the pathophysiology of osteoporosis. N Engl J Med **332:**305–311.
12. Rogers A, Eastell R 1998 Effects of estrogen therapy of postmenopausal women on cytokines measured in peripheral blood. J Bone Miner Res **13:**1577–1586.
13. Sunyer T, Lewis J, Collin-Osdoby P, Osdoby P 1999 Estrogen's bone-protective effects may involve differential IL-1 receptor regulation in human osteoclast-like cells. J Clin Invest **103:**1409–1418.
14. Kimble RB, Srivastava S, Ross FP, Matayoshi A, Pacifici R 1996 Estrogen deficiency increases the ability of stromal cells to support murine osteoclastogenesis via an interleukin-1-and tumor necrosis factor-mediated stimulation of macrophage colony-stimulating factor production. J Biol Chem **271:**28890–28897.
15. Ralston SH 1997 The Michael-Mason-Prize essay 1997—nitric oxide and bone—what a gas. Br J Rheumatol **36:**831–838.
16. Gao YH, Qian WP, Dark K, Toraldo G, Lin ASP, Guldberg RE, Flavell RA, Weitzmann MN, Pacifici R 2004 Estrogen prevents bone loss through transforming growth factor beta signaling in T cells. Proc Natl Acad Sci USA **101:**16618–16623.
17. Cenci S, Toraldo G, Weitzmann MN, Roggia C, Gao YH, Qian WP, Sierra O, Pacifici R 2003 Estrogen deficiency induces bone loss by increasing T cell proliferation and lifespan through IFN-gamma-induced class II transactivator. Proc Natl Acad Sci USA **100:**10405–10410.
18. Friend KE, Hartman ML, Pezzoli SS, Clasey JL, Thorner MO 1996 Both oral and transdermal estrogen increase growth hormone release in postmenopausal women: A clinical research center study. J Clin Endocrinol Metab **81:**2250–2256.
19. Heaney RP, Recker RR, Saville PD 1978 Menopausal changes in calcium balance performance. J Lab Clin Med **92:**953–963.
20. Arjandi BH, Salih MA, Herbert DC, Sims SH, Kalu DN 1993 Evidence for estrogen receptor-linked calcium transport in the intestine. Bone Miner **21:**63–74.
21. Van Cromphaut SJ, Rummens K, Stockmans I, Van Herck E, Dijcks FA, Ederveen A, Carmeliet P, Verhaeghe J, Bouillon R, Carmeliet G 2003 Intestinal calcium transporter genes are upregulated by estrogens and the reproductive cycle through vitamin D receptor-independent mechanisms. J Bone Miner Res **18:**1725–1736.
22. Nordin BEC, Wlshart JM, Clifton PM, McArthur R, Scopacasa F, Need AG, Morris HA, O'Loughlin PD, Horowitz M 2004 A longitudinal study of bone-related biochemical changes at the menopause. Clin Endocrinol (Oxf) **61:**123–130.
23. McKane WR, Khosla S, Burritt MF, Kao PC, Wilson DM, Ory SJ, Riggs BL 1995 Mechanism of renal calcium conservation with estrogen replacement therapy in women in early postmenopause: A clinical research center study. J Clin Endocrinol Metab **80:**3458–3464.
24. Dick IM, Liu J, Glendenning P, Prince RL 2003 Estrogen and androgen regulation of plasma membrane calcium pump activity in immortalized distal tubule kidney cells. Molec Cell Endocrinol **212:**11–18.
25. Duarte B, Hargis GK, Kukreja SC 1988 Effects of estradiol and progesterone on parathyroid hormone secretion from human parathyroid tissue. J Clin Endocrinol Metab **66:**584–587.
26. Greenberg C, Kukreja SC, Bowser EN, Hargis GK, Henderson WJ, Williams GA 1987 Parathyroid hormone secretion: Effect of estradiol and progesterone. Metabolism **36:**151–154.
27. Sokoll LJ, Dawson-Hughes B 1989 Effect of menopause and aging on serum total and ionized calcium and protein concentrations. Calcif Tissue Int **44:**181–185.
28. Aitken JM, Lindsay R, Hart DM 1974 The redistribution of body sodium in women on long-term estrogen therapy. Clin Sci Mol Med **47:**179–187.
29. Minkoff JR, Young G, Grant B, Marcus R 1986 Interactions of medroxyprogesterone acetate with estrogen on the calcium-parathyroid axis in post-menopausal women. Maturitas **8:**35–45.
30. Bayliss DA, Millhorn DE 1992 Central neural mechanisms of progesterone action: Application to the respiratory system. J Appl Physiol **73:**393–404.
31. Orr-Walker BJ, Horne AM, Evans MC, Grey AB, Murray MAF, McNeil AR, Reid IR 1999 Hormone replacement therapy causes a respiratory alkalosis in normal postmenopausal women. J Clin Endocrinol Metab **84:**1997–2001.

