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Maternal-Fetal Calcium and Bone Metabolism During Pregnancy, Puerperium, and Lactation*

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I. Introduction

IN THEIR classic text published in 1948, Albright and Reifenstein (1) noted the presentation of two young women with idiopathic osteoporosis worsened by pregnancy, and they recognized that significant maternal losses of skeletal calcium could occur during both pregnancy and lactation. They speculated that secondary hyperparathyroidism normally develops during pregnancy and lactation to resorb calcium from bone, and they concluded that, in certain cases, these skeletal calcium losses would cause a form of osteoporosis. Since that time, both pregnancy and lactation have been described in various endocrinology texts as states of "physiological, maternal hyperparathyroidism" (2, 3). However, this concept has not been supported by measurements of PTH with newer, more reliable assays.

Although Albright and Reifenstein's theory proved to be incorrect, it is now evident that mineralization of the fetal skeleton and continued skeletal growth in the infant both mandate a series of hormone-mediated adjustments in maternal calcium metabolism during pregnancy and lactation, respectively. These hormone-mediated adjustments normally satisfy the daily calcium needs of the fetus and infant without long-term consequences to the maternal skeleton. In addition, both fetal and neonatal calcium and bone metabolism are uniquely adapted to meet the specific needs of these developmental periods. The fetus must actively transport sufficient calcium across the placenta to meet the large demands of the rapidly mineralizing skeleton, whereas the neonate must quickly adjust to loss of placental calcium transport, while continuing to undergo rapid skeletal growth.

Here we review our present understanding of normal human calcium and bone metabolism during pregnancy, lactation, fetal development, and the neonatal period. We shall also discuss the relevant pathophysiology and management of clinical disorders of calcium and bone metabolism that can occur during these periods. Generally these conditions are due to preexisting disease (*e.g.*, hyperparathyroidism) that is compounded by the alterations in calcium

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and bone metabolism naturally occurring during these reproductive periods.

Although the focus of this review is on human calcium physiology and pathophysiology, the animal literature will be closely considered as well. Many of our models for explaining human physiology are based on these animal studies, particularly since ethical constraints generally prevent all but observational studies from being performed during human pregnancy and fetal development. Where both human and animal data are available, we will point out several significant differences that have been found between the animal and human data. These differences illustrate the difficulty of extrapolating from the animal models in the absence of human data.

The literature reviewed in this paper was obtained from computerized searches of the MEDLINE database, manual searches of *Index Medicus* before 1966, and the bibliographies of individual articles and texts.

II. Maternal Physiology and Pathophysiology During Pregnancy

A. Maternal adaptive goals during pregnancy

Measurements of calcium in ashed human abortuses determined that the normal total accumulation of calcium in a fetus at term is 21 g (range, 13–33 g) (4). Approximately 80% of this calcium accumulates during the third trimester, when the fetal skeleton is rapidly mineralizing (4, 5). Therefore, although maternal adaptations designed to meet the calcium needs of the fetus might begin early in pregnancy, they are most needed in the third trimester. Such adaptations could theoretically involve increased intestinal absorption of calcium, decreased renal excretion of calcium, and increased resorption of calcium from the maternal skeleton. The studies reviewed later in this section indicate that the major adaptive process in human and animal pregnancy is a 2-fold increase in the intestinal absorption of calcium, mediated by increases in 1,25-dihydroxyvitamin D and other mechanisms.

The pregnant rat has typically been used as a model for studying calcium metabolism during pregnancy, but the adaptive strategies of the rat differ importantly from those of the human (Table 1). These differences probably reflect the large litter size (six to 12 fetuses) and the short gestational period (22 days) of the rat; the rat must deliver 12 mg of calcium per fetus between day 17 of gestation and term (6).

TABLE 1. Important differences between calcium physiology of human and rodent pregnancy

Factor	Human pregnancy	Rat pregnancy
Blood ionized calcium	Stable	Reduced in late pregnancy
PTH	Low to low-normal from early pregnancy	Increased
1,25-D	Increased in early pregnancy	Increased in late pregnancy
Intestinal calcium absorption	Increased; follows rise in 1,25-D	Increased; precedes rise in 1,25-D

1,25-D, 1,25-Dihydroxyvitamin D.

B. Mineral ions and calcitropic hormones

The changes that occur in human maternal serum calcium, phosphate, and calcitropic hormone levels are schematically depicted in Fig. 1.

1. Calcium. Early studies of blood calcium levels during pregnancy in humans found a significant decrease in the total serum calcium as pregnancy progressed (7, 8). These early results seemed to confirm that the fetus was "draining" the maternal calcium and thereby creating a state of secondary hyperparathyroidism in the mother, as postulated by Albright and Reifenstein (1). The pregnancy-related fall in total serum calcium was later found to be the consequence of a fall in the serum albumin, and, thereby, the albumin-bound fraction of the total calcium (9). The intravascular fluid volume is greatly expanded during pregnancy, leading to the decreased serum albumin and hemodilution of pregnancy. Measurements of the ultrafiltrable fraction of serum calcium (representing complexed and free calcium) showed no significant change over prepregnancy values (10). More recent measurements of serum ionized calcium, using ion-specific electrodes, demonstrated that the mean ionized calcium level was maintained at the nonpregnant level throughout gestation in most cross-sectional (11–13) and longitudinal studies (14–20).

In contrast, the serum total and ionized calcium have been reported to fall during the last several days of pregnancy in the rat (21). Maternal losses of calcium to a litter of rapidly growing fetuses may exceed the maternal capacity to maintain a normal serum calcium level. Indeed, larger litter sizes correlated with lower serum calcium in pregnant rats (22). In white-tailed deer, the corrected serum calcium falls in the last 1 to 2 weeks of gestation (23). Pregnant ewes have a mild decrease in total serum calcium over the last 6 weeks of pregnancy, likely due to the fall in serum albumin (24); moreover, in one study, about 13% of Awassi fat-tail ewes were found to develop signs and biochemical evidence of hypocalcemia in the last month of pregnancy (25). Therefore, data from several animal models suggest that maternal blood calcium regulation may be disrupted by fetal demands in late pregnancy.

2. Phosphate. Serum phosphate levels are normal throughout pregnancy in humans and animals, as is the renal tubular reabsorption of phosphate (14, 17, 26–29).

3. PTH. The bulk of published human data on PTH levels in pregnancy was obtained from studies that used early-generation PTH RIAs (18, 26, 30–40); some of the more frequently cited studies reported high maternal serum levels of PTH in the latter half of pregnancy (18, 30–34, 39). These data must now be reinterpreted, because it is now known that these PTH RIAs were insensitive and heterogeneously measured multiple different fragments of PTH, most of which were biologically inactive (41, 42).

With the advent of sensitive two-site immunoradiometric (IRMA) PTH assays that accurately determine the level of

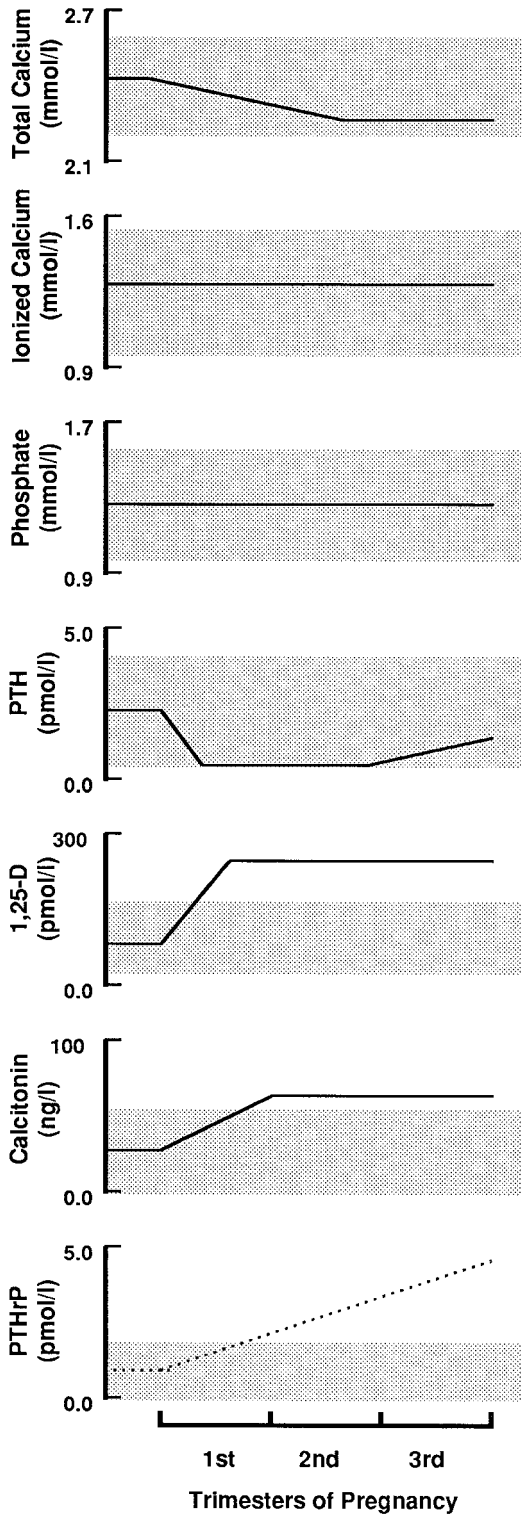

FIG. 1. Schematic illustration of the longitudinal changes in calcium, phosphate, and calcitropic hormone levels that occur during human pregnancy. Normal adult ranges are indicated by the *shaded areas*. Data have been compiled from the following sources: total calcium (9), ionized calcium (14–19), phosphate (14, 17, 26, 27), PTH (11, 14–16, 27, 44), 1,25-dihydroxyvitamin D (15, 41, 57–59), calcitonin (14, 34, 36, 37, 86–89), and PTHrP (44, 111). The progression in PTHrP levels has been depicted by a *dashed line* to reflect that the data are less complete.

intact PTH (42), PTH levels have been typically found to be low-normal in the serum of pregnant women in all three trimesters (11, 12, 17, 19, 20, 27, 43). Five prospective, longitudinal studies found that the mean PTH level was in the low-normal range (*i.e.*, <50% of the mean nonpregnant value) during the first trimester but increased steadily to the mid-normal range by the end of pregnancy (14–16, 27, 44). These findings have been independently validated by reports of normal nephrogenous cAMP levels (12, 26, 44) and low to normal PTH-like bioactivity (11) throughout human pregnancy (although this may be confounded by synthesis of nephrogenous cAMP due to the effects of PTHrP). Studies in primates suggest that the parathyroid glands may have less secretory reserve as pregnancy progresses; the incremental PTH response to acute EDTA-induced hypocalcemia in rhesus monkeys decreased across the trimesters (39).

In contrast to humans, rats develop secondary hyperparathyroidism late in pregnancy. Normally, in late pregnancy, both maternal levels of intact immunoreactive (45) and bioactive (46) PTH rise to exceed the normal range, and the maternal ionized and total calcium levels decline slightly (21). The parathyroid gland volume has also been reported to increase during normal rat pregnancy (47, 48). *In vitro* studies in pregnant rats indicate that the parathyroids secrete more PTH at a given extracellular calcium concentration, when compared with parathyroid cells taken from nonpregnant rats (49). The PTH levels begin to rise earlier in gestation, and peak at higher levels, in pregnant rats fed a modestly calcium-restricted diet (21, 50). This increase in PTH during late pregnancy is critical for normal maternal calcium homeostasis; parathyroidectomized pregnant rats can exhibit signs of tetany in the last 2–4 days of gestation and death during the birthing process (51–54). In parathyroidectomized pregnant rats, dietary intake and weight gain decline, while serum 1,25-dihydroxyvitamin D and intestinal calbindin$_{9K}$-D levels fall (52–54). Maternal tetany coincides with the time onset of rapid fetal accretion of calcium (6); therefore, the parathyroidectomized pregnant rat has compromised dietary intake and intestinal calcium absorption at the time of peak fetal demand for calcium. The calcium abnormalities can be completely prevented when the rats are fed a high-calcium, low-phosphorus diet. Taken together, these observations indicate that rats (but not humans) normally develop a form of secondary hyperparathyroidism during late pregnancy in response to the fall in the maternal serum calcium level. Rats may be more dependent on PTH-mediated bone resorption and PTH-induced 1α-hydroxylase up-regulation during late pregnancy, at a time when the combined calcium need of a litter of fetuses is at its peak.

In summary, immunoreactive and bioactive PTH levels are in the low-normal range during early human pregnancy and are in the mid-normal range at term; in contrast, immunoreactive and bioactive PTH levels in rats are normal in early pregnancy but exceed the normal range in late gestation.

4. 1,25-Dihydroxyvitamin D. Cross-sectional studies have found that the serum level of 1,25-dihydroxyvitamin D more than doubles early in the first trimester in human pregnancy (12, 27, 36, 37, 55–58). Longitudinal studies have found that

the levels of both free and bound 1,25-dihydroxyvitamin D are doubled, and that this increase is maintained until term (15, 20, 41, 57–59). Although clearance of 1,25-dihydroxyvitamin D has not been studied during human pregnancy, in pregnant rats, sheep, and rabbits the increased 1,25-dihydroxyvitamin D levels were due to increased production, and not decreased metabolic clearance, of 1,25-dihydroxyvitamin D (60–63). *In vitro* measurements in homogenates of maternal kidney from rabbits and guinea pigs show that the renal 1α-hydroxylase may be up-regulated 2- to 5-fold (64, 65). The increase in the 1,25-dihydroxyvitamin D level begins while the PTH level is in the low-normal range in humans (*Section II.B.3*, above); this may indicate that PTH does not mediate the up-regulation of the maternal renal 1α-hydroxylase during early human pregnancy. Furthermore, parathyroidectomy in pregnant sheep reduces, but does not eliminate, the pregnancy-related increase in 1,25-dihydroxyvitamin D (66). Other potential direct or indirect regulators of the 1α-hydroxylase include PTHrP (*Section II.B.6*, below), estradiol, PRL, and placental lactogen. Estradiol (67), PRL (68, 69), and placental lactogen (69) acutely stimulate the 1α-hydroxylase *in vitro*, and placental lactogen (but not PRL) raised the serum 1,25-dihydroxyvitamin D levels in hypophysectomized, nonpregnant rats (70). The effect of estradiol on the 1α-hydroxylase has been confirmed *in vivo* by the observation that estrogen replacement in postmenopausal women increases the free and total serum 1,25-dihydroxyvitamin D level (71). However, an effect of PRL *in vivo* has not been confirmed, since hyperprolactinemic patients showed no alteration in 1,25-dihydroxyvitamin D levels (72). Further, in pregnant women, the high 1,25-dihydroxyvitamin D levels of pregnancy did not correlate with serum PRL, estrogens, or human placental lactogen (73).

In addition to the renal 1α-hydroxylase, 1α-hydroxylase activity found in maternal decidua, placenta, and fetal kidneys may also add 1,25-dihydroxyvitamin D to the maternal circulation during pregnancy (59, 74–77). To test this hypothesis, [^{3}H]25-hydroxyvitamin D was administered to pregnant rats after bilateral maternal nephrectomy (74, 78). Newly synthesized (*i.e.*, tritiated) 1,25-dihydroxyvitamin D appeared in the maternal circulation of nephrectomized pregnant rats (but not in nonpregnant nephrectomized rats). Although this study indicates that extrarenally produced 1,25-dihydroxyvitamin D can reach the maternal circulation (74), the specific extrarenal sites and the amounts of 1,25-dihydroxyvitamin D produced could not be ascertained. Data from the Hannover pig model (autosomal recessive 1α-hydroxylase deficiency) indicate that the amounts contributed by these extrarenal sites may be insignificant. In pregnant sows homozygous for absence of 1α-hydroxylase activity, serum levels of 1,25-dihydroxyvitamin D were very low, comparable to the nonpregnant values (79). The presence of heterozygous fetuses did not increase the circulating level of 1,25-dihydroxyvitamin D in the homozygous sows (79). The same gene controls renal and decidual 1α-hydroxylase activity in this model (77). A single case report of a human patient on chronic hemodialysis found 1,25-dihydroxyvitamin D levels of 10–15 pg/ml during pregnancy; these levels were higher than the nonpregnant level in the same patient, but were far lower than in normal pregnancy (80). It is, therefore, likely that increased maternal production of 1,25-dihydroxyvitamin D is mainly due to increased activity of maternal, renal 1α-hydroxylase and not to large contributions from extrarenal sites.

Again, the pregnant rat model differs somewhat from the human, in that the maternal rise in 1,25-dihydroxyvitamin D level does not occur in rats until the time of fetal skeletal mineralization in late gestation (22, 45, 45, 81, 82), at which time the serum PTH levels rise above normal (22, 45) and serum ionized calcium levels fall (21, 22). Larger litter sizes correlate with higher maternal 1,25-dihydroxyvitamin D levels (22). These studies suggest that the effect of PTH on the renal 1α-hydroxylase may dominate the production of 1,25-dihydroxyvitamin D during late pregnancy in the rat.

Serum 25-hydroxyvitamin D levels are unchanged in human pregnancy, and 24,25-dihydroxyvitamin D levels are lower in pregnant women than in controls (35). Supplementation with 1000 IU of vitamin D_3 daily after the first trimester in humans did not affect maternal calcium, phosphate, PTH, and 1,25-dihydroxyvitamin D levels; this suggests that the changes in calcitropic hormone levels observed in human pregnancy are not the result of occult vitamin D deficiency (83). Maternal vitamin D deficiency in the rat has been associated with reduced fertility and smaller litter sizes, and up to 20% of pregnant, vitamin D-deficient rats may die of hypocalcemia near term (84, 85).

In summary, free and total 1,25-dihydroxyvitamin D levels rise early in human pregnancy to peak at twice the normal range, while in rats the 1,25-dihydroxyvitamin D level does not rise until late gestation. These increases appear to be due to increased production of 1,25-dihydroxyvitamin D by the maternal kidneys, with possibly small contributions from maternal decidua, placenta, and fetal kidneys. PTH may be less important during pregnancy in humans compared with rats in mediating this rise in 1α-hydroxylase activity.

5. Calcitonin. Serum calcitonin levels in human pregnancy have generally been reported to be higher than nonpregnant values, with at least 20% of values exceeding the normal range (14, 34, 36, 37, 86–89). Several human studies reported that calcitonin levels were not elevated in pregnancy (15, 18, 33, 35); however, these studies were flawed by the use of improper controls. For example, in some of these studies, postpartum measurements in the same women were used as the baseline, and it has since been shown that calcitonin is also elevated in the postpartum period (see *Section IV.B.5*, below). Similar data from monkeys (39), sheep (77, 90, 91), deer (23), goats (77), and rats (92) have confirmed that the maternal calcitonin level is elevated during pregnancy. No clearance data are available for humans or other animals, but the increased level of calcitonin is generally thought to reflect increased synthesis.

Thyroidal C cells, breast, and placenta are sites of calcitonin synthesis during pregnancy (93, 94). It is not surprising, therefore, that a rise in calcitonin is found in totally thyroidectomized women, most likely due to calcitonin synthesized by the placenta and breast (93, 94). In pregnant rhesus monkeys, acute calcium infusions led to a progressively greater calcitonin response across the trimesters, which may indicate greater secretory reserve of the thyroidal C cells and placenta (39).

