During pregnancy and lactation, female physiology must adapt to meet the added nutritional demands of the fetus and neonate. How much of a demand is there for delivery of calcium and other minerals? What adaptations are invoked during pregnancy and lactation to meet these demands? Do these adaptations alter normal biochemical and hormonal parameters of mineral and skeletal metabolism? What impact do these adaptations have during pregnancy and lactation on preexisting disorders of mineral and bone metabolism? Are there any long-term beneficial or adverse consequences of pregnancy and lactation on the skeletal health of women? All of these questions are addressed in this review.

The fetal requirement for mineral, which the pregnant woman must supply, has been determined by measuring the ash weight and mineral content of fetal cadavers between 24 wk and term. Multiple studies have demonstrated that the average full-term fetus has ~30 g calcium (77, 196, 289, 332, 868, 930, 982–984, 1022), 20 g phosphorus (289, 984, 1022), and 0.80 g magnesium (289, 332, 984, 1022). However, that fetal mineral content is not obtained at a constant rate during pregnancy; instead, at least 80% of the calcium, phosphorus, and magnesium present in a human term fetus is accreted during the third trimester; calcium transfers at 300-350 mg/day during the final 6 wk. The neonate requires 200 mg calcium daily from milk during the first 6 mo, and 120 mg calcium from milk during the second 6 mo (additional calcium comes from solid foods). Calcium transfers can be more than double and triple these values, respectively, in women who nurse twins and triplets. About 25% of dietary calcium is normally absorbed in healthy adults. Average maternal calcium intakes in American and Canadian women are insufficient to meet the fetal and neonatal calcium requirements if normal efficiency of intestinal calcium absorption is relied upon. However, several adaptations are invoked to meet the fetal and neonatal demands for mineral without requiring increased intakes by the mother. During pregnancy the efficiency of intestinal calcium absorption doubles, whereas during lactation the maternal skeleton is resorbed to provide calcium for milk. This review addresses our current knowledge regarding maternal adaptations in mineral and skeletal homeostasis that occur during pregnancy, lactation, and post-weaning recovery. Also considered are the impacts that these adaptations have on biochemical and hormonal parameters of mineral homeostasis, the consequences for long-term skeletal health, and the presentation and management of disorders of mineral and bone metabolism.

I. INTRODUCTION

II. SKELETAL AND MINERAL PHYSIOLOGY

III. DISORDERS OF BONE AND MINERAL

IV. SKELETAL AND MINERAL PHYSIOLOGY

V. DISORDERS OF BONE AND MINERAL

VI. CONCLUSIONS
The neonatal requirement for mineral, which may be supplied through breastfeeding, has largely been inferred from studies in which healthy neonates are weighed before and after each feed; a few studies have measured change in weight of the mother or the volume of milk that can be manually expressed. The calcium intake has been the main parameter considered in these calculations. Such studies reveal an average intake of 780 ml of human milk per day (20, 138, 376, 653), which, combined with an average calcium content in milk of 260 mg/l during the first 6 mo postpartum (41), reveals a total calcium intake of ~200 mg/day. Estimates of fractional calcium absorption from metabolic balance studies have shown that neonates absorb ~60–70% of calcium from human milk (5, 8, 289, 382), thereby making ~120–140 mg of calcium available for the skeleton to potentially take up each day. Fractional calcium absorption is proportional to intake and facilitated by lactose in neonates and infants (479, 480), and can be as low as 30–40% when formula (which has twice the calcium content as human milk) is consumed (6).

Estimates of neonatal mineral accretion are another indication of how much mineral is truly required, but the results have been much more variable due to different techniques, variable body sizes, ethnicity, cross-sectional versus longitudinal studies, small sample sizes, and other factors. For example, direct measurements of ash weight and mineral content have been made in vivo in longitudinal studies of healthy neonates and infants. One widely quoted study lacked any neonatal cadavers, but extrapolated from the mineral content of newborn, childhood, and adult cadavers to infer that the calcium content (the most abundant mineral in bone) increased 50 g by the end of the first year, which corresponds to a daily accretion rate of ~140 mg (546). However, other assessments of cadaveric specimens have suggested lower accretion rates of 30–50 mg/day during the first 6 mo (289, 487). When mineral content has been inferred from peripheral radiographs or by dual X-ray absorptiometry (DXA), the daily accretion rates have ranged from 80 mg (317, 976) to an unrealistic 380 mg (870); the latter study indicates problems in calibrating DXA against true mineral content of neonates and infants.