Chapter 12. Age-Related Osteoporosis

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INTRODUCTION

Age-related fractures are the most common manifestation of osteoporosis and are responsible for the greatest proportion of the morbidity and mortality from this disease. Over the next quarter century, as the population ages, fracture prevalence will also rise. Biochemical, biomechanical, and nonskeletal factors contribute to fragility fractures in the elderly. In this overview, we will focus on the quantitative and qualitative changes in the skeleton as well as the nonskeletal pathways that contribute to osteoporotic fractures in older individuals.

AGE-RELATED CHANGES IN BONE QUALITY AND QUANTITY THAT CONTRIBUTE TO FRACTURE RISK

Numerous studies have documented a progressive reduction in BMD at nearly every skeletal site with aging[1]; however, fracture risk also climbs with age, independent of BMD.[2] Therefore, other skeletal and nonskeletal factors must contribute to overall fracture risk. Recent advances in imaging technology and the availability of more longitudinal studies reveal significant changes in trabecular architecture and connectivity that can be linked to bone strength and ultimately fracture risk in the elderly.[3] Other qualitative factors that are influenced by age include the degree of mineralization, microcrack number and frequency, anisotropy, skeletal geometry, and the periosteal response to trabecular bone loss. The latter is particularly intriguing because the loss of trabecular elements may result in a compensatory increase in the cortical shell diameter.[4] This characteristic is more pronounced in the aging male rather than female and probably serves to protect the skeleton during active bone loss. Finally, recent attention has focused on the role of marrow fat in the bone marrow compartment because adipogenesis at this site increases with aging.[5] . These age-related changes can be visualized by MRI and could have structural consequences, although neither the function nor the fate of marrow adipocytes is known.[6]

Currently. it is difficult, if not impossible, to clinically measure qualitative characteristics of bone; however, risk factors such as age and previous fracture capture some of these qualitative determinants of fracture risk. In contrast to the limited ability to measure qualitative changes in bone, quantifying BMD and loss of BMD can be easily assessed by DXA measurements. BMD changes with aging contribute to the risk of future fracture. For example, over a lifespan, women lose ~42% of their spinal and 58% of their femoral bone mass.[1] Surprisingly, rates of bone loss in the eighth and ninth decades of life may be comparable with or even exceed those found in the immediate peri- and postmenopausal period of some women.[7,8] This is caused by uncoupling in the bone remodeling cycle of older individuals, resulting in a marked increase in bone resorption but no change or a decrease in bone formation.[7,9] The latter scenario is particularly intriguing because aging is associated with a significant increase in stromal cell differentiation into the fat lineage and greater marrow adiposity, which may be associated with fewer stromal cells committed to the osteoblast lineage. Alterations in bone turnover can be detected by biochemical markers of bone remodeling that include bone resorption indices (e.g., urinary and serum breakdown products of type I collagen) and bone formation markers (e.g., osteocalcin, procollagen peptide, bone specific alkaline phosphatase). In general, bone turnover markers are significantly higher in older rather than younger postmenopausal women, and these indices are inversely related to BMD.[10] For example, in the EPIDOS trial of elderly European females, the highest levels of osteocalcin, N-telopeptide, C-telopeptide, and bone-specific alkaline phosphatase were noted for those in the lowest tertile of femoral BMD.[11] Also, increased bone resorption indices were associated with a greater fracture risk independent of BMD.[11] For those women in EPIDOS with low BMD and a high bone resorption rate, there was a nearly 5-fold greater risk of a hip fracture. Similar findings have been noted in other cohorts composed of elderly individuals.[12]

In contrast to consistently high bone resorption indices, bone formation markers in the elderly are more variable. Serum osteocalcin levels are high in elderly individuals, but this may be indicative of an increase in bone turnover rather than reflecting a true rise in bone formation.[11] On the other hand, bone-specific alkaline phosphatase and procollagen peptide levels have been reported to be high, normal or low in elderly men and women.[13] Bone histomorphometric indices in the elderly are also quite variable. Morphologically, the age-associated increase in marrow fat may or may not contribute to the heterogeneity in formation markers. Thus, although there is strong evidence for an age-associated rise in bone resorption, changes in bone formation are inconsistent. Notwithstanding the limitations of biochemical markers, it is generally assumed there is uncoupling of the remodeling unit that leads to bone loss, altered skeletal architecture, and an increased propensity to fractures.