It has been speculated that elevated calcitonin protects the maternal skeleton from excessive resorption of calcium, a hypothesis that has been difficult to prove. Indeed, the physiological role of calcitonin in human calcium and skeletal metabolism has not been established (95). No adequate model of experimental calcitonin deficiency has been created, partly because the extrathyroidal sites of calcitonin synthesis were not appreciated at the time. All models used total thyroidectomy with parathyroid gland autotransplantation and thyroid hormone replacement in pregnant goats or rats (53, 91, 96–98). In none of these models was the serum calcitonin or TSH measured to determine whether a calcitonin-deficient, euthyroid state had been attained. Thus, although these models suggested that an intact thyroid gland protected the maternal skeleton from loss of bone mineral during pregnancy, these findings remain to be confirmed by more rigorously controlled models.

In summary, calcitonin levels are increased during pregnancy in humans and animals, partly due to extrathyroidal synthesis in the placenta and breast. The possible role of calcitonin in protecting the maternal skeleton from increased resorption during pregnancy needs more study.

6. PTH-related protein (PTHrP). PTHrP was originally identified in 1987 as the cause of humoral hypercalcemia of malignancy (99). PTHrP has been postulated to be a prohormone, which is processed into several different circulating fragments or hormones, each of which, in turn, may have different functional roles and specific receptors (100). PTHrP has partial homology in its first 13 amino acids to PTH (101–103) and activates the common PTH/PTHrP receptor (42). Amino-terminal forms of PTHrP (PTHrP 1–34, 1–86, or 1–141) resemble PTH in their actions on kidney and bone (104) and can inhibit acetylcholine-induced uterine contractions in the rat (105). Levels of PTHrP decreased acutely in the amnion and myometrium at the time of onset of labor in humans (106). It has been suggested that amino-terminal forms of PTHrP may, therefore, have a role in regulating the onset of labor (106). A midmolecular form of PTHrP stimulates placental calcium transport in the fetus (*Section III.C*, below), although its possible role in the mother is unclear. The carboxyl-terminal portion of PTHrP, termed "osteostatin," is able to inhibit osteoclastic bone resorption in some *in vitro* assays (107, 108) and in rats *in vivo* (109); therefore, this fragment of PTHrP could have a role in protecting the maternal skeleton during pregnancy.

The development of RIAs for PTHrP has concentrated on detecting the PTH-like amino-terminal fragments of PTHrP and has thus far largely ignored the detection of other fragments that might be biologically active. Therefore, no data are available on the levels of midmolecular or carboxyl-terminal fragments of PTHrP during pregnancy compared with controls. An early RIA that used an antibody to PTHrP 1–34 found no elevation of PTHrP in pregnancy (110). Newer, more sensitive two-site immunoradiometric assays that measure forms of PTHrP that encompass amino acids 1 through 86 have found a significant increase in the maternal PTHrP level, beginning as early as weeks 3 to 13 of human pregnancy (44, 111). This increase is not due to any change in the clearance of PTHrP 1–34, 1–86, or 1–141 during pregnancy, as determined in sheep (112, 113). The increase in amino-terminal PTHrP, by activating the PTH/PTHrP receptor in kidney and bone, may well explain (at least in part) the increase in 1,25-dihydroxyvitamin D and ionized calcium, and the decrease in PTH levels, found during human pregnancy.

The source of PTHrP in the maternal circulation during pregnancy is not established, but several candidate sites are known. PTHrP is produced by the placenta (114), amnion (106), decidua (106), umbilical cord (115), and fetal parathyroid glands (116) and potentially might reach the maternal circulation. PTHrP produced by the breast tissue is detectable in human colostrum (117), and it is produced as early as day 14 of pregnancy by the mammary glands of the rat (118).

Overproduction of PTHrP by the breast might explain the development of hypercalcemia at 24 weeks of gestation in a woman with massive (4.5 kg) mammary hyperplasia of pregnancy, associated with hypercalciuria, hypophosphatemia, and undetectable PTH levels (119). Bilateral mastectomies in the second trimester of that same pregnancy corrected the hypercalcemia and the suppressed PTH level (119).

In summary, PTHrP may be made available to the maternal circulation by several different maternal and fetal sources. PTHrP fragments encompassing amino acids 1–86 are increased in the maternal circulation during pregnancy and may contribute to the elevations in 1,25-dihydroxyvitamin D and blood calcium, and suppression of PTH, noted during pregnancy. The true quantitative importance of PTHrP in maternal physiology needs to be established.

7. Other hormones. Pregnancy induces a dramatic rise in other hormones, including the sex steroids, PRL, and placental lactogen. The possibility that each of these, in turn, may have direct or indirect effects on calcium and bone metabolism during pregnancy has been largely unexplored. There is some evidence to suggest that PRL and placental lactogen may increase the intestinal transport of calcium (70, 120, 121), reduce urinary calcium excretion (122, 123), and stimulate synthesis of PTHrP (124) and 1,25-dihydroxyvitamin D (68, 69). This is discussed in more detail in the relevant sections.

C. Intestinal absorption of calcium

Calcium is absorbed throughout the small intestine, a small portion by active transport in the duodenum and proximal jejunum, and the major portion by passive mechanisms in the distal jejunum and ileum (125). Mineral balance and calcium kinetic studies in humans using stable isotopes of calcium (^{48}Ca, ^{44}Ca, ^{42}Ca) have consistently found a positive calcium balance and a doubling of the intestinal absorption of calcium during human pregnancy from as early as 12 weeks of gestation (the earliest time point studied) (27, 126, 127). By studying the effect of an oral calcium load on serum calcium and urine calcium excretion, other investigators indirectly confirmed that intestinal calcium absorption must be increased in all trimesters (12, 128). The results of these studies led to speculation that the increase was mediated by 1,25-dihydroxyvitamin D, and this appeared to be confirmed when elevated levels of 1,25-dihydroxyvitamin D were found during human pregnancy (*Section II.B.4*, above). 1,25-

Dihydroxyvitamin D probably stimulates intestinal calcium absorption by increasing the synthesis of proteins, including the intestinal vitamin D-dependent calcium-binding protein, calbindin$_{9K}$-D. Protein and mRNA levels of calbindin$_{9K}$-D increase in the intestines of mice and rats during pregnancy and plateau when both maternal 1,25-dihydroxyvitamin D levels and the efficiency of intestinal calcium absorption are at peak levels (129–131). Maternal vitamin D deficiency in rodents reduces the rise in the intestinal expression of calbindin$_{9K}$-D (132, 133), while 1,25-dihydroxyvitamin D administration can restore it (133).

The rise in intestinal absorption of calcium occurs by midpregnancy in rats, before the onset of rapid skeletal mineralization in the fetus (45). The doubling of intestinal absorption persists in parathyroidectomized rats (134) and may, therefore, be independent of PTH regulation. The early increase in intestinal calcium absorption may allow the pregnant mother to accrete calcium (probably in the maternal skeleton), before the peak fetal demand for calcium in late pregnancy. Consistent with this hypothesis, it has been estimated from isotope studies in the pregnant rat that 92% of fetal skeletal calcium content was absorbed from the maternal diet at some point during pregnancy (135). Further, several investigators have found that pregnant rats normally store calcium during the first half of pregnancy (136), such that by the end of pregnancy, the calcium content of the femurs is unchanged (137). Inadequate accretion of calcium early in pregnancy may lead to a net loss of maternal skeletal calcium later in pregnancy. For example, under dietary calcium restriction, pregnant rats (138, 139) and goats (98) have reduced calcium content in their long bones by the end of gestation. Similarly, maternal vitamin D deficiency has been found to cause maternal skeletal demineralization by the end of pregnancy (140).

PRL treatment of pregnant, vitamin D-deficient rats resulted in an increase in the intestinal absorption of calcium; PRL might, therefore, have an effect on the intestine independent of 1,25-dihydroxyvitamin D (120). This is further supported by studies in everted gut sacs of nonpregnant, hypophysectomized rats, where PRL and placental lactogen stimulated the intestinal transport of calcium (70, 121). Also in rats, the increase in duodenal calcium absorption has been found to precede the rise in the 1,25-dihydroxyvitamin D level by 1 week, suggesting that the intestinal effect is not dependent solely on vitamin D (45, 141). Even in the absence of vitamin D, pregnancy in rats is associated with hypertrophy of the small intestine and a doubling of intestinal absorption of calcium (141, 142). Furthermore, rats hypocalcemic from vitamin D deficiency developed a progressive rise in serum calcium levels during pregnancy, despite unchanged serum PTH levels (143). However, an independent effect of PRL on intestinal calcium absorption could not be demonstrated in studies on humans. Hyperprolactinemic patients showed no alteration in the intestinal absorption of calcium (72).

In summary, intestinal calcium absorption is increased 2-fold early in human and rat pregnancy, probably through a 1,25-dihydroxyvitamin D-mediated increase in intestinal calbindin$_{9K}$-D and other proteins. PRL and placental lactogen (or possibly other factors) may mediate part of the normal pregnancy-related increase in intestinal calcium absorption. The early rise in intestinal calcium absorption may allow the maternal skeleton to store calcium in advance of the peak fetal demands later in pregnancy. The increased intestinal calcium absorption appears to be a major maternal adaptation to meet the fetal need for calcium.

D. Renal handling of calcium

Pregnancy is associated with an increase in creatinine clearance and glomerular filtration rate (144, 145). The 24-h urine calcium excretion is increased as early as the 12th week of gestation (the earliest time point studied), and averages 300 ± 61 mg in the third trimester (levels in the hypercalciuric range are not uncommon) (12, 14, 20, 27, 146, 147). Since fasting urine calcium values are normal or low, the increase in 24-h urine calcium reflects the increased intestinal absorption of calcium (absorptive hypercalciuria) (12, 28, 44). A similar 2-fold increase in urinary calcium excretion has been observed in the pregnant rat from the second week of gestation (148). Although PRL and placental lactogen have been shown to reduce urinary calcium excretion in nonpregnant rabbits *in vivo* (122, 123), the effect (if any) of either hormone on the kidneys of pregnant humans and rats must be very modest.

Interestingly, preeclampsia and pregnancy-induced hypertension (PIH) have been associated with hypocalciuria (147, 149–152). Further studies have found the hypocalciuria to be associated with low 1,25-dihydroxyvitamin D levels (149–152), but to be independent of PTH, calcitonin, or ionized calcium levels (147, 149–151). The finding of hypocalciuria prompted a large trial of calcium supplementation in pregnant women, which recently reported no benefit in preventing preeclampsia or PIH (153). These abnormalities in 1,25-dihydroxyvitamin D and urine calcium excretion are, therefore, probably secondary to a primary renal tubular defect occurring in preeclampsia and PIH and are likely not the primary cause of the hypertension (149).

E. Skeletal calcium metabolism

1. Bone formation and resorption. Histomorphometric parameters of both bone formation and osteoclast-mediated resorption are increased during pregnancy in rats (154). Pregnant beagle dogs also show histomorphometric evidence of increased bone turnover in iliac trabecular bone (155). Despite evidence of increased turnover, bone mineral content during pregnancy in rats does not change (137, 140, 154, 156). In contrast, pregnant ewes have a 20% decrease in skeletal calcium content during gestation (157).

Comparable histomorphometric data are not available for human pregnancy, but markers of bone formation and resorption have been assessed. Generally speaking, such indices are more reliable for measuring changes in bone resorption than bone formation (158, 159). Several markers of bone resorption (tartrate-resistant acid phosphatase, deoxypyridinoline/creatinine, pyridinoline/creatinine, and hydroxyproline/creatinine) are low in the first trimester but rise steadily to peak at values up to twice normal in the last trimester (27, 44, 160, 161). In contrast, osteocalcin, a marker

of bone formation, is low or undetectable early in gestation and sometimes rises to normal levels by term (15, 27, 161–163). Other markers of bone formation (procollagen I carboxypeptides, bone-specific alkaline phosphatase) are low in the first trimester and have been found to remain low (44) or rise to normal or above in the last trimester (27, 160). Total alkaline phosphatase rises early in pregnancy due to contributions from the placental fraction, and, therefore, is not a useful marker of bone formation in pregnancy (14, 44).

Taken together, the histomorphometric data from animals, and the changes in the markers of bone formation and resorption in humans, indicate that bone turnover is probably low in the first half of pregnancy, but may be increased in the third trimester. The third trimester increase in bone turnover corresponds to time of the peak rate of calcium transfer to the fetus and may result from mobilization of skeletal calcium stores (which contain 99% of the body's stores of calcium) to help supply the fetus.

2. Bone density. Concerns about fetal radiation exposure have resulted in few studies of changes in maternal bone mass during pregnancy; these studies used techniques that are far less precise or reproducible than the current standard, dual x-ray absorptiometry (DXA) (164, 165). Of the scant data available, an early study used x-ray spectrophotometry of the radius and femur to demonstrate a progressive decrease in trabecular bone density during pregnancy (166). Using more modern techniques, four prospective studies of bone density during pregnancy did not find a significant change in cortical or trabecular bone density, as respectively determined by single photon absorptiometry (SPA) and/or dual-photon absorptiometry (DPA) (28, 146, 167, 168). Another study found a significant decrease in bone mineral density of the femoral neck and radial shaft, but no change in lumbar bone density, by comparing preconception SPA and DPA measurements to those taken 6 weeks postpartum (169). Most recently, cross-sectional (170) and longitudinal studies (161, 171) have found a progressive decrease during pregnancy in indices thought to correlate with bone mineral density, as determined by ultrasonographic measurements of the os calcis in all three trimesters.

The majority of retrospective, epidemiological studies of pre- and postmenopausal women have found no association of parity with bone density or fracture risk (172–191). In contrast, several other studies found increased parity to be beneficial, as indicated by a slightly greater lumbar (192, 193), femoral (194), or radial bone density (194–196) and decreased hip fracture risk (197, 198). Four remaining studies linked parity to somewhat decreased lumbar bone density (199–201) or increased hip fracture risk (202). An epidemiological study of healthy women aged 21 to 95 found divergent effects of parity at different anatomical sites. Femoral neck bone mineral density was decreased in parous women by 1.5% per live birth, while lumbar spine bone density was not influenced by parity (203). Among the studies that found no significant association of parity, several reported that a first pregnancy as an adolescent was associated with decreased bone density (178, 190, 195), possibly because the fetal calcium demands of pregnancy reduce the peak bone mass that is eventually achieved in the adolescent. Overall, many of these epidemiological studies had significant methodological limitations, specifically the difficult problem of retrospectively separating out the effects of parity from those of lactation. Nevertheless, it may be reasonable to conclude from these studies that if parity has either a positive or a negative effect on bone density or fracture risk, it must be only a very modest effect.

Therefore, although changes in serum and urine markers of bone formation and resorption have indicated that bone turnover may be increased in the third trimester, it is impossible to determine from the available bone density data whether there is any acute change in bone mineral during human pregnancy. Further, it is also unknown whether any such acute change has a long-term effect on the calcium content or fragility of the maternal skeleton.

3. Osteoporosis in pregnancy. The rare presentation of idiopathic osteoporosis in a woman of child-bearing age has often been associated with a recent pregnancy, as noted by Albright and Reifenstein (1) and other early case reports (204–206). The exact prevalence of the condition is uncertain. The theory that pregnancy might cause osteoporosis (as proposed by Albright and Reifenstein) was disputed by an early observational study of five women with symptomatic, severe osteoporosis presenting in a first pregnancy (207). In subsequent pregnancies, these women were found to have no worsening of their condition, but the parameters used (new pain or fracture, worsening of osteopenia on plain roentgenograms) were crude and insensitive by methods available today (207). Despite better documentation of the absence of known causes of decreased bone density in more recent case reports (208–212), it has not been possible to exclude the possibility that low peak bone mass and/or an accelerated bone resorptive state preceded the pregnancy and simply became clinically obvious in pregnancy. In addition, some reported cases of osteoporosis in pregnancy have been clearly confounded by the presence of other recognizable causes of secondary osteoporosis, such as chronic heparin, anticonvulsant, or corticosteroid therapy (209, 212). In two documented cases of osteoporosis diagnosed in pregnancy, the female progeny were found at age 10 to also have low bone mineral density (213). This finding suggested that a shared genetic or environmental factor (and not pregnancy) was the cause of osteoporosis in the mothers and daughters. The limited data from bone biopsy typically show no evidence of osteomalacia, but only mild osteoporosis or normal architecture (208, 212). It remains intriguing to speculate that some of these rare cases of osteoporosis presenting in pregnancy may result from excessive resorption of calcium from the maternal skeleton, perhaps in the setting of inadequate intake of calcium, low stores of 25-hydroxyvitamin D, or an excessive rise in PTHrP in the maternal circulation (see also the discussion of osteoporosis in lactation, *Section IV.E.3*, below). Nevertheless, these rare cases may simply represent idiopathic osteoporosis occurring in pregnant women by mere chance.

A second (also rare) form of pregnancy-associated osteoporosis is a focal, transient osteoporosis of the hip (214–217). Typically these patients present with unilateral or bilateral hip pain, limp, and/or hip fracture in the third trimester (214,

216, 218, 219). Radiolucency of the femoral head and neck was recognized on plain radiographs taken in early reports of this condition (215, 220); and more recently, DXA measurements have shown that the bone density of the symptomatic femoral head and neck is reduced (218). Magnetic resonance imaging (MRI) of the affected femoral head in one patient showed a joint effusion and images suggesting increased water content of the femoral head and marrow cavity (221). Routine serum chemistries are typically normal (222). Alkaline phosphatase and urine hydroxyproline have been reported to be elevated (215, 218, 223); however, the interpretation of these findings is uncertain, since control measurements from normal pregnant women were not compared. Intriguingly, the decreased bone mineral density of the femoral head and neck typically resolves within 2 to 6 months postpartum (214, 218, 219), including the MRI findings (221). Patients generally require only pain relief and continued mobilization for this self-limited condition. The fact that this rare condition is typically localized to one or both femoral heads, and not the rest of the skeleton, suggests that it is not the result of a generalized increase in skeletal resorption. Several theories have been proposed to explain this condition, including femoral venous stasis due to pressure from the pregnant uterus, a form of Sudeck's atrophy, or reflex sympathetic dystrophy (causalgia), ischemia, trauma, viral infections, marrow hypertrophy, immobilization, and fetal pressure on the obturator nerve (214–216, 220). As yet, the etiology of transient osteoporosis of the hip in pregnancy remains unclear; its association with pregnancy may be not causal but incidental. In any case, it appears likely that this disorder is not a manifestation of altered calcitropic hormone levels or mineral balance during pregnancy.

F. Primary hyperparathyroidism

The presentation of primary hyperparathyroidism in pregnancy raises important diagnostic and management considerations. Many cases are asymptomatic, detected by routine prenatal biochemical tests or after the presentation of hypocalcemia in the neonate. Several normal pregnancy-related changes in calcium and PTH physiology (noted above) may obscure the diagnosis of mild primary hyperparathyroidism. These include the fall in total serum calcium, the rise in the corrected serum calcium (111, 224), the fall in the intact PTH level (14–16, 44), and the rise in the 24-h urinary excretion of calcium, often into the hypercalciuric range (14, 27) (see also *Sections II.B and II.D*, above).

Although maternal primary hyperparathyroidism in pregnancy is probably a rare condition (there are no data available on its prevalence), it has been associated in the literature with an alarming rate of adverse outcomes in the fetus, including a 30% rate of spontaneous abortion or stillbirth (225, 226). In the neonatal period, a 50% rate of tetany and a 25% rate of neonatal death has been reported (225, 227). These adverse outcomes are thought to result from suppression of the fetal parathyroid glands; this suppression may occasionally be prolonged for months (228, 229). PTH cannot cross the placenta (230–232); therefore, the fetal parathyroid suppression is thought to result from increased net calcium flux across the placenta to the fetus, facilitated by maternal hypercalcemia. Evidence from animal models has confirmed that acute elevations in maternal serum calcium cause an increase in fetal serum calcium, and a fall in fetal PTH level (233). However, whether chronic maternal hypercalcemia has the same effect on fetal serum calcium, or placental calcium transport, has not been determined.