A general consensus is that average milk output, and its average calcium content, are more robust indicators of the average neonatal requirement for calcium (430). The Institute of Medicine used these calculations to determine the estimated average requirement (EAR) for calcium intake, and concluded that breastfeeding requires that an average of 200 mg calcium be provided daily through milk to a singleton during the first 6 mo (430). From this intake the neonatal skeleton is expected to accrete ~100 mg of calcium daily (430). However, the output of milk on an individual basis is determined by the suckling demands of the neonate and can certainly markedly exceed these values. Women who nurse twins and triplets can have, respectively, more than double and triple the milk output of women nursing singletons (233, 792). Individual cases of women nursing singletons have documented milk output of up to 2.4–3.1 liters per day that was sustained for more than 12 mo of lactation (583, 838). The composition of milk is similar between women with average and high outputs (233, 792), and so producing more milk will cause greater maternal losses of calcium.

Between 6 and 12 mo of age, more infant nutrition comes from solid food despite continued breastfeeding. Fewer studies have examined this time frame, so the data are less robust. The average calcium content of human milk is somewhat lower at 200 mg/l (41), and the intake is less at ~600 ml/day (246), which means that the infant has an estimated calcium intake of 120 mg/day from human milk. An additional 140 mg/day of calcium is estimated to come from solid foods to bring the total infant calcium intake to ~260 mg/day (430). Of previously cited cadaveric studies, one indicated a continued daily accretion rate of ~140 mg during the 7th to 12th months (546), whereas the other suggested that calcium accretion is ~50 mg/day during this time (289). An expected average accretion of ~100 mg/day has been assumed by the Institute of Medicine from the available data (430).

Overall, these studies indicate that pregnant women do not provide much calcium or other minerals to their fetuses until the third trimester, when the peak rate of calcium transfer exceeds 300 mg/day on average. Data for lactating women and their babies are more variable, but suggest that the average calcium requirement is more modest, at ~200 mg daily during the first 6 mo, and ~120 mg daily during the second 6 mo. All of these values, from 300 mg daily during the third trimester to 120 mg daily during late lactation, may seem achievable given normal intake of calcium and normal efficiency of intestinal calcium absorption. However, fractional calcium absorption is normally ~25% of intake in healthy adults who consume adequate calcium (420). If normal efficiency of intestinal calcium absorption were relied upon, pregnant women would have to consume an extra 1,200 mg/day during the third trimester, whereas lactating women would have to consume an extra 800 mg daily during the first 6 mo and 480 mg daily during the second 6 mo.

These estimated calcium demands during pregnancy and lactation should also be considered in the context of data from the United States National Health and Nutrition Examination Survey (NHANES) and Statistics Canada, in which actual calcium intakes have been determined for American and Canadian women. Between ages 18 and 50,
the 50th percentile for calcium intake ranges from 800-1,000 mg daily in both populations (430), for an expected fractional absorption of 200–250 mg daily. The 25th percentile of intake is ~600–700 mg daily, while the 5th percentile is about 400 mg daily, in both populations (430). Consequently, if normal intestinal calcium absorption were relied upon, most women do not consume sufficient calcium to meet the combined needs of their babies and themselves.

However, increased intakes are not required in women who consume adequate calcium because of several adaptations that are invoked during pregnancy and lactation. During pregnancy, the efficiency of intestinal calcium absorption doubles to meet the fetal requirement for calcium, whereas during lactation, skeletal resorption increases to provide calcium to milk. It is unclear why different adaptations are invoked during these two reproductive periods. These hormone-mediated adaptations normally meet the daily mineral requirements of the fetus and infant without adverse long-term consequences to the maternal skeleton. However, exceptions do occur, especially in women with habitually very low intakes of mineral, or preexisting disorders that cause skeletal fragility.

Having established the magnitude of the demands for mineral that are placed on pregnant and breastfeeding women, the body of this review will address our current stage of knowledge regarding the maternal adaptations in mineral and skeletal homeostasis that occur during pregnancy, lactation, and post-lactation recovery. Also considered are the impacts that these adaptations have on normal biochemical and hormonal parameters of mineral homeostasis, the presentation and management of preexisting disorders of mineral and bone homeostasis, and long-term skeletal health of women.