FACTORS THAT CONTRIBUTE TO AGE-RELATED BONE LOSS

Nutritional

Increased bone resorption in older individuals can be attributed to a number of factors including calcium and/or vitamin D deficiency. Both are very common in the elderly and are caused by a number of conditions, including abrupt dietary changes, lack of sunlight exposure, malabsorption, use of certain drugs, cachexia, and anorexia. The result of low calcium intake is persistent secondary hyperparathyroidism, which in turn leads to increased bone resorption, often accentuated by occult vitamin D deficiency, especially in women living in northern latitudes.[14] It is estimated that upward of 80% of elderly postmenopausal women may have vitamin D deficiency as defined by a 25-hydroxyvitamin D of <20 ng/ml (50 nM).[15,16] Low vitamin D levels could result not only in reduced bone mass but also altered muscle function.

In a meta-analysis of clinical trials of calcium supplementation for postmenopausal women, lumbar BMD was shown to be slightly increased in women who were supplemented.[17] Calcium treatment alone, however, does not reduce vertebral or nonvertebral fractures and debate continues about whether vitamin D alone, in doses of $\geq$800 IU/day, can prevent fractures.[18–20] Certainly there is some evidence to suggest that vitamin D supplementation can improve muscle function, thereby indirectly reducing fracture risk.[20] The National

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Academy of Science recommends a minimal daily requirement for calcium intake in people >65 years of age to be 1500 mg/day and for vitamin D to be 600 IU/day.[21] It is likely that this 600 IU/day recommendation for vitamin D will be increased to 800 IU/day in the coming years.

A balanced diet that includes micro- and macronutrients is essential for the overall health of the older individual. Besides calcium and vitamin D, other nutritional factors may play a role in age-related osteoporosis. Although total protein intake seems to be beneficial to the skeleton, there is still a debate as to whether high animal protein intake creates an acidic environment in bone, leading to loss of calcium from the skeleton.[22–24] Protein/calorie malnutrition stimulates bone resorption and impairs bone formation both directly and through other mechanisms such as reduced serum IGF-I.[25] Vitamin K deficiency may contribute to an increased risk of osteoporotic fractures, possibly through effects on the carboxylation of bone proteins such as osteocalcin.[26] Other micronutrients such as the B vitamins and vitamin C have been linked to osteoporotic risk in some but not all studies. At least one observational study suggested that increased vitamin A intake might be associated with low BMD.[18,27] A case control study showed a significant relationship between excessive intake of vitamin A and age-related fractures.[28]

Hormonal

Estrogen deficiency has long been recognized as a major cause of bone loss in the first decade after menopause. More recently, investigators have identified a strong relationship between endogenous estrogen and bone mass in elderly men and women. In one prospective study, Slemenda et al.[29] noted that both estrogens and androgens were independent predictors of bone loss in older postmenopausal women. In both the Rancho Bernardo cohort and the Framingham Cohort, estradiol levels were strongly related to BMD at the spine, hip, and forearm.[30,31]

Males also suffer from age-related bone loss, and evidence suggests that absolute estrogen levels, rather than testosterone concentrations, are essential for maintenance of BMD. In the Rancho Bernardo cohort, serum estradiol levels in elderly men correlated closely with bone mass at several sites,[30] and low estradiol levels in men were associated with increased risk of hip fracture. Recently, Falahati et al.[32,33] showed that small amounts of estradiol were essential for preventing bone resorption in men, in part by upregulating osteoprotegerin (OPG). Endogenous testosterone also plays a role in regulating bone turnover, possibly more on the formation side than in respect to resorption. Serum testosterone levels decline with age at a rate of ~1.2%/year, whereas sex hormone binding globulin (SHBG) levels rise. Males treated with androgen antagonists or gonadotropin agonists for prostate cancer metastases rapidly lose bone mass and may be at high risk for subsequent osteoporotic fractures.[34] Overall, it seems likely that both androgens and estrogens are important in the elderly male. Whether changes in male hormone levels are causally related to age-related bone loss in men will have to await large scale prospective studies.