Surgical correction of primary hyperparathyroidism during the second trimester, to prevent fetal and neonatal complications, has been almost universally recommended (226, 234–236). Several case series have found elective surgery to be well tolerated and to dramatically reduce the rate of adverse events when compared with the earlier cases reported in the literature (234, 235, 237, 238). However, many of the women in those early cases were symptomatic and had nephrocalcinosis or renal insufficiency. Those early case reports may also have reflected reporting bias of adverse fetal and neonatal outcomes. Whether the milder, asymptomatic form of primary hyperparathyroidism commonly seen today has the same risk of adverse fetal or neonatal outcomes has not been determined. In several case reports, mild elevations in maternal serum calcium were followed without operative intervention, and no adverse fetal or neonatal outcome occurred (239, 240). However, in other cases the mild hypercalcemia of both asymptomatic primary hyperparathyroidism and familial hypocalciuric hypercalcemia has been reported to cause neonatal parathyroid suppression and tetany (241–243). Nevertheless, it is probably reasonable to follow cases of asymptomatic primary hyperparathyroidism with mild hypercalcemia conservatively and to reserve surgery in the second trimester for patients that are symptomatic or have more severe hypercalcemia. If surgery is deferred, the neonate must be monitored closely for the development of hypocalcemia.

G. Hypoparathyroidism and pseudohypoparathyroidism

As described earlier (*Section II.B.4*), free and bound 1,25-dihydroxyvitamin D levels normally double during human pregnancy in the presence of low-to-normal intact PTH levels, and, therefore, it is likely that PTH does not mediate the pregnancy-related rise in 1,25-dihydroxyvitamin D production. Other hormones of pregnancy, such as estrogen, PTHrP, and perhaps placental lactogen and PRL, may regulate the increased production of 1,25-dihydroxyvitamin D by maternal kidney and decidua. Also, placenta and fetus may contribute to the maternal increase in 1,25-dihydroxyvitamin D.

In multiple case reports, pregnant hypoparathyroid women have been found to have fewer hypocalcemic symptoms, a rise in the serum calcium, and decreased dependence on supplemental calcitriol to maintain a normal serum calcium (244–252). This finding 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. The literature on hypoparathyroidism in pregnancy is not entirely consistent on this point, since in other case reports the calcitriol dosage was increased for a variety of reasons (some incompletely documented) (253–257). Despite these contrasting views on the natural history of hypoparathyroidism in pregnancy, there is general agreement (244, 245, 248, 253, 254,

258) that in *late* pregnancy and the puerperium, hypercalcemia may result unless the calcitriol is discontinued, or the dosage is decreased below the prepartum requirement. Since this effect is even more pronounced in those who breast-feed, and since PTHrP is found at high concentrations in the breast during late pregnancy and lactation (further discussed in *Section IV.B.6*, below), the pregnancy-related rise in 1,25-dihydroxyvitamin D production may be regulated by PTHrP (secreted from the breast) in these hypoparathyroid women.

Calcitriol (rather than vitamin D or calcifediol) has typically been prescribed for hypoparathyroidism in pregnancy, and the dosage needed may range from 0.5–3.0 μg daily. Chronic maternal hypocalcemia must be avoided because it has been associated with the development of intrauterine hyperparathyroidism and death in the fetus (*Section III.F*, below).

Further illumination of the role of PTH in pregnancy has come from cases of pseudohypoparathyroidism in pregnancy. Pseudohypoparathyroidism is a heterogeneous group of genetic syndromes characterized by hypocalcemia due to PTH resistance (259). Although the data are limited, Breslau and Zerwekh (260) noted a normalization of serum calcium levels in two pregnant women with pseudohypoparathyroidism (probably type 1b). Before pregnancy the patients had hypocalcemia, markedly elevated PTH levels, and low 1,25-dihydroxyvitamin D levels. During four pregnancies (two for each patient), the serum calcium levels were normal, their PTH levels were halved, and the 1,25-dihydroxyvitamin D levels increased 2- to 3-fold. Contributions of 1,25-dihydroxyvitamin D from placental and fetal sources might have accounted for these findings; Zerwekh and Breslau (261) noted elsewhere that the placental production of 1,25-dihydroxyvitamin D was no different between placentas obtained from pseudohypoparathyroid women and controls. Alternatively, it is possible that the hormonal milieu of pregnancy lessened the renal resistance to PTH and PTHrP and thereby increased the formation of 1,25-dihydroxyvitamin D. It is apparent from Breslau's observations that estrogens alone cannot be the explanation for such an improvement during pregnancy, because the same two pseudohypoparathyroid women were not improved by treatment with an oral contraceptive. In any case, calcitriol supplementation in these patients should be monitored carefully and adjusted during pregnancy. The progeny of these pregnancies are also at risk of intrauterine, fetal hyperparathyroidism (262, 263), perhaps because of relative maternal hypocalcemia during pregnancy.

H. Summary

The fetal demand for calcium, which largely occurs during the third trimester, is met by a doubling of free and bound maternal 1,25-dihydroxyvitamin D levels, which, in turn, partly mediate a doubling of the intestinal absorption of calcium. Some of the increased intestinal calcium absorption may be mediated by PRL or other hormones of pregnancy. Further, the increase in 1,25-dihydroxyvitamin D may be largely independent of changes in PTH, since PTH levels are typically low or normal at the time of the increase in 1,25-dihydroxyvitamin D. The increased calcium intake and absorption leads to a marked increase in renal calcium excretion (absorptive hypercalciuria). The serum ionized calcium is normal, despite a fall in total serum calcium caused by a reduction in the albumin-bound fraction. Calcitonin and PTHrP are both elevated, particularly in the latter half of gestation, but the physiological importance of these hormones in pregnancy is not known. The typical changes in calcium and calcitropic hormone levels during pregnancy are depicted schematically in Fig. 1.

Bone resorption is increased during late pregnancy, as evidenced by a rise in the levels of serum and urine markers of bone resorption in the third trimester, and this may indicate that maternal skeletal calcium stores are mobilized during the time of rapid fetal accretion of calcium. As noted at the beginning of *Section II.E.2*, bone density studies during pregnancy have been of insufficient precision to determine whether this increased bone resorption results in significant loss of skeletal calcium during pregnancy or the third trimester. Retrospective epidemiological studies (although not definitive) have generally found no effect of parity on the risk of osteoporosis or fractures in later life. Uncommonly, pregnancy may be associated with osteoporosis and fractures, particularly if the woman enters pregnancy with a low peak bone mass. A distinct disorder, focal, transient osteoporosis of the hip in pregnancy, is not likely due to altered calcitropic hormone levels and calcium physiology.

Primary hyperparathyroidism in pregnancy has been classically associated with adverse fetal or neonatal outcomes, but the milder, asymptomatic form of primary hyperparathyroidism most often seen today may not share such outcomes. Maternal hypoparathyroidism may be improved in pregnancy by increased intestinal absorption of calcium, possibly mediated by increased production of 1,25-dihydroxyvitamin D caused by PTHrP or some other non-PTH factor. A similar improvement in biochemical indices has been seen in pregnant women with pseudohypoparathyroidism. In both hypoparathyroid and pseudohypoparathyroid women, maternal hypocalcemia may adversely affect the fetus and must be avoided.

The pregnant rat model differs from the human condition in several important respects (Table 1). The rat normally develops a form of secondary hyperparathyroidism in the last several days of pregnancy, prompted by a fall in the maternal serum-ionized calcium at the time of rapid fetal accretion of calcium. 1,25-Dihydroxyvitamin D increases late in gestation in rats, approximately 1 week after the rise in intestinal calcium absorption. This indicates that mechanisms independent of 1,25-dihydroxyvitamin D may contribute to the increased intestinal calcium absorption in rats.

III. Fetal-Placental Physiology and Pathophysiology

A. Fetal adaptive goals

With respect to calcium physiology, the fetal-placental unit has two main adaptive goals. One is to provide sufficient calcium to mineralize the skeleton, and the other is to maintain an extracellular level of calcium that is physiologically appropriate for fetal tissues (*i.e.*, for cell membrane stability, blood coagulation, etc). A human fetus typically accumulates

21 g of calcium by term, and 80% of this calcium is accumulated in the third trimester alone, necessitating an average daily transfer of 200 mg calcium (4). Similarly, the fetal rat accretes less than 0.5 mg calcium in the first 17 days of gestation, and about 12 mg calcium in the remaining 5 days of gestation (6). To attain the required amount of calcium and regulate the fetal calcium level, the fetus makes use of the placenta, kidneys, bone, and intestine. The studies reviewed herein will demonstrate that the fetal-placental unit functions relatively independently of the mother, such that it is capable of mineralizing the fetal skeleton and maintaining a normal blood calcium, even in the presence of significant maternal hypocalcemia and vitamin D deficiency. In addition, this section will show that PTHrP is a major regulator of placental calcium transport, while PTHrP and PTH may both act on fetal bone and kidneys to regulate the blood calcium.

Human handling of placental calcium transport must be largely inferred from data that have been obtained from studies in sheep, pigs, rats, and mice. Therefore, it must be emphasized that mice and rats have hemochorial placentas that are structurally very similar to those of humans (264–267). In contrast, the epitheliochorial placentas of sheep and pigs differ significantly in structure from the human hemochorial placenta, and may, therefore, be functionally different as well (266).

B. Mineral ions and calcitropic hormones

1. Calcium. In humans, rodents, sheep, cattle, monkeys, and other mammals, the fetal blood calcium (total and ionized) is maintained at a higher level than in the maternal circulation (268–275). This elevation is mainly due to an increase in the ionized calcium level (274). Ionized calcium is approximately 80% of the total calcium in fetal rodents (276); only a small fraction is bound to albumin.

In fetal rats, there is a progressive rise in total and ionized calcium over the last week of gestation, corresponding to the time of a progressive decline in fetal pH (277–279). Data are lacking on precisely how early in gestation the fetal blood calcium begins to exceed the maternal. In sheep, fetal hypercalcemia has been detected as early as the 35th day of gestation (280, 281). In humans, fetal hypercalcemia was documented at 15–20 weeks of gestation (by fetoscopy) (282) and at delivery of preterm singleton and twin pregnancies (mean gestational age 33 weeks) (283).

Two physiological models could explain fetal hypercalcemia: either the fetus maintains a fixed positive gradient of calcium with respect to the maternal level, or the fetus maintains a high, fixed level of calcium. Evidence from rat and mouse models indicates that the fetus sets its blood calcium at a higher level *independently* of the maternal calcium level. For example, in rats, the fetal blood calcium is unchanged in the presence of severe maternal hypocalcemia due to a calcium-restricted diet (284), vitamin D deficiency (29, 84, 285), or thyroparathyroidectomy (46, 134). The calcium gradient from mother to fetus is increased in these fetuses because the maternal blood calcium is lower. When both the pregnant rat and its fetus are thyroparathyroidectomized, the fetus still maintains a higher blood calcium level than the mother (286, 287). Also, in genetically engineered mice, maternal hypercalcemia due to heterozygous ablation of the calcium-sensing receptor (CaSR) gene does not affect the blood calcium level set by normal fetuses (288). Similarly, heterozygous calcium-sensing receptor knockout fetuses establish a constant, abnormally high fetal blood calcium level, regardless of whether the mother is heterozygous (and therefore hypercalcemic) or normal (288). The apparent "calcium gradient" is lower in offspring of these heterozygous mice, due to maternal hypercalcemia. Finally, acute alterations in the maternal blood calcium of rodents and primates (by calcium, 1,25-dihydroxyvitamin D, calcitonin, PTH, or EDTA infusions) are not reflected by much perturbation in the fetal blood calcium (232, 289–292).

Others have reported a fall in the fetal blood calcium after maternal parathyroidectomy in rats (52–54). The fetal blood calcium was normal between the 12th and 17th day of gestation, but fell during the period of rapid fetal skeletal calcium accretion. Therefore, these data indicate that the ability of the fetal rat to set its blood calcium may break down during the time of rapid accretion of calcium by the skeleton, if the mother has been parathyroidectomized.

In summary, from early pregnancy, mammalian fetuses have higher levels of blood calcium than their mothers, mainly due to an increase in the ionized calcium level. The fetus does not establish a particular calcium gradient with respect to the maternal blood calcium; instead, it establishes a particular fetal blood calcium level, irrespective of the ambient maternal blood calcium level. This ability persists in the presence of significant maternal hypocalcemia of various causes, but may be impaired during the time of rapid accretion of calcium by the skeleton. The physiological importance of fetal hypercalcemia is not known.

2. Phosphate. Fetal phosphorus levels are higher than maternal in rats (279) and humans (32, 270, 273). This suggests that phosphate may be actively transported across the placenta, but the regulators of this active transport are unknown (293). PTHrP and PTH do not stimulate placental transport of phosphate in sheep (294); vitamin D may have a role (295).

3. PTH. Fetal parathyroid glands of rats and sheep contain PTH mRNA (114, 116), and PTH immunoreactivity is present in human fetal parathyroid glands as early as 10 weeks of gestation (296). These findings indicate that fetal parathyroid glands are capable of synthesizing PTH early in gestation. Furthermore, PTH detected in the fetal blood likely derives from fetal sources alone. Intact PTH does not cross the placenta of nonhuman primates, sheep, and rodents (230–232) and probably does not cross the human placenta.

The following evidence indicates that fetal parathyroid glands appear to contribute to calcium homeostasis, by secretion of PTH or PTHrP. Fetal thyroparathyroidectomy in sheep and fetal decapitation in rats caused hypocalcemia (52, 297, 298), and mice lacking the PTH/PTHrP receptor gene are hypocalcemic *in utero* (299). PTH can be regulated by the ambient fetal blood calcium, since EDTA-induced fetal hypocalcemia has been found to induce a rise in fetal PTH levels in rats (300), cattle (275), and rhesus monkeys (301), although another study in rhesus monkeys found no fetal PTH re-

sponse (271). Removal of a maternal parathyroid adenoma was followed by a rise in amniotic fluid PTH levels and a decline in the amniotic fluid calcium level during a human pregnancy (302). Since maternal PTH cannot cross the placenta, the findings in this case have been interpreted to indicate that fetal PTH secretion can be influenced by the maternal blood calcium (302).

In fetal humans and other animals, immunoreactive PTH blood levels have been found to be undetectable or very low (*i.e.*, <0.5 pmol/liter) with respect to maternal PTH level near the end of gestation (17, 32, 35, 38, 43, 110, 124, 268, 275, 303–310). Little information is available on PTH levels earlier in gestation. One study in fetal rats found that the PTH level declined in the last several days of gestation as the serum ionized calcium rose (277), while two cross-sectional studies in preterm humans found that the fetal PTH level was not lower than the maternal PTH level (283, 308).

In summary, the available evidence suggests that the fetal parathyroids are capable of synthesizing PTH. Since blood levels of PTH have been typically found to be low in late gestation at a time when the fetal blood calcium is high, other factors must determine the fetal blood calcium level. The precise role of PTH in normal fetal calcium homeostasis will be clarified by ablating the PTH gene in mice.

4. 1,25-Dihydroxyvitamin D. Although maternal vitamin D deficiency reduces fertility and litter size in the rat (84, 85), evidence from several animal models indicates that 1,25-dihydroxyvitamin D is not necessary for normal fetal calcium and bone metabolism. In pregnant rats, sheep, and pigs that were hypocalcemic due to severe vitamin D deficiency, the fetuses maintained completely normal blood calcium and phosphate levels and had fully mineralized skeletons at term, as determined by total weight, ash weight, and calcium content of femurs (29, 84, 284, 285, 311). Each of these studies is limited by the possibility that low levels of vitamin D might have reached the fetus.

Further evidence that 1,25-dihydroxyvitamin D is not needed for normal fetal calcium and bone homeostasis comes from the 1α-hydroxylase-deficient Hannover pig model, in which the fetuses of homozygous 1,25-dihydroxyvitamin D-deficient sows also maintained completely normal blood calcium and phosphate levels and fully mineralized their skeletons (79). Nephrectomy of fetal rats did not affect fetal blood calcium or phosphate levels when measured 48 h later, even though fetal 1,25-dihydroxyvitamin D levels fell (286). Also in fetuses of vitamin D-deficient rats, normal levels of calbindin$_{28K}$-D and calbindin$_{9K}$-D were found in the placenta, intestine, and other tissues (132, 311, 312). In addition, fetal mice that lack the gene encoding the receptor for 1,25-dihydroxyvitamin D are born with normal skeletons (313, 314).

Some data from humans lend support to the observation that 1,25-dihydroxyvitamin D is not needed for normal fetal calcium and bone metabolism. At term, the cord blood calcium and skeletal mineralization is completely normal in the offspring of vitamin D-deficient mothers (315–317). It is only in the first or second week after birth that hypocalcemia develops; skeletal demineralization and other rachitic changes are typically not detectable until 1 or 2 months of age (see *Section V.E*, below).

These observations of a minimal effect of vitamin D deficiency on fetal calcium and skeletal metabolism do not mean that 1,25-dihydroxyvitamin D is inactive or has no role in fetal life. In rats, the receptor for 1,25-dihydroxyvitamin D appears on day 13 of gestation in the mesenchyme that will subsequently condense to form the skeletal tissues, and by day 17 of gestation it is expressed in proliferating and hypertrophic chondrocytes, and osteoblasts of limb buds and the vertebral column (318). The widespread expression of the vitamin D receptor early in fetal skeletal development suggests an important role for its ligand in fetal bone development, but evidence for this postulated role has not yet been found. Further studies manipulated the 1,25-dihydroxyvitamin D level in fetal animals to test the role of this hormone. Infusion of antibody to 1,25-dihydroxyvitamin D decreased the ovine fetal blood calcium level (66). 1,25-Dihydroxyvitamin D given to pregnant guinea pigs increased the fetal calcium and phosphate levels (319). Bilateral nephrectomy in fetal sheep resulted in reduced ionized and total calcium and increased phosphate and PTH levels; these changes could be reversed by administration of 1,25-dihydroxyvitamin D to the fetus (295). Since these changes could be attributable to uremia and not loss of the renal 1α-hydroxylase enzyme, additional fetuses underwent bilateral ureteral sectioning alone. This surgical procedure allowed urine to drain into the fetal peritoneal cavity while retaining functional kidneys *in situ*. In these fetuses, ureteral sectioning had no effect on fetal calcium or calcitropic hormone levels. Thus, at least in the absence of normal renal function, 1,25-dihydroxyvitamin D may have a substantial influence on fetal mineral ion homeostasis.

1,25-Dihydroxyvitamin D does not readily cross the placenta in rats (320); consequently, circulating levels of 1,25-dihydroxyvitamin D in the fetus are largely derived from fetal sources. The fetal kidneys and placenta possess the 1α-hydroxylase enzyme and convert 25-hydroxyvitamin D to the active form (1, 25-dihydroxyvitamin D) (75, 76). The contribution of the fetal kidneys must be significant, since fetal nephrectomy reduced the fetal 1,25-dihydroxyvitamin D levels in sheep and rats (66, 286). Fetal blood levels of 1,25-dihydroxyvitamin D are typically lower than maternal levels in humans (37, 56, 304, 321), but umbilical artery levels of 1,25-dihydroxyvitamin D are slightly higher than umbilical venous levels, confirming the contribution of the fetal kidneys (37). 25-Hydroxyvitamin D levels have been found to be roughly equal to maternal levels (37, 56); this is not surprising since 25-hydroxyvitamin D readily crosses the placenta in rats (322). Levels of 24,25-dihydroxyvitamin D correlate with, but are typically lower than, maternal levels at term in humans (283, 307, 321).