This paper is a companion to a recent review of fetal and neonatal mineral and skeletal homeostasis (492), which may be consulted for additional details and references that are pertinent to mineral and bone homeostasis of the offspring. This paper is also a substantial update and rethinking on the topic since the previous review in 1997 (499).

In the subsequent sections, animal data are discussed first and followed by relevant human data. Although the focus is on understanding human mineral and skeletal physiology during reproduction, it is necessary to discuss animal data because ethical and practical considerations prevent certain aspects of the reproductive time frame from being studied in women. As will be made clear, much of what has been found in the animal models has been directly or indirectly confirmed in human studies, but there remain important areas where this has yet to be done or where it may never be possible to do so.
A. Changes in Mineral Ions and Calciotropic and Phosphotropic Hormones

Progressive changes in serum calcium, phosphorus, and calciotropic hormone levels during human pregnancy are schematically depicted in **FIGURE 1**. Important differences among human, rat, and mouse pregnancies are listed in **TABLE 1**.

1. Calcium and phosphorus

A) ANIMAL DATA. Serum calcium declines during late pregnancy in rats (90, 105, 117, 313, 315, 425, 519, 714, 735), guinea pigs (958), sheep (21, 257, 269), goats (21), cows (21), and white-tailed deer (170). It has commonly been measured without correcting for any change in the protein or albumin concentration, even though a progressive 10–25% fall in protein and albumin has been found in pregnant rats (44, 315, 519), guinea pigs (958), and sheep (257). The drop in serum albumin causes a fall in serum calcium; however, a decrease in serum calcium does not necessarily indicate a change in ionized calcium, which is the physiologically important fraction of extracellular calcium. One study in rats consuming a 0.75% calcium diet found that the ionized calcium progressively increased while the serum calcium was falling (735), whereas rats given a 0.4% calcium diet were found to have a significant fall in the ionized calcium during late pregnancy (90). The observed declines in serum calcium during pregnancy generally represent the effect of reductions in the albumin-bound fraction, while the ionized calcium may be unchanged unless a lower calcium diet is consumed (such as 0.4% calcium).

A confounding problem in published rat studies during pregnancy and lactation is the calcium content of diet. The original 1976 and subsequently reformulated 1993 standard rodent diets of the American Institute of Nutrition indicate a 0.50% content of calcium (749). In older studies, it is typical to see diets with 0.4, 0.45, and 0.5% calcium. However, a 1% calcium diet is now more commonly used in rats, and in most studies of mice, and that impacts such parameters as serum calcium, parathyroid hormone (PTH), bone turnover, etc., as will be discussed.

Mice may differ from rats in their blood calcium parameters during pregnancy. In multiple studies wherein wild-type

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**FIGURE 1.** Schematic depiction of longitudinal changes in calcium, phosphorus, and calciotropic hormone levels during human pregnancy. Shaded regions depict the approximate normal ranges. PTH does not decline in women with low calcium or high phytate intakes and may even rise above normal. Calcidiol (25OHD) values are not depicted; most longitudinal studies indicate that the levels are unchanged by pregnancy, but may vary due to seasonal variation in sunlight exposure and changes in vitamin D intake. FGF23 values cannot be plotted due to lack of data. The data from which this summary figure has been derived are cited in section II A.
(WT) mice consumed a standard 1% calcium diet, there was no change in the ionized calcium or the serum calcium during pregnancy (296, 477, 478, 992, 994). One study measured the ionized calcium every other day in the same mice from the time of mating and noted no change during pregnancy (827). Fewer studies have assessed the serum albumin, but it was unchanged in pregnant mice compared with nonpregnant controls and did not decline during the last 7 days of pregnancy (154, 175, 197).

Physiologically important hypocalcemia can occur during pregnancy when the fetal demand for calcium exceeds the mother’s ability to maintain her own serum calcium. This may be especially true in rats where the hourly fetal demand for calcium almost equals the amount of calcium present in the maternal circulation (196). Consistent with this, increased litter number and weight are associated with nonpregnant controls and did not decline during the last several days before delivery (321). A low-calcium diet similarly provoked hypocalcemia in pregnant ewes compared with consumption of a usual and a high-calcium diet (69). In pregnant or lactating cows, such hypocalcemia is termed milk fever when it occurs near parturition or during lactation and provokes symptoms such as paresis (21). During milk fever the serum calcium can be 50% of normal or even lower despite a compensatory rise in PTH (613); the calcium demand of the fetuses is the precipitating factor. Sudden deaths during late pregnancy from presumed severe hypocalcemia have occurred in rats rendered severely vitamin D deficient (359, 361–363), and in Vdr null (296, 502) and Cyp27b1 null mice (330) maintained on a normal 1% calcium diet.