Changes in other circulating factors may be related to bone loss in the elderly. For example, growth hormone (GH) secretion declines 14% per decade and is the principle cause for low serum IGF-I concentrations in both elderly men and women.[35] Serum IGF-I concentrations in some studies but not others are directly related to BMD, and in one study, low IGF-I was an independent predictor of hip fracture.[36] Similarly, the adrenal androgens, dehydroepiandrosterone (DHEA) and DHEA-S, also decline precipitously with age and are 10–20% of young adult serum levels.[37] OPG levels are also lower in the elderly than in younger postmenopausal woman, but whether this can lead to increased bone loss has yet to be shown.[2] Aging is also associated with a generalized cytokinemia, including greater serum levels of interleukin-6 (IL-6) and TNF, as well as C-reactive protein (CRP). Whether these changes are a function of other disease processes and therefore may contribute to bone loss in the elderly have not been established.

Heritable and Environmental Factors

Age-related bone loss can be dramatic in some individuals, and this decline cannot be attributed solely to hormonal or nutritional factors. Several investigators have hypothesized that there is genetic programming, which when triggered by environmental factors, may lead to bone loss, especially in the elderly. Some animal models, but not others, have shown a heritable component to age-related bone loss.[38] Recently, Bouxsein et al.[39] showed that, after ovariectomy, inbred strains of mice lost bone at very different rates. This would suggest that genetic programming may be operative in determining the rate of bone loss with estrogen deprivation. In humans, the multiplicity of environmental factors makes the determination of fracture heritability complicated, although recent publications also suggest a genetic component.[40,41] On the other hand, environmental factors, including inactivity and loss of muscle mass, smoking, alcohol, and medications such as glucocorticoids and anticonvulsants, may contribute to an excessive rate of bone loss in some elderly.

FRACTURES AND FALLS IN THE ELDERLY

In elderly individuals, decreased bone strength, as reflected by BMD, is only one of many important contributors to overall hip fracture risk. Other factors include propensity to fall, inability to correct a postural imbalance, characteristics of the faller such as height and muscle activity, the orientation of the fall, adequacy of local tissue shock absorbers, and characteristics of the impact surface. The resistance of a skeletal structure to failure (i.e., fracture) depends on the geometry of the bone, the material properties of the calcified tissue, and the location and direction of the loads to which the bone is subjected (i.e., during a fall or other activities). Estimations of the forces generated within the bone in response to a given load can be estimated using basic engineering principles. Those forces can be compared with the strengths of the tissue. The ratio of the impact force expected during a fall to the force required to cause the bone to fail incorporates the two major determinants of fracture risk. When this ratio is close to or more than 1, the structure is at great risk of failure.

In the elderly, this ratio is 0.3 at the femoral neck for simple stance and normal ambulation. For stair climbing, it is about 0.6. In falls, the ratio ranges from 1 to >70.[42] These calculations are complicated by considerable uncertainty about the loads to which hips are actually subjected during falls. For example, skeletal structures at high risk for age-related fracture, such as the hip, change their geometry with aging and bone remodeling, making it difficult to ascertain the true force of failure in vivo. Most of the energy from a fall dissipates before actual injury, and yet the residual force at impact remains two orders of magnitude greater than the energy required to fracture elderly femurs. This would suggest that a simple fall is easily capable of fracturing the proximal femur.

Falls in older people are rarely due to a single cause. Falls usually occur when a threat to the normal homeostatic mechanisms that maintain postural stability is superimposed on underlying age-related declines in balance, ambulation, and cardiovascular function. In some cases, this may involve an acute illness such as a fever or infection or an environmental stress such as a newly initiated drug or an unsafe walking surface. Regardless of the nature of the stress, an elderly person may not be able to

compensate because of either age-related declines in function or severe chronic disease. It is unlikely for an extrinsic stress to completely explain the circumstances of a fall. Older persons, by virtue of their age alone, experience declines in physiologic function, have greater numbers of chronic diseases, acute illnesses, and hospitalizations, and use multiple medications. Superimposed on these age-related characteristics, challenges to postural control may have a greater impact in aged persons according to their risk-taking behavior and opportunity to fall. Thus, those individuals who are completely immobile may not be at risk of falling despite multiple predisposing factors. On the other hand, persons who are either vigorous or only slightly frail may be at higher risk compared with individuals in between those extremes, due in part to more risk taking and inability to compensate for postural changes. Despite the importance of falls, BMD still remains a major predictor of fracture risk[2] regardless of age.[43] Recent evidence also implicates low vitamin D as an important contributor to the risk of falls and fractures and to lower extremity function.[20,44,45] Moreover, vitamin D supplementation may reduce fracture risk by enhancing muscle strength, particularly in the lower extremity.