In summary, evidence from animal models indicates that deficiency of 1,25-dihydroxyvitamin D impairs neither fetal skeletal formation and calcification nor the ability of the fetus to maintain a normal blood calcium. Although these data suggest a limited role for 1,25-dihydroxyvitamin D in the fetus, fetal production of 1,25-dihydroxyvitamin D and the vitamin D receptor mandate a continued search for fetal roles for 1,25-dihydroxyvitamin D. 25-Hydroxyvitamin D readily

crosses the placenta and can be 1α-hydroxylated by the fetal kidneys. However, 1,25-dihydroxyvitamin D does not cross the placenta, and fetal blood levels of 1,25-dihydroxyvitamin D are low.

5. Calcitonin. Immunoreactive calcitonin can be detected in human fetal thyroid glands from as early as the 15th week of gestation (323), and fetal calcitonin levels are maintained at higher levels than maternal (35, 37, 86, 88, 89, 269, 273, 304, 324). Maternal calcitonin cannot cross the placenta (325). The increased fetal levels of calcitonin are thought to reflect increased synthesis, but the metabolism and clearance of calcitonin have not been studied in fetal animals.

Several acute experimental perturbations suggest a role for calcitonin in fetal calcium homeostasis. Infusion of calcitonin antiserum to fetal rats at day 21.5 of gestation slightly increased the fetal blood calcium 1 h later (326), while fetal injection of calcitonin caused hypocalcemia and hypophosphatemia (327). However, fetal thyroidectomy with subsequent T_4 replacement did not affect the fetal blood calcium in sheep, indicating that fetal thyroidal C cells alone may not affect the regulation of the blood calcium level (298). Therefore, the precise role of calcitonin in fetal calcium homeostasis and skeletal metabolism has not yet been established.

6. PTHrP. Studies of PTH bioactivity in human umbilical cord blood (as determined by an *in vitro* cytochemical bioassay) found high PTH-like bioactivity, while immunoreactive PTH was simultaneously found to be undetectable or low (38, 303, 328). Subsequently, it has been recognized that human cord blood PTHrP levels are significantly higher than the simultaneous maternal levels at term (43, 310). When both PTH 1–84 and PTHrP 1–86 were simultaneously measured by two-site immunoradiometric assays [and expressed in equivalent units (picomoles/liter)], human cord blood PTHrP levels were 2–4 pmol/liter, up to 15-fold higher than the levels of PTH (0.2–0.5 pmol/liter) (43, 110, 124). It has yet to be confirmed that PTHrP accounts for the high PTH-like bioactivity in human cord blood; however, studies in fetal pigs (329) and sheep (116, 330) found that the levels of PTHrP and PTH-like bioactivity were tightly correlated in late gestation and the neonatal period.

As noted earlier (*Section II.B.6*), PTHrP may be a prohormone that is processed into separate circulating fragments, each of which may have different functional roles and receptors (100). Although the structures of these fragments have been deduced from studies of tumor cell lines transfected with the PTHrP gene, it has yet to be determined which of these fragments normally circulate in fetal life. Full-length PTHrP has twice the molecular weight of PTH; since PTH cannot cross the placenta, PTHrP probably does not either. PTHrP 1–86 did not cross the placentas of sheep and goats (113); however, the possibility that smaller, biologically active fragments of PTHrP might cross the placenta has not been evaluated.

PTHrP is produced in many sites throughout the developing embryo and fetus, including the fetal parathyroid glands (116, 331), skeletal growth plate (332, 333), trophoblast cells of the placenta (114, 331), amnion (106, 334), chorion (334), umbilical cord (115), and many other organs. All of these sites may contribute to the circulating level of PTHrP in the fetus and may thereby be relevant to fetal calcium and bone metabolism. Since venous umbilical PTHrP levels were higher than umbilical arterial levels in pigs, the placenta may be an important source of systemically circulating PTHrP in the fetus (329). Due to local production of PTHrP by the umbilical cord (115), the level of PTHrP in cord blood might not accurately reflect the systemic level of PTHrP, but this has not been tested.

PTHrP has multiple possible roles during embryonic and fetal development (335). PTHrP gene-ablated mice have abnormalities of chondrocyte differentiation (336) and aberrant breast development (337). PTHrP may also be an important regulator of the fetal blood calcium. PTHrP levels correlate with the fetal ionized calcium levels in pigs (329). In genetically engineered mice, homozygous ablation of the PTHrP gene results in a fetal blood calcium no higher than that of the mother (299). In sheep, fetal parathyroidectomy causes hypocalcemia that can be reversed by PTH or PTHrP infusion (297, 298, 338). Since PTH normally circulates at low or undetectable levels in the fetus near term (*Section III.B.3*, above), it is possible that the hypocalcemic effect of fetal parathyroidectomy is at least partly due to the loss of PTHrP produced by the parathyroids. In the next section (*III.C*), the unique role of PTHrP in stimulating placental calcium transport will be discussed.

In summary, PTHrP is produced by diverse fetal tissues and circulates in fetal blood at levels higher than adult levels. PTHrP appears to regulate the fetal blood calcium as well as fetal-placental calcium transport.

C. Fetal-placental calcium transport

Calcium is actively transported across the placenta (339, 340). The site of active transport is likely at the fetus-facing basement membrane of the syncytiotrophoblast cells in the human and at the trophoblast cells and the basal surface of the endoderm of the intraplacental yolk sac in rodents (341, 342). The active transport of calcium may be mediated by a Ca^{2+}-ATPase present at these same sites (339, 342). This enzyme's activity can be inhibited by dinitrophenol, ouabain, quercetin, and antibody to the human erythrocyte plasma membrane calcium pump (339, 342). Calcium-binding proteins in the placenta and yolk sac are also thought to be involved in active placental calcium transport. The placental calbindin$_{9K}$-D mRNA and protein levels increase over the last week of gestation in rats (129, 343) and mice (130, 133) and are unaffected by maternal vitamin D deficiency (132, 311). Transplacental transport of calcium is generally found to be a one-way process, *i.e.*, fetal-to-maternal flow of calcium is typically less than 1% of the forward (maternal-to-fetal) flow (344, 345). In rhesus monkeys, backflux was reported to be 80% of the forward flow (345); it is not certain whether this represented a true species difference or methodological differences. It has not been determined when active transport of calcium begins in gestation, due to technical difficulties involved in studying placental physiology early in gestation. However, active transport of calcium must be underway by the third trimester in humans, which is the time of rapid skeletal mineralization and peak fetal calcium requirement.

1. Maternal hormones. Maternal hormones might influence the fetal-placental calcium transport process by raising or lowering the ambient maternal calcium level, and by direct effects on the placenta. However, several lines of evidence from animal experiments indicate that fetal-placental calcium transport and net maternal-fetal calcium transfer are maintained relatively independently of maternal hypocalcemia or hormone deficiencies. In pregnant sheep, maternal hypocalcemia due to parathyroidectomy or dietary calcium restriction did not affect the rate of fetal-placental calcium transfer as directly assessed in placental perfusion experiments (297, 346); in addition, the fetal blood calcium, phosphate, PTH, and 1,25-dihydroxyvitamin D levels were all unchanged by maternal hypocalcemia (284, 347). The finding of a "normal" rate of calcium transfer across the placenta indicates that the fetal-placental unit must be working harder to extract the normal amount of calcium from a reduced amount of maternal calcium presented to the placenta. Indeed, the following observation from intact fetal rats confirmed that the rate of placental calcium transfer is up-regulated in response to parathyroidectomy-related chronic maternal hypocalcemia. In this experiment, a maternal calcium infusion caused a marked, acute rise in the blood calcium of fetuses from parathyroidectomized rats, but had no effect on the fetuses of normal rats (348). Such an up-regulation in placental calcium transfer may compensate for a low ambient maternal blood calcium and permit a normal amount of calcium to be transferred by the end of gestation.

Several additional studies have examined only indirectly the effect of other maternal hormone deficiencies on placental calcium transfer. In these studies, net fetal accumulation of calcium at term was used as an index of placental calcium transfer during pregnancy in vitamin D-deficient rats (311), thyroidectomized, T_4-supplemented ("calcitonin-deficient") sheep (349, 350), and in sheep that received daily administration of PRL and/or bromocriptine (351). Since placental calcium transport was not directly assessed in these studies, conclusions cannot be drawn about the effect of maternal vitamin D, calcitonin, and PRL deficiency on placental calcium transport.

2. Fetal hormones. The role of 1,25-dihydroxyvitamin D in fetal-placental calcium transport has not been thoroughly studied. Receptors for 1,25-dihydroxyvitamin D are present in the placentas of humans, rats, and sheep and might, therefore, have a role in placental calcium physiology (352–355). In placental perfusion models in rats, guinea pigs, and sheep, pharmacological doses of 1,25-dihydroxyvitamin D or 1α-cholecalciferol increased the fetal blood calcium, transport of calcium across the placenta, and mineral content of ashed fetuses (319, 356, 357). Also, prior nephrectomy of fetal sheep reduced calcium transfer in the placental perfusion model, and this effect could be partly restored by administering 1,25-dihydroxyvitamin D (339). Thus, the evidence indicates a possible role for 1,25-dihydroxyvitamin D in fetal calcium homeostasis, but this role is not yet well defined.

The role of fetal calcitonin is even less well established. In intact fetal sheep, calcitonin has been found to reduce the PTHrP-mediated increases in the apparent rate of calcium transfer (349, 358). In contrast, fetal thyroidectomy with subsequent T_4 replacement did not alter placental calcium transfer in sheep, indicating that loss of fetal calcitonin does not perturb the placental calcium pump (298).

The role of the parathyroid gland in fetal calcium metabolism has been extensively studied. Fetal thyroparathyroidectomy in sheep and fetal decapitation in rats resulted in a lower fetal blood calcium, such that the maternal-fetal calcium gradient was obliterated (297, 298, 359). In addition, when the thyroparathyroidectomized sheep or rat fetuses were removed so that the placentas could be artificially perfused *in situ*, active transport of calcium across these experimentally perfused placentas was found to be reduced (297, 298, 359, 360). These findings indicate that the parathyroid glands have a critical role in maintaining the fetal blood calcium and the active transport of calcium across the placenta. Infusion of autologous blood from fetuses with intact parathyroid glands restored calcium transport across the perfused placentas of thyroparathyroidectomized fetal sheep (298). However, PTH failed to restore the active transport of calcium in fetal sheep or rats under these conditions (338, 359, 361).

The placenta may be able to transport calcium actively to some extent even in the absence of fetal parathyroid glands. When rat dam and fetus were both thyroparathyroidectomized, the fetus maintained a higher blood calcium level than the dam (286, 287). Although calcium transport was not measured, the fact that relative fetal hypercalcemia was maintained may indicate that some capability for active transport of calcium persists after fetal parathyroidectomy.

In the studies of thyroparathyroidectomized fetal sheep, synthetic PTHrP molecules of amino acid lengths 1–141, 1–86, and 67–86 were found to stimulate placental calcium transport in the experimentally perfused placentas (338, 361–363). These results suggested that PTHrP, perhaps produced by the parathyroid glands, stimulates active transport of calcium across the placenta. In contrast, PTHrP 1–34, which contains only the PTH-like amino-terminus, failed (like PTH) to stimulate calcium transport in this model. Studies in genetically engineered mice support the hypothesis that PTHrP stimulates placental calcium transport. A reduction in blood calcium to the maternal level and reduced placental transfer of calcium has been found in homozygous PTHrP-gene knockout fetal mice (299). The placental transfer of calcium was acutely increased in the homozygous fetuses by treatment with PTHrP 1–86 or PTHrP 67–86, but not by PTHrP 1–34 or intact PTH (299). These data suggest that PTHrP increases placental calcium transport by acting through a receptor distinct from the PTH/PTHrP receptor, since the PTH/PTHrP receptor is stimulated equally by amino-terminal PTH and PTHrP. This hypothesis is further supported by the studies of fetal mice homozygous for deletion of the PTH/PTHrP receptor gene. These mice are hypocalcemic, but placental calcium transport in these fetuses is increased (299).

These data on the effects of PTHrP in parathyroidectomized fetal sheep and genetically engineered mice are not supported by the following observations in fetal rats. In the perfused placentas obtained from intact or decapitated fetal rats, active transport of calcium was found to be stimulated slightly by amino-terminal PTH or PTHrP, but not by frag-

ments of PTHrP that do not contain the amino terminus (359, 360). This could reflect a true species difference or methodological differences.

Taken together, the studies in genetic knockout mice, and those in thyroparathyroidectomized fetal sheep, suggest that in fetal life, PTHrP is necessary to maintain the normal fetal hypercalcemia and at least part of the active transport of calcium across the placenta. This transport is regulated in part by a midmolecular portion of PTHrP acting on a novel (as yet uncloned) receptor distinct from the PTH/PTHrP receptor. Recent evidence suggests that the structure of this midmolecular fragment of PTHrP may encompass amino acids 38–94 (100, 364), but this has yet to be confirmed by RIAs of fetal blood. The studies in sheep suggested that the parathyroid glands secrete the PTHrP that controls placental calcium transport; however, direct evidence to support this hypothesis is not yet available. Finally, the physiological importance of the actions of fetal 1,25-dihydroxyvitamin D and calcitonin require further exploration.

D. Renal handling of calcium and the amniotic fluid

The fetal kidneys may play a role in regulating the fetal blood calcium level, by adjusting the relative reabsorption and excretion of calcium and phosphate by the renal tubules in response to the filtered load and other factors, such as PTHrP and/or PTH. The fetal kidneys may also participate by synthesizing 1,25-dihydroxyvitamin D. However, little hard data are available on fetal kidney function and its relative importance in regulating the fetal blood calcium. As noted previously, nephrectomy in fetal lambs resulted in hypocalcemia, hyperphosphatemia, and reduced placental calcium transfer; these effects were attributed to loss of renal production of 1,25-dihydroxyvitamin D (295, 339). The fetal renal tubular function may be under the control of PTHrP or PTH in fetal life, since thyroparathyroidectomy in fetal sheep resulted in an increase in fractional excretion of calcium by the fetal kidneys and reduced phosphate excretion (365, 366). These effects were reversed by treatment with amino-terminal fragments of either PTH or PTHrP (365, 366). Thyroparathyroidectomy also reduced placental calcium transport in fetal sheep (*Section III.C.*, above); therefore, the hypocalcemia in thyroparathyroidectomized fetuses likely results from the combined effects of reduced influx of calcium across the placenta, increased excretion of calcium by the fetal kidneys, and possible effects of the loss of PTHrP and/or PTH on the fetal skeleton and renal 1α-hydroxylase.

In fetal life, renal excretion of calcium does not necessarily represent a permanent loss of calcium for the fetus. Fetal urine excretion is probably the major source of fluid and solute in the amniotic fluid, while fetal swallowing is likely the major pathway for clearance of amniotic fluid and is a pathway by which excreted calcium can be made available again to the fetus (367). The volume and composition of amniotic fluid have been used as indirect measures of fetal renal function. The amniotic fluid ionized calcium level has been found to be constant between 14–15 weeks of gestation and term in humans, while the total calcium and phosphate levels decline over the same interval (13). This may indicate that renal excretion of calcium is equally balanced by fetal swallowing and intestinal reabsorption of calcium. However, other sources may contribute to the amniotic fluid, including secretions from the respiratory tract, and exchange of fluid and/or solute across the fetal skin, fetal membranes (amnion, chorion and chorionic plate), and umbilical cord (367). Little is known about the relative contribution of these sites to the volume and composition of the amniotic fluid, and hence uncertainty remains about how accurately the amniotic fluid composition reflects renal function. Nevertheless, amniotic fluid represents a pathway by which excreted calcium may be recirculated to the fetus.

E. Skeletal calcium metabolism

The fetal skeleton may well have two interdependent roles — substantial growth during late fetal life and participation in fetal calcium homeostasis. Several lines of evidence indicate that the fetal skeleton participates in fetal calcium homeostasis and that skeletal calcium may be mobilized in response to reduced transfer of calcium from mother to fetus. Maternal hypocalcemia due to thyroparathyroidectomy or calcitonin infusion increased the basal level of bone resorption in subsequently cultured fetal rat bones (368, 369). These effects were blocked by prior fetal decapitation, which is thought to mimic the effects of thyroparathyroidectomy (368, 369). Further, the fetal parathyroid glands enlarge in response to maternal hypocalcemia in the rat (47, 370), and fetal femur length and mineral ash content are subsequently reduced (53). Additional, recent observations in genetically engineered mice suggest a role for the skeleton in fetal calcium homeostasis. The ionized calcium of fetal mice that lack the PTH/PTHrP receptor gene is lower than that of fetal mice that lack the PTHrP gene, despite the fact that placental calcium transport is supranormal in PTH/PTHrP receptor knockout fetuses and subnormal in PTHrP knockout fetuses (299). Lack of bone responsiveness to amino-terminal PTH and PTHrP may well, therefore, contribute to the hypocalcemia in mice without PTH/PTHrP receptors.

Intact fetal parathyroid glands are needed for normal skeletal development, since fetal thyroparathyroidectomy in sheep caused decreased ash content and rachitic changes in the fetal skeleton by term (371, 372). These effects could be partly reversed or prevented by fetal calcium and phosphate infusions; thus, much of the effect of fetal parathyroidectomy was caused by a decrease in blood levels of calcium and phosphate (372). However, although bone formation parameters were corrected by the calcium and phosphate infusion, bone resorption parameters remained abnormal (reduced resorption cavities, reduced osteoclast numbers). Therefore, functioning fetal parathyroids (and, therefore, parathyroid gland-produced PTH and/or PTHrP) are required for normal fetal bone resorption and mineralization.

PTHrP is clearly important for growth plate development, because absence of PTHrP (in the PTHrP gene-knockout mouse) results in a chondrodysplasia characterized by accelerated differentiation of growth cartilage and adjacent endochondral bone (336). This effect of PTHrP must be mediated by the PTH/PTHrP receptor, because mice homozygous for the absence of the PTH/PTHrP receptor have a similar skeletal phenotype (373). However, these gene

knockout experiments could not determine whether systemically delivered PTHrP adds to the likely role of PTHrP produced in the growth plate itself.

In summary, normal mineralization of the fetal skeleton requires intact fetal parathyroid glands and adequate delivery of calcium to the fetal circulation. The fetal skeleton can participate in the regulation of fetal calcium homeostasis, probably through actions of PTHrP and/or PTH. In addition to its effects on regulating placental calcium transport, PTHrP is required for normal skeletal development. Evidence from experimental vitamin D deficiency and the vitamin D receptor knockout indicates that 1,25-dihydroxyvitamin D may not be required by the developing skeleton.

F. Fetal response to maternal hyper- or hypoparathyroidism

As discussed earlier (*Section II.F*), in humans, maternal hypercalcemia due to primary hyperparathyroidism may suppress the fetal parathyroid glands, since hypocalcemia can be present at birth (225, 235). The fetal parathyroid glands may also be suppressed when the mother has hypercalcemia due to familial hypocalciuric hypercalcemia (241–243, 374).