Serum phosphorus remains normal during pregnancy in rats (117, 314, 718) and mice (477, 478, 580), but may be modestly reduced in guinea pigs (958), sheep (21, 57), goats (21, 55), and cows (21). It is especially low during milk fever of cows (613). One study of pregnant rats differed by reporting a reduction in serum phosphorus (425). Serum magnesium has been less commonly measured but is unaltered in pregnant rats (315, 425), mice (580), and goats (55).

### Human Data

The earliest studies in pregnant women found a progressive 5–10% decrease in serum calcium such that the mean value fell below the normal range, thereby indicating biochemical hypocalcemia (641, 674). Such results confirmed what had already been observed in animal models, and suggested that the human fetus drains calcium from the maternal circulation in sufficient amounts to provoke maternal hypocalcemia and secondary hyperparathyroidism (15). It was not until later that it was better appreciated that the serum albumin falls during pregnancy due to normal expansion of the intravascular volume, thereby provoking a decline in the albumin-bound fraction of serum calcium (720). The decline in the albumin-bound fraction of serum calcium is physiologically unimportant.

When it became possible to measure the ultrafiltrable fraction of serum calcium (which includes both complexed and ionized calcium, but not albumin-bound calcium), no change was seen when prepregnancy and pregnancy measurements were compared (471). Later innovations resulted in the ability to measure the ionized calcium with ion-specific electrodes. Both cross-sectional (210, 227, 324) and longitudinal studies (221, 294, 721, 739, 791, 809, 813) have shown that the ionized calcium does not change during pregnancy in pregnant women.

### Table 1. Key differences in mineral physiology of human and rodent pregnancy

<table>
<thead>
<tr>
<th></th>
<th>Human</th>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium</td>
<td>Low</td>
<td>Low</td>
<td>Normal</td>
</tr>
<tr>
<td>Albumin-adjusted calcium</td>
<td>Normal</td>
<td>Low</td>
<td>Normal</td>
</tr>
<tr>
<td>Ionized calcium</td>
<td>Normal</td>
<td>Low</td>
<td>Normal</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Normal</td>
<td>Low</td>
<td>Normal</td>
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<tr>
<td>Magnesium</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>PTH</td>
<td>Low or low-normal*</td>
<td>Increased</td>
<td>Low or normal</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>PTHrP</td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Calcitriol</td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>FGF23</td>
<td>No data</td>
<td>No data</td>
<td>Increased</td>
</tr>
<tr>
<td>Estradiol</td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Intestinal calcium absorption</td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Urinary calcium excretion</td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Change in bone mass</td>
<td>Slight loss</td>
<td>Small loss</td>
<td>Increased or no change</td>
</tr>
</tbody>
</table>

*Women with low calcium or high phytate intakes have normal or increased PTH.
human pregnancy, even though the mean serum calcium simultaneously drops ~10% into the hypocalcemic range (221).

The albumin-corrected serum calcium is a reasonable substitute for the ionized calcium, as several longitudinal and cross-sectional studies have shown it to remain stable across all three trimesters, despite the concurrent fall in serum albumin (85, 599, 632, 646, 778).

Twin pregnancy does not impair the ability of a woman to maintain a normal serum calcium, as shown in a comparative study of women bearing twins versus singletons (646).

Serum phosphorus shows no change in most studies during human pregnancy (33, 207, 221, 294, 468, 632, 641, 674, 791) and was similarly unchanged during twin pregnancies (646). There is also no change in the renal tubular reabsorption of phosphorus (207, 329, 468). Serum magnesium is similarly unaltered during pregnancy (33, 207, 221, 294, 721).

C) SUMMARY. Expansion of the intravascular volume during normal human pregnancy causes a fall in serum albumin and, thereby, the serum calcium. However, the ionized calcium and albumin-corrected serum calcium do not change, confirming that the physiologically relevant circulating fraction of calcium concentration remains normal. Either the ionized calcium or the albumin-corrected serum calcium may be reliably used to assess adequacy of the blood calcium concentration during pregnancy. Animal models show a similar albumin-related fall in serum calcium, whereas both the serum and ionized calcium levels appear to stay constant in pregnant mice, likely because albumin does not decline. Ionized calcium is more likely to fall in rodents when challenged by larger litters, low calcium diets, or disorders of vitamin D physiology.