In one large study for hip fracture risk in elderly women, the subjects were grouped into three categories according to number of risk factors for fracture other than BMD. Across all three risk groups, BMD remained an important predictor for fracture.[2]

APPROACH TO FRACTURE PREVENTION IN THE ELDERLY PATIENT

Because older persons have lower BMD to start, are continuing to lose bone, and are in the age group most likely to fracture, interventions would be expected to be most cost-effective when initiated in these individuals. The interventions can be divided into two groups: (1) those that reduce the applied load to the skeleton (fall prevention, passive protective systems) and (2) those that preserve or increase BMD.

Interventions That Reduce the Applied Load

Interventions to prevent falls must be predicated on an assessment of fall risk. This should include a history of falls because a history of falls is the single most important risk factor for a subsequent fall. If that history is positive, additional information can be obtained surrounding the events of the fall, because this information may identify important factors for targeting risk factor modification strategies. The physical assessment of fall risk should include orthostatic vital sign measurement, a test of visual acuity, hearing, cardiac exam, extremity exam, and a test of the postural stability system as a whole using any of several recently developed assessment tools such as the "Get Up and Go" test[46,47] and the Short Portable Physical Performance Battery.[48] Because some of the unfavorable outcomes of major fractures such as hip fractures are highly dependent on the premorbid status of an older patient, fracture prevention efforts should include a thorough assessment of underlying disability and frailty of the older person, because these factors influence long-term outcomes.[49]

For fall prevention interventions, the pooled results from several studies suggest that an intervention in which older people are assessed by a health professional trained to identify intrinsic and environmental risk factors is likely to reduce the fall rate (OR = 0.79; 95% CI, 0.65–0.96).[50,51] Because falls to the side that impact on the hip are the primary determinant of hip fracture,[52] protective trochanteric padding devices have been developed. Over the past 5 years, randomized, controlled trials have largely confirmed that hip protectors can reduce hip fracture, but subject compliance has been low.[53]

Interventions That Preserve or Increase BMD

In addition to the attention to adequate basic nutritional factors, the use of therapeutic agents in the treatment of osteoporosis in the elderly person may be useful. Because older persons are at the greatest risk of fracture and because fracture reduction has been shown for the bisphosphonates,[54–56] estrogen therapy,[57,58] nasal calcitonin,[59] risedronate,[55] and PTH,[60] potentially fewer elderly persons would have to be treated for less duration to prevent fractures than a younger population at lower risk of fracture.[61–64]