On the other hand, maternal hypoparathyroidism in human pregnancy has been associated with the development of intrauterine, fetal hyperparathyroidism. This condition 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, and low birth weight (246, 375–378). Spontaneous abortion, stillbirth, and neonatal death have also been associated with this condition (379–381). Similar skeletal findings have been reported in the fetuses and neonates of women with pseudohypoparathyroidism (262, 263), renal tubular acidosis (382), and chronic renal failure (383). Although these skeletal changes have been interpreted to indicate fetal hyperparathyroidism, no serum measurements of intact PTH (or PTHrP) have been reported for this condition, and the serum calcium level has been generally reported to be normal.

G. Integrated fetal calcium homeostasis

Previous sections have demonstrated that the fetus maintains a blood calcium higher than the maternal level, and that the placenta and bone, and perhaps fetal kidney and intestine, transport calcium into and out of the bloodstream. However, the need for fetal hypercalcemia and the mechanisms by which it is maintained are not fully understood.

The need for an increased fetal blood calcium level is uncertain. It may well not be necessary for normal accretion of calcium by the developing skeleton, since, despite obliteration of the normal fetal-maternal calcium gradient (299), homozygous PTHrP-null fetal mice do not have a deficit in skeletal mineral content, as assessed by alizarin red staining and ash mineral content (336, 384).

In adult life, the CaSRs on the parathyroid glands and kidneys set the ambient serum calcium level, mainly by controlling the synthesis and secretion of PTH by the parathyroid glands and regulating renal tubular handling of calcium (385, 386). Inactivating or loss-of-function mutations of this receptor raise the set point for PTH secretion and increase renal calcium retention; these actions lead to hypercalcemia (385, 386). If the parathyroid gland CaSR were responsible for the elevated fetal calcium level, the set point would have to be different in fetal life, and one would expect to find increased or inappropriately normal PTH levels in the presence of the increased fetal calcium level. Instead, PTH in humans is normally low or undetectable at term (*Section V.B.3*), and the fetal blood calcium and PTH level are negatively correlated (387). Therefore, it is likely that neither the parathyroid CaSR nor PTH is responsible for the high fetal blood calcium; instead, the normally functioning CaSR suppresses the parathyroids in response to a high fetal calcium that is maintained by other processes. Further evidence that factors other than PTH derived from the parathyroids have a role in regulating the fetal blood calcium come from studies of thyroparathyroidectomized rat fetuses, which maintain a higher blood calcium than their simultaneously thyroparathyroidectomized mothers (286, 287). Such factors might include PTHrP (derived from the placenta, parathyroids, and other tissues), among other factors that might still be undiscovered.

That PTHrP has a role in maintaining the fetal blood calcium is suggested by the finding of a reduction in the fetal calcium to a level equal to that of the mother, in the PTHrP gene knockout fetuses (299). PTH levels are sharply increased in the PTHrP-null fetus (388), indicating that, in the absence of PTHrP, the parathyroids respond to regulate the fetal calcium level. Indeed, other evidence indicates that eliminating the amino-terminal actions of PTH, in addition to those of PTHrP, has a greater effect on reducing the fetal blood calcium than removing the PTHrP gene. The ionized calcium level of PTH/PTHrP receptor-null fetuses is *lower* than the ambient maternal level, despite the presence of a supranormal rate of placental calcium transfer (299).

Since the ionized calcium level of PTHrP-null fetuses is not higher than the maternal calcium level, this might indicate that PTH cannot make up for lack of PTHrP (including the effect of PTHrP to stimulate placental calcium transport); alternatively, in the absence of PTHrP, the fetal parathyroid CaSR may then control the regulation of the blood calcium, by stimulating PTH and setting the ionized calcium at the normal adult level. Further work is needed to determine whether either of these proposed mechanisms is correct.

Thus, an integrated model of normal fetal calcium homeostasis proposes that the fetal blood calcium is set at a level higher than maternal through the actions of PTHrP (among other potential factors). The parathyroid CaSR responds appropriately to this increased level of calcium and suppresses the parathyroids. 1,25-Dihydroxyvitamin D synthesis and secretion are, in turn, suppressed due to the effects of low PTH and high blood calcium and phosphate. PTHrP may be autonomously produced by the placenta and other tissues, or its production may be regulated. This model further proposes that the fetal blood calcium is maintained not only by flux of calcium across the placenta from the mother, but by contributions from fetal skeleton and kidney.

This tentative model needs to be tested further, and the physiological benefit of fetal hypercalcemia requires clarification.

H. Summary

The fetal-placental unit has adapted to rapidly extract calcium from the maternal blood stream in sufficient amounts to mineralize the fetal skeleton in late gestation. Fetal blood calcium and active transport of calcium across the placenta are regulated relatively independently of the levels of maternal calcium and calcitropic hormones. The fetus has a higher blood calcium than the ambient maternal calcium level. PTH and 1,25-dihydroxyvitamin D, the traditional calcitropic hormones, are present at low levels in the fetal circulation and may have a limited role in fetal calcium physiology. Calcitonin levels are elevated in the fetal circulation, but the role of calcitonin in fetal calcium homeostasis is uncertain. PTHrP is critical for maintaining normal fetal hypercalcemia and active transport of calcium across the placenta, although it is likely that other factors regulate placental calcium transport, as well. A midmolecular form of PTHrP stimulates placental calcium transport, through actions on a receptor that is distinct from the cloned PTH/PTHrP receptor.

The fetal-placental unit has a remarkable ability to meet its needs irrespective of maternal calcium or vitamin D levels. However, maternal hypercalcemia due to primary hyperparathyroidism or familial benign hypercalcemia can suppress the fetal parathyroid glands. In turn, maternal hypocalcemia due to hypoparathyroidism and pseudohypoparathyroidism (and other causes) can cause fetal parathyroid gland enlargement and increased resorption in the fetal skeleton.

The fetus does not set a calcium gradient against the maternal circulation; instead, the fetus sets its blood calcium at a particular level independently of the maternal value. The physiological role of this relative hypercalcemia is uncertain. The normal setting of this level requires the presence of a normal calcium-sensing receptor. The fetal blood calcium is not simply determined by the influx of calcium across the placenta. Rather, other important factors include fluxes of calcium in and out of the developing skeleton, and (probably to a lesser extent) renal tubular reabsorption and excretion of calcium, and reabsorption of calcium from swallowed amniotic fluid.

IV. Maternal Physiology and Pathophysiology During Lactation

A. Maternal adaptive goals during lactation

Albright and Reifenstein (1) reported that maternal losses of calcium during 9 months of lactation are 4-fold higher than the losses occurring during pregnancy. More specifically, the typical daily loss of calcium in breast milk has been estimated to range from 280–400 mg (389, 390), although daily losses as great as 1000 mg calcium have been reported (391). Accurate estimates of the calcium content of breast milk are complicated because the content varies within and between feedings (392, 393), between breasts of the same person (393), and among different mothers and ethnic groups (392). Although the calcium content of milk is lower at 6 months compared with 3 months postpartum (390, 392, 394, 395), the volume of milk produced at 6 months tends to be greater (390), and therefore the daily maternal loss of calcium may be greater when lactation extends to 6 months and beyond (390).

In a classic study in 1931, Donelson *et al*. (396) carefully measured dietary calcium intake and losses of calcium in feces, urine, and breast milk in a group of lactating women who exclusively pumped breast milk and did not permit their infants to suckle. Despite adequate vitamin D and calcium intake (in the form of cod liver oil and yeast), calcium balance was negative in these women throughout lactation (396). A similar study of three lactating women found a negative calcium balance (net loss of 1 g calcium) during the interval of greatest milk production, despite ingestion of supplemental calcium and phosphorus; only during the time of lessened milk production (weaning) did the calcium balance return to normal (391).

To compensate for the calcium requirements of lactation, the maternal adaptations could, in theory, include increased intestinal absorption of calcium, renal conservation of calcium, and increased resorption of calcium from the skeleton. In fact, the studies reviewed in this section indicate that a temporary demineralization of the skeleton is the main mechanism by which lactating humans and animals meet these calcium requirements. This demineralization does not appear 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.

B. Mineral ions and calcitropic hormones

The changes that occur in maternal calcium, phosphate, and calcitropic hormone levels during lactation, weaning, and postweaning are schematically depicted in Fig. 2.

1. Calcium. From the earliest measurements of total calcium to the newer reports of ionized calcium determinations in lactation, the blood calcium of lactating humans has been found to be normal or slightly increased (7, 397–399). More recently, with larger sample sizes, it has been shown that the mean ionized calcium level of exclusively lactating women is higher than that of normal controls (400, 401). Also, mothers nursing twins have been found to have significantly higher total calcium levels than mothers nursing singletons (402). Furthermore, occasionally substantial hypercalcemia may develop during lactation and resolve only at weaning (403).

In contrast, the data from lactating rats are conflicting: two reports found lactating rats to be slightly hypocalcemic compared with nonlactating controls (50, 404); another found normal calcium levels that could be decreased by feeding a low calcium diet (405), while a fourth found mild hypercalcemia (406). More intensive lactation (as determined by the relative rate of weight gain in the pups of a litter or by the number of pups in a litter) correlated with a lower maternal serum calcium (22, 50, 407, 408). Deer have also been found to have higher corrected serum calcium levels during lactation than after weaning (23). With abrupt weaning, the serum calcium of lactating rats typically rebounds into the hypercalcemic range (81, 409).

2. Phosphate. Serum phosphate levels are typically higher during lactation in humans, and, in some cases, exceed the

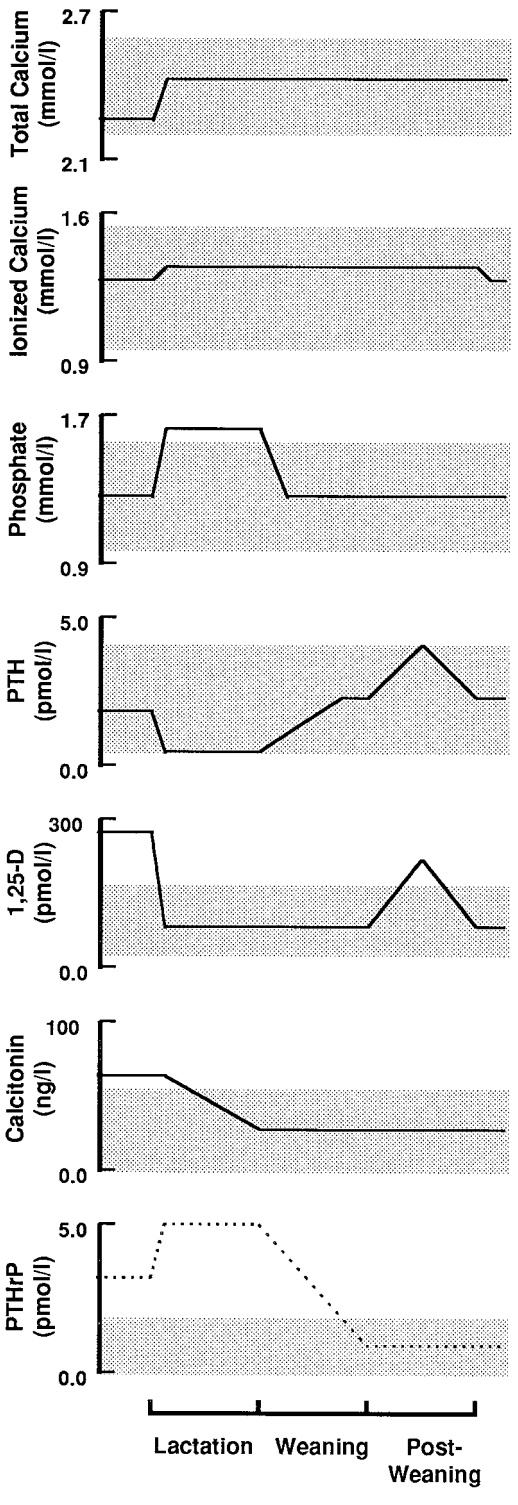

FIG. 2. Schematic illustration of the longitudinal changes in calcium, phosphate, and calcitropic hormone levels that occur during lactation and postweaning in humans. Normal adult ranges are indicated by the *shaded areas*. Data have been compiled from the following sources: total calcium (7, 397–399), ionized calcium (400, 401), phosphate (14, 398, 401, 410–412), PTH (27, 160, 398–401, 410, 411), 1,25-dihydroxyvitamin D (27, 58, 160, 397, 398, 410, 414), calcitonin (14, 397, 402, 420), and PTHrP (399–401, 411, 435). The progression in PTHrP levels has been depicted by a *dashed line* to reflect that the data are less complete.

normal range (14, 28, 398, 401, 410–412); similar results have been reported for lactating rats (50) and deer (23). Reabsorption of phosphate by the kidneys may be increased, although few measurements of tubular reabsorption of phosphate have been made (27, 160, 410, 411), and the data are conflicting. 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 (*Sections IV.E.1* and *IV.E.2*, below) in the setting of decreased renal phosphate excretion (*Section IV.D*, below).

3. PTH. Lactation had been described as a state of secondary hyperparathyroidism by Albright and Reifenstein (1) and, as with pregnancy, early PTH assay results appeared to confirm this hypothesis (413) [although some early assays reported normal levels (30, 55, 397, 414)]. However, intact PTH, as determined by a two-site IRMA, has been found to be reduced 50% or more in lactating women in both cross-sectional (399, 401) and longitudinal studies (27, 160, 398, 400, 411, 415–417). The intact PTH level remains low compared with that of nonlactating postpartum women, rising to normal (160), or above normal (27, 398, 410) after weaning. This postweaning increase in intact PTH level may be sustained for 2–3 months (27, 398). This elevation corresponds to the time when bone mineral is restored to the skeleton (see discussion on bone density during lactation, *Section IV.E.2*, below) (27, 410).

PTH, measured with a two-site IRMA, rises during lactation in rats (45, 49, 50). Under the stress of a low calcium diet, urine cAMP levels sharply increase (405), and PTH levels (N-terminal assay) rise even higher (21, 50). Similar effects have been seen in dams nursing larger litters (50, 408). However, a functioning parathyroid gland is not necessary to provide calcium to the milk of the lactating rat (418). After weaning in rats, PTH levels were found to fall within 6 h (as determined by an N-terminal assay) and were normal by 24–48 h (21).

These data suggest that PTH levels are likely subnormal or normal during lactation in humans; the original hypothesis of hyperparathyroidism in lactation has not been substantiated. The metabolism of lactating rats, on the other hand, may represent a functionally hyperparathyroid state, in response to a fall in maternal blood calcium during lactation.

4. 1,25-Dihydroxyvitamin D. In contrast to the high 1,25-dihydroxyvitamin D levels of pregnancy that contribute to the doubling of intestinal calcium absorption, within days of parturition, maternal free and bound 1,25-dihydroxyvitamin D levels fall to normal (20, 55, 58, 73, 160). The levels remain normal throughout lactation and postweaning (27, 58, 160, 397, 398, 410, 414, 417), although one study reported that the levels rose above the normal range when lactation continued beyond 6 months (414), and another found an elevation after weaning (398). Women nursing twins had higher 1,25-dihydroxyvitamin D levels than women nursing singletons (402). Serum 25-hydroxyvitamin D levels are typically unchanged by lactation (410).

In contrast to humans, lactating rats have elevated levels of 1,25-dihydroxyvitamin D (22, 81), and more intense lactation correlated with higher levels (22, 407). The lactational

rise in 1,25-dihydroxyvitamin D seen in rats was prevented by a small increase in the calcium content of the diet and was exacerbated by a low-calcium diet (419). Parathyroidectomy in the lactating rat caused a 70% decrease in the level of 1,25-dihydroxyvitamin D (81). Therefore, 1,25-dihydroxyvitamin D levels are elevated in rats in response to a fall in blood ionized calcium and a rise in serum PTH.

5. Calcitonin. In lactating humans, high calcitonin levels that do not vary with suckling have been reported (14, 420), while normal levels 6 weeks to 6 months postpartum have been found by others (397, 402, 417). In these reports the serum calcium was not different between lactating women and controls. Nursing of twins resulted in higher calcitonin and corrected serum calcium levels compared with nursing of singletons, although the levels remained in the normal range (402). Calcitonin is secreted into breast milk at concentrations 45 times that of maternal plasma (93); its functional role within the breast is unknown. Since a rise in serum calcitonin during lactation persists in totally thyroidectomized women, the breast may be an important extrathyroidal source of calcitonin during lactation (93).

In sheep, despite constancy of serum calcium levels, calcitonin levels fall to nonpregnant levels at parturition, rise over the period of lactation, and fall again at weaning (324). A similar rise in serum calcitonin has been found in deer, which also manifest a mild increase in the corrected serum calcium during lactation (23). In contrast, the data from lactating rats are conflicting. Investigators have reported low calcitonin levels in the presence of mild lactational hypercalcemia (406), while others have found high calcitonin levels until weaning in the presence of a normal or low serum calcium (421).

In the "calcitonin-deficiency" model previously described (*Section II.B.5*), rats made calcitonin-deficient during pregnancy lost more mineral content of their femurs than normal after 3 weeks of lactation (96, 97). Similarly, calcitonin-deficient goats fed a calcium-deficient diet lost more bone mineral by day 60 of lactation than control animals (77). Lactating, thyroparathyroidectomized rats had a prompt (1.8 mg/dl) rise in blood calcium after eating, while lactating control (intact) rats had no change in blood calcium (422). These findings indicate that in some species, during lactation, calcitonin protects the maternal skeleton from excessive resorption and regulates the maternal blood calcium level, particularly in response to meals. The relevance of these findings to humans has not been determined.

6. PTHrP. PTHrP has been detected in breast milk of humans and other animals at concentrations exceeding 10,000 times the level in the blood of patients with hypercalcemia of malignancy or normal human controls (110, 117). The primary role of PTHrP in the mammary glands or milk is not clear. A paracrine action of PTHrP in mammary tissue has been suggested because PTHrP concentrations in the milk are positively correlated with total milk calcium content of the human (423) and cow (424) [although no such correlations were found in rats (118, 425)], and administration of bromocriptine reduces both the PTHrP and calcium level in the milk of goats (426). However, a direct effect of PTHrP on the transport of calcium into the breast and breast milk has not been established. PTHrP has been found to have vasodilatory effects on mammary vessels and, therefore, may regulate mammary blood flow (427, 428). PTHrP has also been shown to have an essential role in mammary development (337).

PTHrP immunoreactivity in milk and PTHrP mRNA levels in mammary tissue have been observed to rise over the first few days postpartum in rats (118). Suckling induces PTHrP (mRNA and protein) locally in rat mammary glands (425, 429), and this response appears to be mediated by PRL and not oxytocin (429) and is blocked by bromocriptine (429). Milking a mammary gland in goats caused a marked increase in the PTHrP concentration in milk from that gland, but not in the milk of the contralateral (unstimulated) mammary gland (430). This result suggests that the synthesis and/or secretion of PTHrP by the mammary glands is under the control of local factors rather than the systemic level of PRL alone (430). Further, in rats, PTHrP is higher in milk at day 21 of lactation, after the PRL level has fallen, indicating that factors other than PRL may stimulate continued production of PTHrP by the mammary glands (431).

Studies in lactating rats have found that suckling induces phosphaturia and an increase in nephrogenous cAMP, which persists in parathyroidectomized, lactating rats (432). This result suggests that PTHrP (but not PTH) is released into the maternal circulation in physiologically relevant amounts in response to suckling. A similar study in cows found a milking-induced phosphaturia that could be blocked by $(Tyr)^{34}$-bovine PTH(7–34)-NH_2, a PTH/PTHrP receptor antagonist (433). Further, a significant venous-arterial concentration gradient of PTHrP was found across the mammary gland of the goat (426), consistent with PTHrP reaching the maternal circulation from the mammary glands. However, chronic, passive immunization of the lactating mouse with antibodies to amino-terminal PTHrP, sufficient to eliminate PTHrP bioactivity in the breast tissue, failed to affect maternal calcium or phosphate levels (434). Thus, PTHrP is not the sole determinant of mineral homeostasis in lactating rats. Perhaps PTH, which is elevated in lactating rats (*Section IV.B.3*), can substitute for PTHrP in this circumstance.