REFERENCES

1. Riggs BL, Wahner HW, Seeman E, Offord KP, Dunn WL, Mazess RB, Johnson KA, Melton LJ III 1982 Changes in bone mineral density of the proximal femur and spine with aging. Differences between the postmenopausal and senile osteoporosis syndromes. J Clin Invest **70:**716–723.
2. Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, Cauley J, Black D, Vogt TM 1995 Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. N Engl J Med **332:**767–773.
3. Bouxsein ML 2003 Bone quality: Where do we go from here? Osteoporos Int **14**(Suppl 5)**:**118–127.
4. Ahlborg HG, Johnell O, Turner CH, Rannevik G, Karlsson MK 2003 Bone loss and bone size after menopause. N Engl J Med **349:**327–334.
5. Justesen J, Stenderup K, Ebbesen EN, Mosekilde L, Steiniche T, Kassem M 2001 Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis. Biogerontology **2:**165–171.
6. Wehrli FW, Hopkins JA, Hwang SN, Song HK, Snyder PJ, Haddad JG 2000 Cross-sectional study of osteopenia with quantitative MR imaging and bone densitometry. Radiology **217:**527–538.
7. Ensrud KE, Palermo L, Black DM, Cauley J, Jergas M, Orwoll ES, Nevitt MC, Fox KM, Cummings SR 1995 Hip and calcaneal bone loss increase with advancing age: Longitudinal results from the study of osteoporotic fractures. J Bone Miner Res **10:**1778–1787.
8. Hannan MT, Felson DT, Dawson-Hughes B, Tucker KL, Cupples LA, Wilson PW, Kiel DP 2000 Risk factors for longitudinal bone loss in elderly men and women: The Framingham Osteoporosis Study. J Bone Miner Res **15(4):**710–720.
9. Ross PD, Knowlton W 1998 Rapid bone loss is associated with increased levels of biochemical markers. J Bone Miner Res **13:**297–302.
10. Dresner-Pollak R, Parker RA, Poku M, Thompson J, Seibel MJ, Greenspan SL 1996 Biochemical markers of bone turnover reflect femoral bone loss in elderly women. Calcif Tissue Int **59:**328–333.
11. Garnero P, Hausherr E, Chapuy MC, Marcelli C, Grandjean H, Muller C, Cormier C, Breart G, Meunier PJ, Delmas PD 1996 Markers of bone resorption predict hip fracture in elderly women: The EPIDOS Prospective Study. J Bone Miner Res **11:**1531–1538.
12. Garnero P, Sornay-Rendu E, Claustrat B, Delmas PD 2000 Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: The OFELY study. J Bone Miner Res **15:**1526–1536.
13. Bollen AM, Kiyak HA, Eyre DR 1997 Longitudinal evaluation of a bone resorption marker in elderly subjects. Osteoporos Int **7:**544–549.
14. Chapuy MC, Schott AM, Garnero P, Hans D, Delmas PD, Meunier PJ 1996 Healthy elderly French women living at home have secondary hyperparathyroidism and high bone turnover in winter. EPIDOS Study Group. J Clin Endocrinol Metab **81:**1129–1133.
15. Heaney RP 2000 Vitamin D: How much do we need, and how much is too much? Osteoporos Int **11:**553–555.
16. Rosen CJ 2005 Clinical practice. Postmenopausal osteoporosis. N Engl J Med **353:**595–603.
17. Cranney A, Wells G, Willan A, Griffith L, Zytaruk N, Robinson V, Black D, Adachi J, Shea B, Tugwell P, Guyatt G 2002 Meta-analyses of therapies for postmenopausal osteoporosis. II. Meta-analysis of alendronate for the treatment of postmenopausal women. Endocr Rev **23:**508–516.
18. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE 1997 Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. N Engl J Med **337:**670–676.
19. Recker RR, Hinders S, Davies KM, Heaney RP, Stegman MR, Lappe JM, Kimmel DB 1996 Correcting calcium nutritional deficiency prevents spine fractures in elderly women. J Bone Miner Res **11:**1961–1966.
20. Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, Staehelin HB, Bazemore MG, Zee RY, Wong JB 2004 Effect of Vitamin D on falls: A meta-analysis. JAMA **291:**1999–2006.
21. 2001 Osteoporosis prevention, diagnosis, and therapy. JAMA **285:**785–795.