Although one less sensitive single-site assay found normal levels of PTHrP in maternal blood during human lactation (110), studies that used a more sensitive RIA, or two-site IRMAs, have found PTHrP levels to be significantly higher than in nonpregnant controls (399–401, 411, 435). In addition, a small rise in the systemic level of PTHrP can be demonstrated after suckling (400, 436). PTHrP was not elevated in the first 3 days postpartum, at which time lactation is not fully established (437); PTHrP immunoreactivity rises steadily in the breast milk over the first few days postpartum and subsequently declines as lactation wanes (400, 431).

Amino-terminal fragments of PTHrP and PTH are equipotent in activating the human PTH/PTHrP receptor (104), and it is possible that fluctuations in secretion of PTHrP into the maternal circulation from the lactating breast cause resorption of calcium from the maternal skeleton, renal tubular resorption of calcium, and suppression of PTH. In support of this hypothesis, PTHrP levels have been found to correlate negatively with PTH levels and positively with the ionized calcium levels of lactating women (400, 401). Also, PTHrP

levels correlate with loss of bone mineral density during lactation in humans (435). Furthermore, aparathyroid or hypoparathyroid women have been found, while lactating, to be able to activate the 1α-hydroxylase enzyme to synthesize low-to-normal levels of 1,25-dihydroxyvitamin D and maintain normal serum calcium levels while not receiving supplemental calcitriol (244, 245, 258). 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. This consistent finding has resulted in the recommendation that calcitriol supplementation be reduced and the ionized calcium carefully followed in the postpartum period of hypoparathyroid women who plan to breast-feed (254).

PTH-independent hypercalcemia can occur during lactation and may be PTHrP-mediated. A 21-yr-old woman was reported to develop hypercalcemia and hypercalciuria during lactation in the presence of low PTH, low 1,25-dihydroxyvitamin D, and high PTHrP levels (438). These abnormalities resolved within 2 weeks of weaning, except that the elevated PTHrP level declined more gradually (438). A second woman had lactational hypercalcemia associated with low PTH levels and bone biopsy evidence of active cellular resorption, consistent with hyperparathyroidism (439). Another case of PTHrP-mediated hypercalcemia was reported to occur in a lactating woman with mammary hyperplasia and fully resolved only after a reduction mammoplasty was performed (440).

In summary, PTHrP is present at very high concentrations in breast milk and at higher than normal concentrations in the maternal circulation during lactation. PTHrP may affect maternal calcium metabolism, particularly by increasing skeletal resorption of calcium and phosphate, and renal tubular reabsorption of calcium. It may, thereby, be at least partially responsible for the slight increase in ionized calcium that occurs normally in lactating women and may contribute to the occasional occurrences of hypercalcemia and osteoporosis in lactation (*Section IV.E.3*, below).

C. Intestinal absorption of calcium

Intestinal absorption of calcium in lactating humans falls from the high levels of pregnancy to control levels (12, 28, 126–128, 412, 441), coinciding with the corresponding fall in 1,25-dihydroxyvitamin D levels to normal (*Section IV.B.4*, above). Even when women are assigned to different levels of dietary calcium intake, a lactational increase in efficiency of intestinal absorption of calcium cannot be demonstrated (412). Intestinal absorption of calcium does increase slightly in lactating women whose menses have resumed (412). In some women, dietary intake of calcium may be increased during lactation, such that the total amount of calcium absorbed is increased, even though the efficiency of intestinal absorption is not (412). However, other studies have indicated that such dietary calcium supplementation is of uncertain benefit, since it will increase the urine calcium excretion without affecting the calcium content of breast milk or maternal skeletal losses of calcium (160, 442, 443) (see discussion in *Section IV.E.2*, below). After weaning, there is an increase in intestinal absorption of calcium (412), which may facilitate restoration of calcium to the maternal skeleton.

The adaptations in lactating rats differ from those in humans. These may reflect the effect of greater calcium demands due to the larger litter size and far shorter lactational period. Rats demonstrate a 2-fold increase in duodenal calcium absorption in everted gut sacs, similar to the levels found during pregnancy (141, 444); 1,25-dihydroxyvitamin D levels are also elevated (81). Other factors, in addition to vitamin D, may influence calcium absorption, since, even in the setting of vitamin D deficiency, lactating rats exhibited an increase in duodenal calcium absorption and raised their serum calcium (141, 444). This finding suggests that PRL or some other factor present during lactation might stimulate intestinal calcium absorption. The increased intestinal calcium absorption also persisted in the presence of ovariectomy on the postpartum day 2 (445). At weaning, rats normally experience a rebound hypercalcemia (81, 409), and this is still detected in the presence of severe vitamin D deficiency (143), consistent with an increase in intestinal absorption of calcium. However, this rebound hypercalcemia could also be explained by enhanced release of calcium from the skeleton due to active bone resorption. Despite the persistent increase in dietary absorption, rats appear to also mobilize calcium from the skeleton to meet the demands of lactation (*Sections IV.E.1* and *IV.E.2*, below).

Therefore, in summary, intestinal calcium absorption is not increased during lactation in humans, despite calcium requirements similar to those in pregnancy. In contrast, intestinal absorption of calcium is increased in rodents throughout lactation by increases in 1,25-dihydroxyvitamin D and probably other unknown mechanisms.

D. Renal handling of calcium

In humans, the glomerular filtration rate falls from the elevated level of pregnancy, and renal excretion of calcium is typically reduced to levels as low as 50 mg/24 h (12, 14, 27, 28, 44, 128, 146, 410). The low urine calcium in the setting of high calcium in blood suggests that tubular reabsorption of calcium might be increased to account for the reduction in calcium excretion. The reduction in calcium excretion appears to persist after weaning during the period of restoration of bone density to the skeleton (410). Renal calcium excretion has been shown to be similarly reduced in the lactating rat (446).

E. Skeletal calcium metabolism

1. Bone formation and resorption. Bone turnover is increased during lactation in rats, as indicated by changes in histomorphometric parameters of bone (136, 156, 447). Beagle dogs also show histomorphometric evidence of increased bone turnover in iliac trabecular bone (155), proximal femur (448), and lumbar vertebrae (448) during lactation.

In humans, definitive histomorphometric data are lacking, and, therefore, serum and urine markers of bone formation and resorption have been used to assess bone turnover. Markers of bone resorption (tartrate-resistant acid phosphatase, deoxypyridinoline/creatinine, hydroxyproline/

creatinine) are elevated 2- to 3-fold during lactation and are higher than the levels attained in the third trimester (27, 28, 160, 161, 400, 410, 415, 449). Although markers of bone formation have been reported in a few studies to be generally normal (27, 162), most studies have found such markers to be high during lactation and increased over the levels attained during the third trimester (28, 160, 161, 400, 410, 415, 449). Total alkaline phosphatase falls immediately postpartum due to loss of the placental fraction, but may still remain above normal due to the elevation in the bone-specific fraction (161, 162).

Overall, the results of these studies in humans of markers of bone formation and resorption are consistent with the histomorphometric data from animal models, indicating that bone turnover is significantly increased during lactation.

2. Bone density. Within 21 days of parturition, lactating rats lose about 35% of bone mineral, primarily from trabecular sites (primarily femurs, tibias, and lumbar vertebrae), as determined by a variety of methods that determined total or trabecular ash weight, ash mineral content, and changes in bone mineral density (2, 50, 139, 140, 405, 447, 450). These losses can be increased to 43% or more of trabecular bone mineral by consumption of a low calcium diet beginning at parturition (50, 405, 408, 451, 452). These changes are sufficient to adversely affect the mechanical properties of bone (strength, stiffness, toughness, and ductility) (408).

The loss of skeletal calcium can be worsened by larger litter sizes (408). The loss is similar in vitamin D-deficient and vitamin D-replete rats (137), although another study found a 2-fold greater loss of calcified bone in vitamin D-deficient rats (154). Moreover, vitamin D-replete rats regain the lost mineral after weaning while vitamin D-deficient rats do not; thus vitamin D is needed to restore the lost mineral (137). The lactational loss of bone mineral also persists in the absence of the maternal parathyroid glands (453, 454) and is not affected by ovariectomy, adrenalectomy, or immediate pregnancy (455), unless the animals are simultaneously fed a low calcium (0.1%) diet (445). Therefore, estrogen (and perhaps estrogen deficiency), adrenal hormones, 1,25-dihydroxyvitamin D, and PTH are not needed to mobilize skeletal calcium during lactation. On the basis of these observations, Brommage and DeLuca (455) first proposed that the putative hypercalcemia of malignancy factor, later identified to be PTHrP, might be the mediator of skeletal bone resorption during lactation.

The earliest longitudinal study of bone mineral density during human lactation followed 10 women with serial measurements of the femoral shaft, using a ^{241}Am source, and found a mean loss of 2.2% of bone mineral content over 100 days of lactation (456). More recent studies have followed women with serial measurements of bone density during lactation (by SPA, DPA, or DXA), and a fall of 3–8.0% in bone mineral content has been reported after 2 to 6 months of lactation at trabecular sites (lumbar spine, hip, femur, and distal radius), with smaller losses at cortical sites (28, 146, 160, 166, 169, 400, 410, 415, 417, 449, 456–463); this subject has recently been reviewed in detail elsewhere (390). In contrast, bottle-feeding, postpartum controls do not lose bone density at the lumbar spine over the same interval and may show a net gain in bone density (415, 417, 458, 462, 464). A 15% decrease in cortical bone density determined by SPA of the distal radius was found in teenaged mothers who consumed calcium and phosphate at levels below the recommended daily allowance. This result suggests that poor maternal nutrition might worsen the skeletal changes during lactation (459, 460). This was confirmed in a follow-up study, in which lactating adolescents who consumed calcium in excess of 1600 mg daily had no change in bone mineral density, compared with lactating adolescents who consumed 900 mg calcium daily and lost 10% of bone mineral content over the same interval (465). These teenaged mothers had not yet reached their peak bone mass, which might have been a factor in the responsiveness of the skeleton to dietary calcium supplementation.

Other evidence suggests that skeletal calcium will be preferentially resorbed and that supplementing the diet with calcium will not prevent resorption. For example, consumption of a calcium supplement by lactating Gambian women caused a sharp increase in urinary calcium excretion but had no effect on lactational bone mineral loss when compared with women who consumed less than the recommended daily allowance of calcium (442, 443). In a randomized clinical intervention trial that studied the effect of consuming dietary calcium in excess of the recommended daily allowance (2.4 g daily), lactating women still lost 6.3% of bone mineral density at the lumbar spine and up to 8% from the radius and ulna, as determined by DXA (160). Furthermore, in a preliminary report, the lactational decrease in lumbar spine bone mineral density was not influenced by maternal calcium intake but was negatively and significantly correlated with the breast milk output (466). Therefore, loss of bone mineral from the maternal skeleton appears to be a normal consequence of lactation and may not be preventable by raising the calcium intake above the recommended dietary allowance.

It is not clear whether this loss of bone mineral content is simply due to relative estrogen deficiency of lactation or a more complex, possibly humorally mediated, mechanism. Several studies have suggested that estrogen withdrawal and the intensity and duration of lactation are independent factors in determining the rate and magnitude of bone loss during lactation (218, 416, 462, 464, 467). For example, early resumption of menses (464) or an oral contraceptive (462) can reduce the skeletal losses, but bone density may continue to decrease during extended lactation, even after menses have resumed (218, 467). No published study has adequately addressed the relative role of estrogen withdrawal during lactation in a definitive way, since no study has thus far manipulated estrogen independently of lactation. However, it is evident from studying the effects of acute estrogen deficiency induced by GnRH agonist therapy in young women (given for such diverse conditions as endometriosis, uterine leiomyomata, and premenstrual syndrome) that estrogen deficiency alone is unlikely to account for all of the changes in skeletal calcium metabolism seen during lactation (Table 2). After 6 months of GnRH agonist therapy, the bone mineral content of trabecular-rich sites in the axial skeleton alone is affected and is typically reduced by only 2–4%, as determined by DXA (468–478). At the same time, the serum calcium and

TABLE 2. Comparison of the changes in bone density, calcitropic hormones, minerals, and markers of bone resorption that occur during 6 months of lactation and 6 months of GnRH agonist therapy

	Lactation	GnRH Agonist
Serum calcium	Increased	Increased
Serum phosphate	Increased	Increased
PTH	Decreased	Decreased
1,25-Dihydroxyvitamin D	Normal	Decreased
24-h Urine Ca	Decreased	Increased
Urinary Ca/Cr	Decreased	Increased
Urine HP/Cr	Increased	Increased
BMD changes (DXA)	3–8% at trabecular-rich sites; less at cortical	2–4% at trabecular-rich sites
Recovery of BMD at 6 months	? Complete	? Complete

BMD, Bone mineral density; Ca, calcium; Cr, creatinine, HP, hydroxyproline.

phosphate are increased (469–471, 473, 479), 24-h urine calcium excretion and urinary calcium/creatinine ratio are increased (469–473, 478, 479), but the levels of intact PTH and 1,25-dihydroxyvitamin D are low (469, 473, 479). Therefore, in lactation, the greater losses of bone mineral density (at both trabecular and cortical sites), the normal 1,25-dihydroxyvitamin D levels, and the reduced urinary calcium excretion may be due to the effects of other factors (such as PTHrP) in addition to the effects of estrogen withdrawal. In one recent study of lactating women, higher PTHrP levels were found to correlate with loss of bone mineral density at the lumbar spine and femoral neck, even after accounting for the effects of estradiol levels, PTH, and breast-feeding status (435). In this same study, the high PTHrP levels correlated with breast-feeding status, high PRL levels, and lower estradiol levels (435). Further support for a role of PTHrP in mediating the skeletal changes that occur during lactation comes from the finding that PTHrP is also elevated in the serum of patients with hyperprolactinemia due to pituitary adenomas (401, 480). In these patients, PTHrP correlated positively with the serum PRL and calcium and was negatively correlated with the serum PTH and bone density of the lumbar spine.

The bone density losses of lactation are substantially reversed during weaning, such that the maternal skeleton is able to provide for the calcium requirements of lactation with few, if any, long-term consequences (160, 410, 417, 467). Also, the losses are regained quickly enough that women who breast-feed for at least 6 months, but have a second pregnancy within 18 months, do not have lower bone density after the second pregnancy (463, 481). Only one study has thus far reported failure of the bone density to return to baseline (415). Compared with the studies of GnRH therapy, the longitudinal, prospective studies of lactating women were of insufficient power, however, to eliminate the possibility of modest failure to completely restore bone mass after weaning. The reversibility of bone density losses has also been found in the lactating rat model, where the loss of 35% of trabecular bone mineral content is completely restored at weaning (2, 140, 452).

In the long-term, the consequences of lactation-induced depletion of bone mineral content appear clinically unimportant. The vast majority of epidemiological studies of pre- and postmenopausal women have found that a history of lactation has no adverse effect (172, 174–181, 183, 188–191, 195, 197, 202, 482) or a protective effect (184, 187, 203, 483–487) on peak bone mass, bone density, or hip fracture risk. Only five studies suggest that a history of lactation correlates with reduced radial (196, 488–490) or lumbar (199) bone mineral content. Thus, there appears to be little or no long-term harm caused by the temporary demineralization of the skeleton during lactation.

A similar pattern of decreasing bone density of the lumbar spine during lactation with recovery at weaning has been observed in another primate species, the African green monkey (491). The bone mineral density of the lumbar spine in these monkeys has been observed to fall by about 12% from the baseline value at parturition during 20 weeks of lactation; during an additional 20 weeks of observation after weaning, the bone mineral density increased but did not fully recover to baseline. This model may more closely resemble the human condition, but has not yet been thoroughly studied.

In summary, bone mineral density decreases of 3–8% at trabecular sites occur in the first 6 months of lactation in humans. By comparison, lactating rats lose about 35%; less dramatic losses have been reported in African green monkeys. In all cases, the lost bone mineral content is largely restored after weaning, and there appears to be little or no long-term increased risk of fracture or decreased bone density in women who have lactated. The loss of bone mineral density during lactation may be due to the combined effects of relative estrogen deficiency and PTHrP-induced skeletal resorption; both of these factors may correlate with more intense and prolonged lactation.

3. Osteoporosis of lactation. Rarely, osteoporosis presents during lactation; like the osteoporosis of pregnancy, this may represent a coincidental, unrelated disease. Alternatively, it may represent a continuum of the condition that may present in pregnancy and an exacerbation of the normal degree of skeletal demineralization during lactation (208, 223, 438, 492). In some of these cases, low peak bone mass may have preceded lactation (and pregnancy). Typically these women present 1 to 6 months postpartum with vertebral crush fractures, bone pain, loss of height, and rarely hypercalcemia; the osteopenia may resolve subsequently (438, 492). Bone biopsies show features indistinguishable from normal (223) or may show evidence of increased resorption (492). When measured, PTH is typically normal or reduced, and 1,25-dihydroxyvitamin D is also normal (438, 492). As for the osteoporosis of pregnancy, the difficult diagnostic question remains: when did the condition start? Given the preceding discussion, the possibility arises that excessive PTHrP release from the lactating breast into the maternal circulation could cause excessive bone resorption, osteoporosis, and fractures in these cases (438, 493). PTHrP was high in one case of lactational osteoporosis and was found to remain elevated for months after weaning (438). However, the extent to

which PTHrP contributes to the reduction of bone density during lactation has yet to be established.

F. Hypoparathyroidism and pseudohypoparathyroidism

Calcitriol requirements of hypoparathyroid women fall early in the postpartum period, especially if the woman breast-feeds, and hypercalcemia may occur if the calcitriol dosage is not substantially reduced (244, 245, 248, 253–255, 258, 494). As noted earlier in the discussion of hypoparathyroidism in pregnancy (*Section II.G*), this may result from the activation of the renal 1α-hydroxylase by PTHrP.

The management of pseudohypoparathyroidism has been less well documented. Since 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 can be expected to return to prepregnancy levels and should be unchanged by lactation.

G. Summary

Even when dietary intake of calcium exceeds the recommended daily intake, the calcium demands of lactation in humans are preferentially met by increased skeletal resorption of calcium and probably increased renal conservation of calcium, but not by increased intestinal absorption of calcium. Serum calcium is slightly increased or normal, while phosphate is high normal or frankly elevated. These increases reflect calcium and phosphate entering the circulation from bone in increased amounts and reabsorption of calcium and phosphate by the kidney. PTHrP levels are extremely high in breast milk and increased in the maternal circulation, while PTH levels are generally low during lactation. 1,25-Dihydroxyvitamin D levels are typically normal throughout lactation but may be higher in women nursing twins. Markers of bone resorption and formation are increased, and bone density has been found to reversibly decrease during lactation. These lactational decreases in bone density may not adversely affect the skeleton in the long-term, although occasionally the normal lactation-induced decrease in bone density may be excessive, leading to fractures and a clinical diagnosis of osteoporosis.