22. Dawson-Hughes B, Harris SS 2002 Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. Am J Clin Nutr **75:**773–779.
23. Hannan MT, Tucker KL, Dawson-Hughes B, Cupples LA, Felson DT, Kiel DP 2000 Effect of dietary protein on bone loss in elderly men and women: The Framingham Osteoporosis Study. J Bone Miner Res **15:**2504–2512.
24. Tucker KL, Chen H, Hannan MT, Cupples LA, Wilson PW, Felson D, Kiel DP 2002 Bone mineral density and dietary patterns in older adults: The Framingham Osteoporosis Study. Am J Clin Nutr **76:**245–252.
25. Schurch MA, Rizzoli R, Slosman D, Vadas L, Vergnaud P, Bonjour JP 1998 Protein supplements increase serum insulin-like growth factor-I levels and attenuate proximal femur bone loss in patients with recent hip fracture. A randomized, double-blind, placebo-controlled trial. Ann Intern Med **128:** 801–809.
26. McKeown NM, Jacques PF, Gundberg CM, Peterson JW, Tucker KL, Kiel DP, Wilson PW, Booth SL 2002 Dietary and nondietary determinants of vitamin K biochemical measures in men and women. J Nutr **132:**1329–1334.
27. Promislow JH, Goodman-Gruen D, Slymen DJ, Barrett-Connor E 2002 Retinol intake and bone mineral density in the elderly: The Rancho Bernardo Study. J Bone Miner Res **17:**1349–1358.
28. Michaelsson K, Lithell H, Vessby B, Melhus H 2003 Serum retinol levels and the risk of fracture. N Engl J Med **348:**287–294.
29. Slemenda CW, Longcope C, Zhou L, Hui SL, Peacock M, Johnston CC 1997 Sex steroids and bone mass in older men. Positive associations with serum estrogens and negative associations with androgens. J Clin Invest **100:**1755–1759.
30. Greendale GA, Edelstein S, Barrett-Connor E 1997 Endogenous sex steroids and bone mineral density in older women and men: The Rancho Bernardo Study. J Bone Miner Res **12:**1833–1843.
31. Amin S, Zhang Y, Sawin CT, Evans SR, Hannan MT, Kiel DP, Wilson PW, Felson DT 2000 Association of hypogonadism and estradiol levels with bone mineral density in elderly men from the Framingham study. Ann Intern Med **133:**951–963.
32. Khosla S, Atkinson EJ, Dunstan CR, O'Fallon WM 2002 Effect of estrogen versus testosterone on circulating osteoprotegerin and other cytokine levels in normal elderly men. J Clin Endocrinol Metab **87:**1550–1554.
33. Falahati-Nini A, Riggs BL, Atkinson EJ, O'Fallon WM, Eastell R, Khosla S 2000 Relative contributions of testosterone and estrogen in regulating bone resorption and formation in normal elderly men. J Clin Invest **106:** 1553–1560.
34. Smith MR, Finkelstein JS, McGovern FJ, Zietman AL, Fallon MA, Schoenfeld DA, Kantoff PW 2002 Changes in body composition during androgen deprivation therapy for prostate cancer. J Clin Endocrinol Metab **87:**599–603.
35. Rosen CJ, Donahue LR, Hunter SJ 1994 Insulin-like growth factors and bone: The osteoporosis connection. Proc Soc Exp Biol Med **206:**83–102.
36. Bauer DC, Rosen CJ, Cauley J, Cummings SR 1997 Low serum IGF-I but not IGFBP-3 predicts hip and spine fractures. Bone **21:**561.
37. Barrett-Connor E, Kritz-Silverstein D, Edelstein SL 1993 A prospective study of dehydroepiandrosterone sulfate (DHEAS) and bone mineral density in older men and women. Am J Epidemiol **137:**201–206.
38. Halloran BP, Ferguson VL, Simske SJ, Burghardt A, Venton LL, Majumdar S 2002 Changes in bone structure and mass with advancing age in the male C57BL/6J mouse. J Bone Miner Res **17:**1044–1050.
39. Bouxsein ML, Myers KS, Shultz KL, Donahue LR, Rosen CJ, Beamer WG 2005 Ovariectomy-induced bone loss varies among inbred strains of mice. J Bone Miner Res **20:**1085–1092.
40. Deng HW, Mahaney MC, Williams JT, Li J, Conway T, Davies KM, Li JL, Deng H, Recker RR 2002 Relevance of the genes for bone mass variation to susceptibility to osteoporotic fractures and its implications to gene search for complex human diseases. Genet Epidemiol **22:**12–25.
41. Karasik D, Myers RH, Cupples LA, Hannan MT, Gagnon DR, Herbert A, Kiel DP 2002 Genome screen for quantitative trait loci contributing to normal variation in bone mineral density: The Framingham Study. J Bone Miner Res **17:**1718–11727.
42. Hayes WC 1991 Implications for assessment of fracture risk. In: Mow VC, Hayes WC (eds.) Basic Orthopaedic Biomechanics. Raven Press, New York, NY, USA, pp. 93–142.
43. Nevitt MC, Johnell O, Black DM, Ensrud K, Genant HK, Cummings SR 1994 Bone mineral density predicts non-spine fractures in very elderly women. Study of Osteoporotic Fractures Research Group. Osteoporos Int **4:**325–331.
44. Bischoff-Ferrari HA, Dietrich T, Orav EJ, Hu FB, Zhang Y, Karlson EW, Dawson-Hughes B 2004 Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged > or =60 y. Am J Clin Nutr **80:**752–758.
45. Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B 2005 Fracture prevention with vitamin D supplementation: A meta-analysis of randomized controlled trials. JAMA **293:**2257–2264.
46. Mathias S, Nayak US, Isaacs B 1986 Balance in elderly patients: The "get-up and go" test. Arch Phys Med Rehabil **67:**387–389.
47. Tinetti ME 1986 Performance-oriented assessment of mobility problems in elderly patients. J Am Geriatr Soc **34:**119–126.
48. Guralnik JM, Ferrucci L, Pieper CF, Leveille SG, Markides KS, Ostir GV, Studenski S, Berkman LF, Wallace RB 2000 Lower extremity function and subsequent disability: Consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery. J Gerontol A Biol Sci Med Sci **55:**M221–M231.
49. Leibson CL, Tosteson AN, Gabriel SE, Ransom JE, Melton LJ 2002 Mortality, disability, and nursing home use for persons with and without hip fracture: A population-based study. J Am Geriatr Soc **50:**1644–1650.
50. Gillespie L, Gillespie W, Robertson M, Lamb S, Cumming R, Rowe B 2001 Interventions for preventing falls in elderly people. Cochran Database Syst Rev **3:**CD000340.
51. Gillespie LD, Gillespie WJ, Robertson MC, Lamb SE, Cumming RG, Rowe BH 2003 Interventions for preventing falls in elderly people. Cochran Database Syst Rev **4:**CD000340.
52. Greenspan SL, Myers ER, Kiel DP, Parker RA, Hayes WC, Resnick NM 1998 Fall direction, bone mineral density, and function: Risk factors for hip fracture in frail nursing home elderly. Am J Med **104:**539–545.
53. Parker MJ, Gillespie LD, Gillespie WJ 2003 Hip protectors for preventing hip fractures in the elderly. Cochran Database Syst Rev **3:**CD001255.
54. Black DM, Cummings SR, Karpf DB, Cauley JA, Thompson DE, Nevitt MC, Bauer DC, Genant HK, Haskell WL, Marcus R, Ott SM, Torner JC, Quandt SA, Reiss TF, Ensrud KE 1996 Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Lancet **348:**1535–1541.
55. McClung MR, Geusens P, Miller PD, Zippel H, Bensen WG, Roux C, Adami S, Fogelman I, Diamond T, Eastell R, Meunier PJ, Reginster JY 2001 Effect of risedronate on the risk of hip fracture in elderly women. Hip Intervention Program Study Group. N Engl J Med **344:**333–340.
56. Chesnut IC, Skag A, Christiansen C, Recker R, Stakkestad JA, Hoiseth A, Felsenberg D, Huss H, Gilbride J, Schimmer RC, Delmas PD 2004 Effects of oral ibandronate administered daily or intermittently on fracture risk in postmenopausal osteoporosis. J Bone Miner Res **19:**1241–1249.
57. Cauley JA, Robbins J, Chen Z, Cummings SR, Jackson RD, LaCroix AZ, LeBoff M, Lewis CE, McGowan J, Neuner J, Pettinger M, Stefanick ML, Wactawski-Wende J, Watts NB 2003 Effects of estrogen plus progestin on risk of fracture and bone mineral density: The Women's Health Initiative randomized trial. JAMA **290:**1729–1738.
58. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J 2002 Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results From the Women's Health Initiative randomized controlled trial. JAMA **288:**321–333.
59. Chesnut CH III, Silverman S, Andriano K, Genant H, Gimona A, Harris S, Kiel D, LeBoff M, Maricic M, Miller P, Moniz C, Peacock M, Richardson P, Watts N, Baylink D 2000 A randomized trial of nasal spray salmon calcitonin in postmenopausal women with established osteoporosis: The prevent recurrence of osteoporotic fractures study. PROOF Study Group. Am J Med **109:**267–276.
60. Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster JY, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK, Wang O, Mitlak BH 2001 Effect of parathyroid hormone (1–34) on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med **344:** 1434–1441.
61. Lufkin EG, Wahner HW, O'Fallon WM, Hodgson SF, Kotowicz MA, Lane AW, Judd HL, Caplan RH, Riggs BL 1992 Treatment of postmenopausal osteoporosis with transdermal estrogen. Ann Intern Med **117:**1–9.
62. Overgaard K, Hansen MA, Jensen SB, Christiansen C 1992 Effect of salcatonin given intranasally on bone mass and fracture rates in established osteoporosis: A dose-response study. BMJ **305:**556–561.
63. Black DM, Cummings SR, Karpf DB, Cauley JA, Thompson DE, Nevitt MC, Bauer DC, Genant HK, Haskell WL, Marcus R, Ott SM, Torner JC, Quandt SA, Reiss TF, Ensrud KE 1996 Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Fracture Intervention Trial Research Group. Lancet **348:**1535–1541.
64. Liberman UA, Weiss SR, Broll J, Minne HW, Quan H, Bell NH, Rodriguez-Portales J, Downs RW, Jr., Dequeker J, Favus M 1995 Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. The Alendronate Phase III Osteoporosis Treatment Study Group. N Engl J Med **333:**1437–1443.