Increasing the dietary intake of calcium does not consistently prevent the loss of skeletal calcium from occurring. Relative estrogen deficiency and elevation in PTHrP levels may both play a role in the lactational loss of skeletal calcium. PTH and 1,25-dihydroxyvitamin D elevations occurring after weaning may indicate a role for these hormones in providing the mineral needed to restore the skeleton.

In contrast to the findings in humans, skeletal losses of calcium are far greater in lactating rats, and hypocalcemia may ensue with more intense lactation. In that setting, the dietary absorption of calcium is increased, and both PTH and 1,25-dihydroxyvitamin D levels are elevated. These striking differences may limit the usefulness of the lactating rat as a model for understanding the human condition (Table 3).

Although critical experimental data are missing, a tentative hypothesis for explaining calcium physiology in lactation starts with the dominant role of PRL to suppress gonadotropins (and, therefore, estrogen) and to stimulate PTHrP synthesis. Estrogen withdrawal then leads to bone resorption. If PTHrP were not available, the bone resorption would be expected to suppress PTH levels, which would cause loss of calcium in the urine, decrease in 1,25-dihydroxyvitamin D levels and intestinal calcium absorption, and diminution of net bone resorption (*i.e.*, similar to the changes seen in GnRH therapy, Fig. 3). Such actions would act against the provision of calcium for milk production. In this context, the production of PTHrP can be viewed as the provision of a PTH surrogate, but one that is not under negative feedback control by calcium. Thus, even with low levels of PTH, 1,25-dihydroxyvitamin D levels are then maintained, urinary calcium is reabsorbed, and bone resorption continues (Fig. 3).

In this formulation, the bone resorption initiated by estrogen withdrawal is not a disease, but an intelligent way to assure a supply of calcium for milk, independent of dietary vagaries. At weaning, young bones are apparently able to restore bone mass by as yet uncertain mechanisms. In this context, menopausal osteoporosis can be viewed as an evolutionary accident — a property of estrogen withdrawal that is constructive during the reproductive years, but destructive in the setting of older bones and prolonged estrogen withdrawal.

V. Neonatal Physiology and Pathophysiology

A. Neonatal adaptive goals

As described earlier, the fetus maintains a higher blood calcium than the mother. This level of blood calcium appears to be dependent on the input of calcium across the placenta, an intact PTH/PTHrP receptor, and fetal PTHrP to maintain the fetal blood calcium and regulate placental calcium transport. After the umbilical cord has been cut and the placental calcium infusion (and placental sources of PTHrP) abruptly lost, the neonate becomes dependent on intestinal calcium intake and skeletal calcium stores to maintain a normal blood calcium at a time of continued skeletal growth. Further, the kidney, which postnatally produces urine unavailable for reingestion, takes on a major role in maintaining calcium and, particularly, phosphate homeostasis. If intestinal calcium intake is inadequate, continued growth and mineralization of the skeleton will be compromised. In adjusting to the loss of the placental calcium pump, PTH and 1,25-dihydroxyvitamin D become more important, while PTHrP appears to be less involved in neonatal calcium homeostasis. Therefore, the adaptive goals of the neonate are to quickly turn on PTH and 1,25-dihydroxyvitamin D synthesis, which,

TABLE 3. Important differences between calcium physiology of humans and rodents during lactation

Factor	Human lactation	Rat lactation
Blood ionized calcium	Stable or slightly increased	Reduced
PTH	Low to low-normal	Increased
1,25-D	Normal	Increased
Intestinal calcium absorption	Normal	Increased
Skeletal calcium losses	3–8%	30–35%

1,25-D, 1,25-Dihydroxyvitamin D.

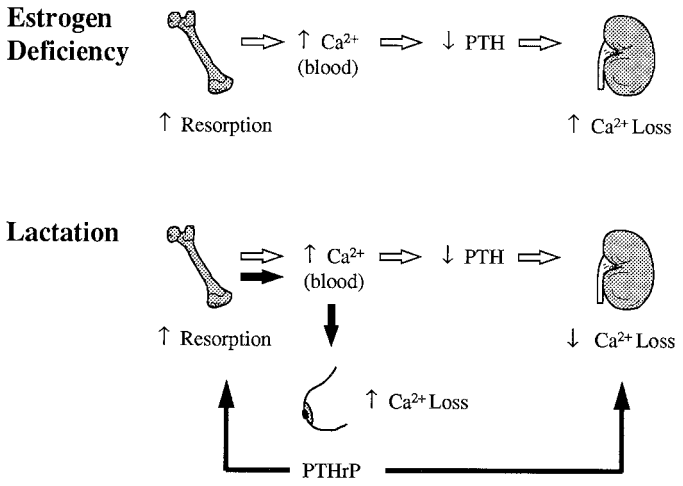

FIG. 3. Schematic illustration of the responses to acute estrogen deficiency alone (*e.g.*, GnRH analog therapy) *vs.* lactation. Acute estrogen deficiency increases skeletal resorption and raises the blood calcium; in turn, PTH is suppressed and renal calcium losses are increased. During lactation, the combined effects of PTHrP (secreted by the breast) and estrogen deficiency increase skeletal resorption and raise the blood calcium, but calcium is directed into breast milk. Although PTH is suppressed during lactation, renal calcium losses are lower than normal.

in turn, up-regulate intestinal calcium absorption, and regulate skeletal and renal handling of calcium and phosphate.

B. Mineral ions and calcitropic hormones

The changes that occur in neonatal calcium, phosphate, and calcitropic hormone levels over the first 4 days after birth are schematically depicted in Fig. 4.

1. Calcium. In rodents, ionized calcium and total calcium fall to about 60% of the high fetal values within 6–12 h of birth (269, 278). Thereafter, the ionized calcium rises over the succeeding 12–36 h to about 80% of the fetal value, and then slowly falls over the following week to normal adult levels (276). In contrast, after reaching a nadir at 6–12 h after birth, the total calcium gradually rises to adult levels over the succeeding 7–14 days (269, 276). The total calcium changes largely reflect alterations in the serum albumin, such that, by the end of the first 2 weeks of life, the albumin-bound fraction is approximately 50% of the total calcium (in fetal life it accounts for only about 20%) (276). Neonatal lambs differ in that the blood calcium may remain at the higher fetal value over the first several months of postnatal life (330).

Although data from umbilical cord and neonatal blood levels are less complete in humans, the progression in ionized and total calcium values appears to be similar to that of rats (268, 270, 495). The ionized calcium in normal neonates has been reported to fall from the umbilical cord level of 1.45 mmol/liter to a mean of 1.20 mmol/liter by 24 h after birth (496). Babies delivered by elective cesarean section were found to have lower blood calcium and higher PTH levels at birth compared with babies delivered by spontaneous vaginal delivery (387). This finding suggests that the mode of delivery can affect early neonatal calcium homeostasis, a variable that has not been controlled in most studies of neonatal calcium physiology.

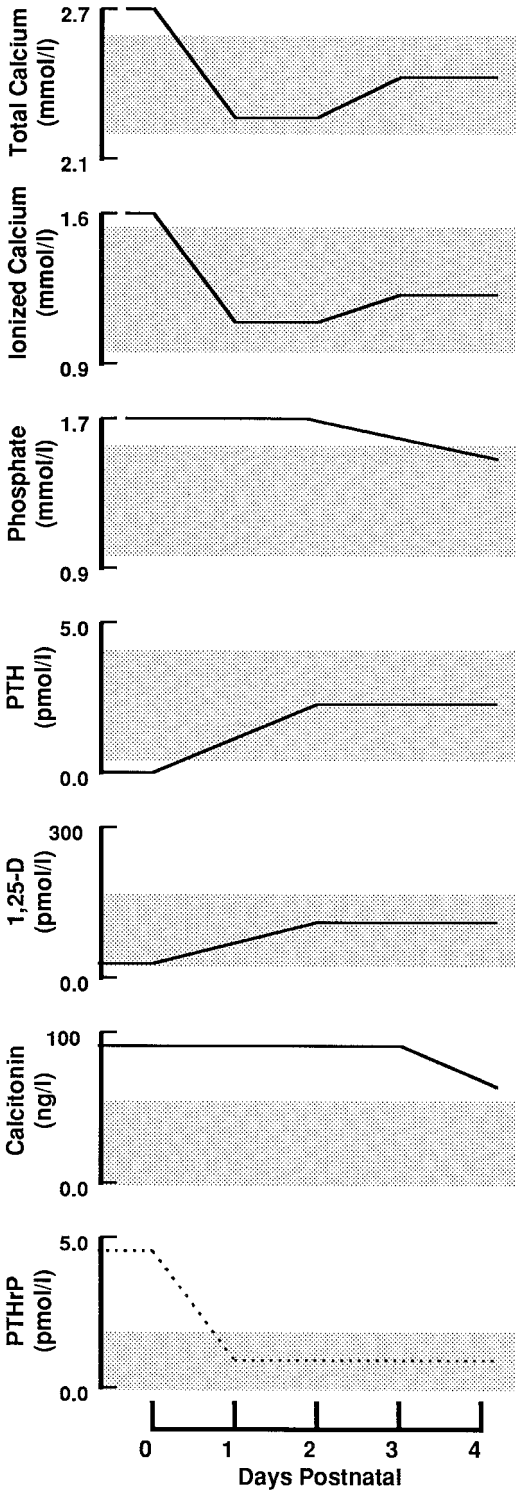

FIG. 4. Schematic illustration of the longitudinal changes in calcium, phosphate, and calcitropic hormone levels that occur during the neonatal period in humans. Normal adult ranges are indicated by the *shaded areas*. Data have been compiled from the following sources: total calcium (268, 270, 495), ionized calcium (268, 270, 495, 496), phosphate (32, 270, 273), PTH (17, 43, 110, 124, 268, 272, 305–307, 387, 495, 497–501), 1,25-dihydroxyvitamin D (11, 37, 40, 56, 304, 321), calcitonin (11, 86, 272, 500), and PTHrP (43, 110, 124, 310). The progression in PTHrP levels has been depicted by a *dashed line* to reflect that it is speculative.

2. Phosphate. Phosphate initially rises over the first 24 h of postnatal life in humans (270) and then gradually declines. The rise in phosphate corresponds to the early postnatal period when the parathyroid glands are still suppressed; phosphate declines as PTH secretion increases (see *Section, V.B.3*).

3. PTH. As discussed earlier (*Section III.B.3*), in fetal life PTH is present at low levels in the circulation and is apparently synthesized at low levels by the parathyroid glands. Based on measurements taken only at birth and 24 h of age in humans, the intact PTH level has been found to rise briskly after birth to within or near the normal adult range (17, 387, 497–501). However, these studies did not ascertain how soon the PTH level begins to rise postnatally and whether the peak level is attained by 24 h or even later. A more detailed time course for PTH was obtained using earlier generation PTH assays; as discussed earlier, these assays were less reliable, and measured fragments that accumulated in the circulation much more slowly than intact PTH. With these caveats in mind, data from these older assays found that PTH levels remained low over the first 12–24 h in humans and did not reach peak levels until 48 h or later (268, 272, 305–307).

Regardless of the exact time course, it is apparent that the increase in PTH levels follows the early postnatal drop in the serum ionized calcium and precedes the subsequent rise in ionized calcium (268, 272, 306, 495) and 1,25-dihydroxyvitamin D (*Section V.B.4*, below). The fall in ionized calcium is probably a consequence of the parathyroid gland suppression seen in late gestation (*Section III.B.3*, above) combined with the sudden loss of placental calcium delivery; the subsequent rise in PTH and the ionized calcium represents the progressive recovery from this suppression. 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 (268, 272, 306, 502). The degree of responsiveness to acute hypocalcemia appears to increase with postnatal age (306, 502); however, the interpretation of these data are limited by the nature of the older PTH assays from which they were obtained.

4. 1,25-Dihydroxyvitamin D. In humans, 1,25-dihydroxyvitamin D rises to adult levels over the first 48 h of postnatal life, likely in response to the rise in PTH (11, 40). Phosphate levels are high at this time and would tend to inhibit a rise in 1,25-dihydroxyvitamin D. Although in humans, rats, sheep, and pigs the fetal blood calcium and skeletal mineralization are unaffected by vitamin D deficiency, in the neonatal period deficiency of vitamin D can become manifest (29, 79, 84, 284, 285). This is further discussed in *Section V.E*, below.

5. Calcitonin. Data from humans have found a rise in the serum calcitonin, 2- to 10-fold over cord blood levels, over the first 24–48 h of life (11, 272, 500). Infants that are premature, asphyxiated, or hypocalcemic have the highest postnatal calcitonin levels (272); consequently, it has been suggested that hypercalcitoninemia may cause neonatal hypocalcemia (86). Consistent with this hypothesis, hypocalcemic preterm infants had higher calcitonin levels than normocalcemic, preterm control infants (503). However, the same study found that the calcitonin levels of hypocalcemic term infants did not differ from those of normocalcemic term infants (503). Therefore, the influence of calcitonin on neonatal calcium homeostasis may vary with gestational age at birth; or, alternatively, have little role in calcium homeostasis. The physiological effect of calcitonin administration to neonates (human or animal) has not been directly tested.

Further evidence suggests that calcitonin levels are not significantly regulated by calcium, or responsive to the serum calcium level, in the neonatal period. In infants of normal and diabetic pregnancies, calcitonin levels increased after birth irrespective of the rate of fall in serum calcium, indicating that the postnatal surge in serum calcitonin may not be the main mechanism of postnatal hypocalcemia (500). Similarly, in very-low-birth-weight, preterm infants, spontaneous hypocalcemia provoked a rise in PTH but no change in calcitonin levels, and a calcium infusion to treat the hypocalcemia reduced the PTH level but did not affect the calcitonin level (504).

After the early surge in calcitonin levels over the first 48 h of life, calcitonin gradually falls to adult levels over the first month after birth in sheep (324) and humans (86).

6. PTHrP. PTHrP secretion from placenta, amnion, and umbilical cord is lost at birth; secretion from the parathyroid glands is also apparently lost, since PTHrP circulates at low to undetectable levels (with currently available assays) during normal adult life in humans and animals (104, 116, 335, 493, 505). The loss of placental sources of PTHrP, in addition to loss of the placental calcium pump, may be one of the reasons that the ionized and total calcium levels fall dramatically at birth. However, a preliminary report in neonatal (1- to 3 day-old) rat pups has found persistently high PTHrP levels, in the range of 20–40 pmol/liter (506). Studies in neonatal lambs have shown a progressive decline in PTHrP immunoreactivity in the parathyroid glands, and serum bioactivity attributable to PTHrP, over the first several months after birth, corresponding to the delayed fall in blood calcium that occurs in this species (116, 330). As noted earlier (*Section III.C*), indirect evidence from fetal sheep suggests that the fetal parathyroid gland may be an important source of PTHrP for normal regulation of the blood calcium and placental calcium transport. However, it is not known whether PTHrP synthesis occurs in the parathyroid glands of neonatal humans, or is turned off at some point in postnatal development.

Although immunoreactive PTHrP in milk can be absorbed into the bloodstream of suckling neonates (and might account for high levels of PTHrP in neonatal plasma) (113, 507), PTHrP may not have a dramatic effect on neonatal mineral homeostasis, since blocking its activity in milk by passive immunization of the mother had no dramatic effect on mouse pup blood calcium, weight gain, or femur mass (434). These negative results, however, might result from compensatory changes in neonatal PTH, calcitonin, and 1,25-dihydroxyvitamin D, which were not measured in this study. Passive immunization of the 2-day-old neonatal mouse with antibodies to amino-terminal PTHrP did not affect neonatal calcium homeostasis (508), but again, compensations may have obscured the role of PTHrP. These findings are consistent

with the observations described above from humans and rats, that PTH dominates the regulation of calcium homeostasis by 48 h of postnatal life. However, since there are no human data, and limited animal data on PTHrP levels in the neonatal period, an important role for PTHrP in calcium homeostasis after birth has not been ruled out.

C. Intestinal absorption of calcium

Calcium is absorbed in the gastrointestinal tract by both active and passive mechanisms (125, 509). Active intestinal transport occurs mainly in the duodenum and is regulated by 1,25-dihydroxyvitamin D, partly through its stimulation of calbindin$_{9K}$-D. Passive transport of calcium appears to be an unregulated, nonsaturable process that occurs throughout the small intestine; the rate of passive transfer of calcium into intestinal cells is directly proportional to the intraluminal concentration of calcium.

In newborn rat pups, intestinal calcium absorption is largely a passive, nonsaturable process that is not dependent on vitamin D (510–512). 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 (513–515). As the pups mature, receptors for 1,25-dihydroxyvitamin D begin to appear in intestinal cells (516), and the mucosal levels of calbindin$_{9K}$-D increase sharply (517). Around the same time, vitamin D-dependent active transport of calcium becomes noticeable (512), while passive transfer of calcium into the intestinal cells declines (510, 511). By the time of weaning, 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 (510–512).

Data from newborn humans is less complete; therefore, the timing of the development of vitamin D-dependent calcium transport in humans is not known. However, normal term and preterm infants exhibit a similar postnatal increase in the efficiency of intestinal calcium absorption (518–520). Studies from preterm infants indicate that passive, non-vitamin D-dependent absorption of calcium may be the dominant route of calcium transfer (518, 521). In addition, the lactose content of breast milk has been shown to increase the efficiency of intestinal calcium absorption in human infants (522, 523). Postnatal supplementation of the preterm infant (mean gestational age, 32 weeks) with vitamin D markedly increased the absorption of calcium by 2–4 weeks after birth, as compared with similar preterm infants that were not supplemented (520). However, the intestinal absorption of vitamin D has been shown to undergo a postnatal age-dependent increase (524); this may limit the efficacy of vitamin D supplementation in the newborn.

In summary, data from newborn rats and humans indicate that intestinal calcium absorption is largely a passive, non-vitamin D-dependent process until near the time of weaning. The high lactose content of milk increases the efficiency of passive absorption of calcium. The normal postnatal maturation of the neonatal intestine may affect the ability of preterm humans to accrete sufficient calcium for skeletal mineralization and to regulate their blood calcium.

D. Renal handling of calcium

Little data are available from animal or human studies on the renal handling of calcium in the first few days of postnatal life (509). In humans, urinary excretion of calcium is low over the first few days of life and rises over the succeeding 2 weeks (498, 525, 526). This rise in renal calcium excretion probably reflects the concurrent 2-fold rise in glomerular filtration rate over the first 2 weeks after birth (525, 527). It may also reflect a greater filtered load of calcium as a consequence of the gradually rising serum calcium, 1,25-dihydroxyvitamin D, and PTH levels and gradually declining serum calcitonin (*Section V.B*, above). The responsiveness of the renal proximal tubules to PTH may increase over the same time period. In addition to evidence from animal studies presented earlier (*Section III.D*), data in preterm and term human infants indicate that the renal tubules are responsive to exogenously administered PTH, as evidenced by a rise in urinary cAMP (528, 529); further, this response to exogenous PTH increases with postnatal age (277, 528, 529).

E. Skeletal calcium metabolism

In humans that receive adequate dietary calcium and phosphate, the neonatal skeleton continues to accrete calcium at a rate of about 150 mg/kg per day, similar to the rate of the late-term fetus (5, 530–532). Having abruptly lost the placental calcium pump, the neonate becomes completely dependent on intestinal intake of calcium and phosphate to continue to meet the demands of the developing skeleton. In turn, the blood calcium will be maintained by calcium obtained from skeletal stores, absorbed by the intestine, and reabsorbed by the kidneys. The effects of vitamin D deficiency during gestation become manifest during the neonatal period because intestinal calcium transport becomes necessary for supply of calcium to the skeleton. As noted earlier, from studies of maternal vitamin D deficiency in rats (29, 79, 84), pigs (79, 285), and sheep (284), the blood calcium is typically normal at birth and the skeleton is fully mineralized, but after birth the neonate will begin to manifest the complications of vitamin D deficiency. In vitamin D-deficient rats and pigs, a slight decrease in blood calcium was detected by 3 days after birth, but it was only after 14 days of neonatal life that absence of 1,25-dihydroxyvitamin D resulted in significant, but still modest, hypocalcemia (84, 285, 311). Thereafter, rat pups failed to thrive and manifested rachitic skeletal growth plates and decreased longitudinal growth (84, 285). These effects became more pronounced after weaning (84, 285). Further, in vitamin D receptor knockout mice, hypocalcemia and rachitic changes in the skeletons developed only after the first three postnatal weeks (313, 314).

The development of vitamin D dependence is similar for humans. In human vitamin D deficiency, hypocalcemia appears late in the first or second week of life, and rickets develops after 2–3 months (316, 317). Vitamin D supplementation during pregnancy is associated with higher neonatal serum calcium levels and a reduced incidence of neonatal hypocalcemia (83, 533, 534). Neonates with rachitic changes in the skull (craniotabes) have lower 25-hydroxyvitamin D levels than those without craniotabes (535), and fewer neo-

nates develop craniotabes if the mothers received a vitamin D supplement during pregnancy (534). Infants in China with lower cord blood 25-hydroxyvitamin D levels were found to have fewer wrist ossification centers at 3–5 days of postnatal age (536); furthermore, the crown-heel lengths of infants born to vitamin D-insufficient Pakistani women were found to correlate positively with the maternal serum calcium and negatively with the maternal PTH level (537). These findings suggest that fetal vitamin D deficiency may be manifest earlier in postnatal life than previously believed (and perhaps even during fetal life) or may be a marker for more global nutritional deficiency.

Although parathyroidectomy of rats at birth results in hypocalcemia and hyperphosphatemia (538, 539), by the time of weaning the neonatal rats still have normal body weight, normal shape and trabecular content of developing long bones, and normal calcium and phosphorus content of bone ash (539). These observations are similar to the finding of normal skeletons in experimental models of vitamin D deficiency, and in the vitamin D receptor knockout mice. These findings suggest that factors other than PTH and vitamin D may be required for normal accretion of calcium by the skeleton in the first several weeks after birth, while the infant is nursing. The lactose content of milk (discussed in *Section V.C*) and the high levels of PTHrP in milk may be important.

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 (315, 316). These premature infants are born with a bone mineral content appropriate for gestation age (as assessed by SPA of the radius), but if untreated, their radial bone mineral content fails to increase as appropriate for their gestational age (540–544). By 2–3 months of age, the physical and radiological signs of rickets of prematurity may develop. These include craniotabes, rachitic rosary, chest deformity, osteopenia, pathological fractures, metaphyseal stippling, and flaring and widening of the epiphyses of long bones (315).

Rickets of prematurity is not due to vitamin D deficiency. 25-Hydroxyvitamin D and 1,25-dihydroxyvitamin D levels are typically normal, and vitamin D supplementation of the mother during pregnancy, or the infant postnatally, does not prevent its development (315, 545, 546). Rickets of prematurity appears 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 (315, 316, 544, 545, 547).

In summary, continued mineralization of the neonatal skeleton is dependent on adequate vitamin D stores and intact intestinal calcium absorption. Preterm infants are prone to develop a form of metabolic bone disease if the immature intestine cannot absorb calcium efficiently enough to make up for the loss of the placental calcium pump.

F. Neonatal response to maternal hyper- or hypoparathyroidism

Although the fetal blood calcium is set independently of the maternal level *in utero*, and PTH does not cross the placenta, it is clear from more than 100 reported cases in humans that maternal hyperparathyroidism adversely affects the neonate (11, 225, 227, 229, 235, 237). Premature labor and stillbirth may result from unrecognized maternal hyperparathyroidism (225, 235). Typically, the parathyroid glands remain suppressed after birth, and complications of neonatal hypocalcemia, tetany, permanent childhood hypoparathyroidism, and even death may result. The mechanism of the prolonged suppression is not known, but is probably due to increased flux of calcium across the placenta when the mother is hypercalcemic (233). However, this hypothesis has not been tested experimentally. A similar suppression has also been observed in normal infants of women with hypercalcemia due to familial hypocalciuric hypercalcemia, who typically have similar elevations in the serum calcium without an increase in serum PTH level (241–243, 374).

Similarly, maternal hypoparathyroidism in humans has been associated in the neonate with parathyroid gland hyperplasia, generalized skeletal demineralization, subperiosteal bone resorption, bowing of the limbs, fractures of ribs and long bones, and low birth weight (246, 375–378). Stillbirth and neonatal death have also been associated with this condition (379–381). The serum calcium level of the neonate has been reported to be normal in most cases, while the PTH level (older assays) has been found to be elevated (263, 376, 548). 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, with or without parathyroid autotransplantation and cryopreservation, may be required to control more severe, autonomous disease (549).

Maternal hypocalcemia of any cause may result in parathyroid gland hyperplasia and hyperparathyroidism in the fetus and neonate (376, 382, 383). In women with pseudohypoparathyroidism, children who do not inherit the genetic disorder are usually normal at birth, although transient neonatal hyperparathyroidism has been reported in some cases (262, 263). Furthermore, children who did inherit the condition may also be normal at birth but may gradually develop the full biochemical features of pseudohypoparathyroidism over the first several years of life (550).

G. Neonatal hypocalcemia

Neonatal hypocalcemia typically presents as seizures, starting between 4–28 days of age (551, 552). The preterm infant is particularly prone to hypocalcemia, having lost the placental calcium pump at a time when the skeleton is rapidly accreting calcium, and the intestinal calcium absorption mechanism is relatively immature. In addition to prematurity, other causes of neonatal hypocalcemia include congenital hypoparathyroidism, magnesium deficiency, maternal diabetes, vitamin D deficiency or resistance, and hyperphosphatemia (Table 4) (552). In many cases the etiology of the

TABLE 4. Causes of neonatal hypocalcemia

Prematurity
Maternal diabetes
Congenital hypoparathyroidism
Maternal hypercalcemia
Magnesium deficiency
PTH resistance
Maternal vitamin D deficiency
Resistance to vitamin D
Anticonvulsants
Hyperphosphatemia
Citrated blood transfusion
Phototherapy
Respiratory alkalosis
Alkali therapy

hypocalcemia is unknown. One study reported that three hypocalcemic, otherwise unremarkable, neonates had high PTH and serum phosphate levels and a subnormal phosphaturic response to PTH infusion; this "neonatal pseudohypoparathyroidism" completely resolved by 6 months of age (551). In addition, the higher phosphate content of infant formula has been associated with increased serum phosphate and decreased serum ionized calcium levels in formula-fed infants, as compared with breast-fed neonates (553).

When older PTH assays were used, preterm infants, infants of diabetic pregnancies, and hypocalcemic neonates were typically found to have even lower or undetectable levels of PTH than normal neonates and took a day or two longer to manifest the rise in PTH (272, 305, 554). This was interpreted to indicate that in preterm infants, and in infants of diabetic pregnancies, the parathyroid glands are less able to regulate the blood calcium and prevent hypocalcemia. However, more recent studies have failed to confirm the earlier impression that parathyroid function is abnormal in preterm infants (17, 504).

Neonatal hypocalcemia can occur as a complication of maternal diabetes in pregnancy in up to 50% of cases (555, 556), although tight control of the maternal glucose during pregnancy reduces the incidence of neonatal hypocalcemia (557). The cause of hypocalcemia in these infants is likely to be multifactorial. As noted above, parathyroid gland secretion may be blunted during the first few days of life in both normocalcemic and hypocalcemic infants of diabetic pregnancies (272, 554, 558). Neonatal hypomagnesemia, which results from maternal wasting of magnesium in association with glycosuria during pregnancy, correlates with the severity of neonatal hypocalcemia and may be the major factor contributing to the sluggish neonatal parathyroid function (557, 559, 560). However, this hypothesis has not been tested by correcting the hypocalcemia through magnesium replacement alone. Although occult vitamin D deficiency has been suggested as a cause of hypocalcemia in these infants (561), supplementation of infants of diabetic pregnancies with vitamin D at 2, 24, 48, and 120 h after birth did not reduce the magnitude or incidence of hypocalcemia as compared with infants that were not supplemented (562).

In addition to the blunted parathyroid function and hypomagnesemia, it is also possible that alterations in maternal calcium homeostasis due to diabetes might predispose the neonate to become hypocalcemic. Although two cross-sectional studies in humans found no effect of maternal diabetes on maternal levels of calcium, phosphate, PTH, and calcitonin (563, 564), a longitudinal study found that pregnant diabetic women had lower 1,25-dihydroxyvitamin D, total serum calcium, and ionized calcium levels in the third trimester, as compared with nondiabetic pregnant women (565). Furthermore, experimental models of diabetes in pregnant rats demonstrate marked maternal hypercalciuria (566), reduced maternal-fetal transfer of calcium and magnesium (567), a 12-fold reduction in placental calbindin$_{9K}$-D levels (567), and reduced calcium content of fetal ash (567). Collectively, the human and animal data indicate that maternal diabetes can affect maternal calcium homeostasis and reduce the placental transfer of calcium. However, whether these effects predispose to the development of hypocalcemia in infants of diabetic pregnancies has not been determined.

H. Summary

In the early neonatal period, the neonate is challenged by the loss of the placental calcium pump and manifests a quick transition, from an environment in which PTHrP plays an important role to a PTH- and 1,25-dihydroxyvitamin D-controlled neonatal milieu. This is reflected in a rapid fall in total and ionized calcium over the first 6 h of life. The calcium level gradually corrects over the following 48 h, after PTH secretion by the parathyroid gland increases, and 1,25-dihydroxyvitamin D levels ascend to adult values. Serum phosphate persists at high levels until the rising PTH levels and increasing renal responsiveness to PTH permit a phosphaturia. Calcitonin levels may remain elevated for several weeks; the physiological importance of this elevation is uncertain. These changes in calcium and calcitropic hormone levels are summarized in Fig. 4. In contrast to the rapid changes in calcitropic hormone levels, intestinal calcium absorption changes gradually from a passive to an active, 1,25-dihydroxyvitamin D-mediated process over the first weeks of life.

Although human and animal fetuses develop remarkably normally in the presence of maternal calcium, PTH, and vitamin D deficiency, the resulting neonates demonstrate abnormalities that are consequences of the prior abnormal maternal calcium homeostasis. Maternal hyperparathyroidism and hypoparathyroidism during pregnancy can be manifest as disturbances of neonatal calcium and bone metabolism. In addition, maternal diabetes during pregnancy can predispose to neonatal hypocalcemia, probably as a consequence of fetal hypomagnesemia induced by maternal renal wasting of magnesium.

When data from fetal and neonatal humans and animals are compared, it is apparent that PTHrP has a significant role in fetal calcium homeostasis and circulates at higher levels than PTH. In contrast, there is as yet no evidence of an important role for PTHrP in normal postnatal calcium homeostasis. PTHrP is normally found at low to undetectable levels in the adult, but the postnatal time point at which PTHrP is lost is not known.

VI. Discussion and Conclusions

Maternal adaptations of calcium homeostasis differ between pregnancy and lactation (Fig. 5). The pregnant woman manifests a 2-fold increase in intestinal calcium absorption that is mediated partly by a 2-fold increase in free and total 1,25-dihydroxyvitamin D levels and partly by mechanisms that are independent of vitamin D. In addition, the increased dietary intake of calcium is offset by increased renal losses of calcium. Skeletal stores of calcium do not appear to be mobilized to any significant degree during pregnancy. PTH levels are low or low-normal for much of pregnancy, and thus the concept of "physiological hyperparathyroidism of pregnancy" is invalid. The increased intestinal calcium absorption results in a gradual, slight increase in the corrected serum calcium and ionized calcium and a marked increase in renal calcium excretion (absorptive hypercalciuria). Bone mineral stores may be increased early in pregnancy in anticipation of the peak fetal demand of the third trimester; by term, the maternal skeleton has no apparent deficit of bone mineral. In contrast, the lactating woman does not increase intestinal calcium absorption, but increases bone turnover and renal tubular reabsorption of calcium to provide adequate calcium for the breast milk. Again, the serum ionized calcium is increased slightly, while the serum phosphate may be frankly elevated. These increases reflect the increased skeletal resorption and decreased renal excretion of these minerals. The lactation-associated fall in estrogen levels, along with secretion of PTHrP, leads to bone resorption. This, combined with PTHrP-mediated calcium reabsorption from the urine, leads to suppression of PTH. Lactation causes a loss of 3–8% of bone mineral content that is restored after weaning; this reversible loss of bone mineral does not appear to adversely affect the skeleton in the long term.

Estrogen deficiency due to menopause is associated with significant, essentially irreversible losses of calcium from the aging skeleton; these losses cause osteoporosis and increase the risk of fractures. During lactation, the effect of estrogen deficiency, in association with PTHrP, increases skeletal resorption to provide calcium for the milk; the younger skeleton appears capable of restoring calcium losses after weaning. In this sense, the occurrence of osteoporosis at menopause may be regarded as the unfortunate consequence of outliving normal ovarian function and inducing permanent estrogen deficiency without the normal bone-restorative factors that are also present during lactation and weaning.

The regulatory mechanisms that direct the adaptive processes that occur in pregnancy and lactation are by no means fully elucidated. The potential roles of reproductive hormones of pregnancy in calcium homeostasis (estrogen, PRL, CG, placental lactogen, etc.) have not been adequately explored, and it is also possible that other calcitropic factors, perhaps specific to pregnancy and lactation, remain to be identified. The results of studies from animal models of pregnancy and lactation must be interpreted carefully, given that there are significant differences between the adaptive strategies for calcium and bone homeostasis seen in humans, rodents, and other animals (Tables 1 and 3). For example, the pregnant and lactating rat both develop secondary hyperparathyroidism, and the lactating rat can lose up to 35% of skeletal calcium before restoring it after weaning. The lactating rat also increases the intestinal absorption of calcium, whereas lactating women do not.

The rare disorders of osteoporosis in pregnancy and lactation may represent chance occurrences of idiopathic osteoporosis, or they may represent a spectrum of one common condition whose time of presentation is determined by the prepregnancy bone mass and the rate of bone resorption subsequent to conception. It seems likely that estrogen deficiency combines with the actions of PTHrP to stimulate the loss of calcium from the skeleton during lactation. The mechanism by which calcium is restored to the skeleton after weaning is not known, but if understood, might be adapted to a bone-restorative therapy for osteoporosis. The postpartum restoration of normal estrogen levels (recognized by the resumption of normal menses) is clearly an important factor,

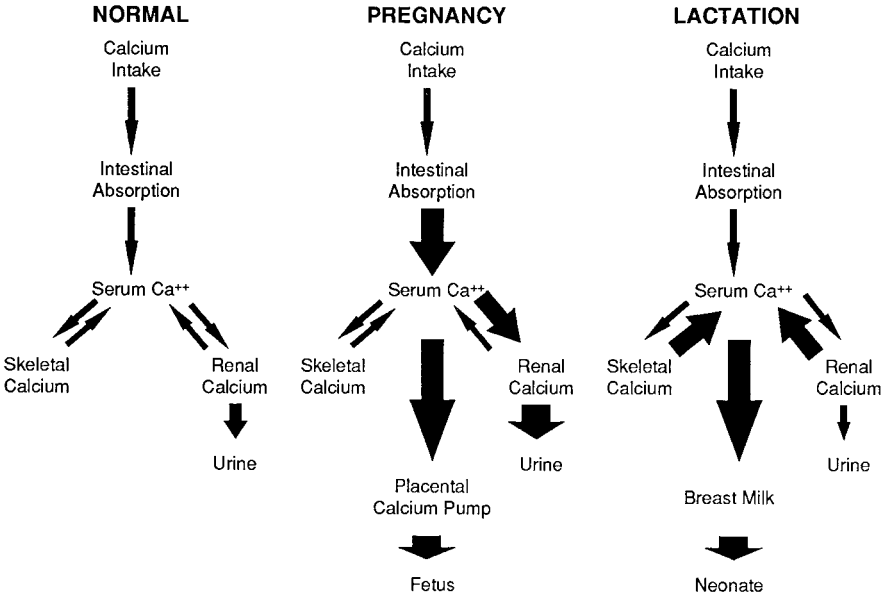

FIG. 5. Schematic illustration contrasting the adaptive processes of calcium homeostasis in human pregnancy and lactation, as compared with normal. The *thickness of arrows* indicates a relative increase or decrease with respect to the normal, nonpregnant state.

but probably not the only one, since part of the restoration of calcium to the skeleton occurs only with weaning.

Fetal calcium homeostasis in late gestation appears to be largely regulated by PTHrP, which stimulates placental calcium transport, resorbs bone, and may stimulate renal calcium reabsorption. PTH can also resorb bone, stimulate renal calcium reabsorption, and stimulate synthesis of 1,25-dihydroxyvitamin D. However, since PTH and 1,25-dihydroxyvitamin D are found at very low levels in the fetal circulation during late gestation, their roles in fetal life are less well defined. The relative importance of PTH and PTHrP earlier in fetal life are unknown. The fetus sets its blood calcium level irrespective of the ambient maternal blood calcium level. The usefulness to the fetus of a blood calcium higher than the mother's is not understood, as it does not appear to be necessary for full mineralization of the skeleton to occur. It is possible that the high fetal calcium levels act as a safety margin at birth, allowing the newborn to experience a postnatal fall in ionized calcium without tetany or convulsions. Alternatively, a higher ionized calcium may be useful for cellular functioning under fetal conditions (low pO_2, low pH, for example) that differ from those in later life. In any case, the fetus is able to maintain a normal blood calcium and fully mineralize its skeleton in the setting of significant maternal calcium and vitamin D deficiencies.

A changeover to a PTH- and 1,25-dihydroxyvitamin D-driven environment occurs during the first 48 h of postnatal life, accompanied by loss of hypercalcemia and high PTHrP levels. Placental calcium transfer may suppress PTH synthesis by the fetal parathyroid glands, and loss of the placenta may, therefore, stimulate PTH synthesis in the neonatal parathyroid glands. Since PTHrP is normally undetectable in the adult circulation, secretion of PTHrP into the circulation by fetal tissues must be lost postnatally; the time of this occurrence has not been determined. The rising PTH and 1,25-dihydroxyvitamin D levels mobilize skeletal calcium to maintain the blood calcium level and increase the efficiency of intestinal calcium absorption to meet the continued demands of the mineralizing skeleton. Preterm infants are compromised by the loss of the placental calcium pump at a gestational age when rapid accretion of calcium by the skeleton normally occurs. They cannot increase their bone mineral content postnatally unless given high amounts of calcium and phosphate parenterally or in their diet. Infants of hypercalcemic mothers may have suppressed parathyroid function in the first month of life, while infants of hypocalcemic mothers may have enlarged, overactive (and occasionally autonomous) parathyroid glands that caused significant skeletal demineralization *in utero*. Although increased mortality has been associated with both conditions, in most instances the disturbance in neonatal calcium homeostasis is self-limited.

As stated, the adaptive strategies of the pregnant woman differ from the lactating woman, in the face of similar calcium demands. The fetal and neonatal adaptations differ, largely because the former utilizes a placental calcium pump, while the source of calcium for the latter is the intestine. If more fully understood, these adaptive mechanisms might be exploited further to treat disorders of calcium and bone metabolism in later life, such as osteoporosis.

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