2. PTH

A) ANIMAL DATA. Rodents have generally been described as developing secondary hyperparathyroidism during normal pregnancy (499), although the extent to which this occurs depends on the calcium content of the diet. One of the earliest studies of pregnant rats found that both COOH-terminal and NH2-terminal PTH concentrations declined between gestational days 16.5 to 21.5 to reach the lower end of the normal range (NH2-terminal values became undetectable) before increasing significantly postpartum (the calcium content of the diet was not specified) (309, 311). However, several other studies in rats used 0.4 to 0.75% calcium diets and found that PTH is modestly increased in late pregnancy compared with nonpregnant rats (90, 105, 735); one of these studies simultaneously found increased PTH bioactivity (105). A more recent study used a 1.18% calcium diet and found lower baseline PTH levels that almost tripled by late pregnancy (920). Parathyroid gland volume is doubled in normal pregnant rats compared with virgins (784, 842), while in vitro studies suggest that, for any level of extracellular calcium, parathyroids from pregnant rats secrete more PTH than those from virgin rats (806). In addition to the obvious effect of the varying calcium content of the rodent diets, there may be additional variability between studies due to rats strains, and differing NH2-terminal or COOH-terminal radioimmunoassays (RIAs) that were designed to detect human PTH. In most of these studies the serum calcium alone was measured without correction for the lower serum albumin. But a 0.4% calcium diet provoked a decrease in the ionized calcium together with an increase in PTH in pregnant rats (90), while more marked hypocalcemia and secondary hyperparathyroidism developed when pregnant rats were challenged by a 0.1% calcium-restricted diet (321).

More recently, pregnant mice consuming a standard 1% calcium diet have been consistently found to have mean PTH values reduced to ~20% of the nonpregnant value, with many individual values being undetectable, as determined by rodent or mouse-specific PTH immunoradiometric assays (IRMAs) and enzyme-linked immunoassays (ELISAs or ELAs) (477, 478, 992, 994). Consumption of a 2% calcium diet resulted in even lower PTH levels in nonpregnant mice that declined slightly but nonsignificantly during pregnancy (296, 330).

These findings confirm that whether the PTH concentration changes during pregnancy, and the direction of that change, are dependent on the rodent’s dietary calcium intake. Rats appear more prone to develop secondary hyperparathyroidism than mice during pregnancy. As will be made clear in subsequent sections, the proportionately greater calcium demand faced by the pregnant rodent mandates that calcium be provided from both intestinal absorption and skeletal resorption. Secondary hyperparathyroidism enables the rodent to upregulate both routes of mineral delivery, especially when the fetal demand is at its peak during late pregnancy.

B) HUMAN DATA. The earliest data on PTH measurements in pregnant women used first-generation radioimmunoassays that yielded inconsistent results (23, 209, 219, 255, 329, 387, 721, 754, 760, 876, 975, 981, 983), with many of these studies finding significantly increased PTH concentrations in the latter half of pregnancy (209, 219, 255, 721, 754, 760, 975). Taken together with the concurrent decline in serum calcium, this resulted in the concept that pregnancy represents a physiological state of secondary hyperparathyroidism. It was not until some years later that it became clear that this conclusion was erroneous. As noted above, the fall in serum calcium is caused by a fall in albumin levels, while the ionized calcium remains normal during human pregnancy. Furthermore, these early, insensitive
PTH radioimmunoassays measured multiple biologically inactive fragments of PTH (725, 959).

Two-site immunoradiometric (IRMA) PTH assays are more sensitive and accurate (725). In cross-sectional studies of North American and European women consuming a diet adequate in calcium, PTH is typically suppressed to the lower end of the normal range during all three trimesters (207, 227, 294, 324, 791, 809, 905). In longitudinal studies from North America, Europe, and Asia, the mean PTH level was suppressed to the lower end of the normal range or below it during the first trimester, and either remained suppressed or increased to mid-normal by the end of pregnancy (33, 85, 207, 221, 304, 632, 739, 813). Twin pregnancy did not change the serum PTH value compared with women bearing singletons (646).

Newer “bio-intact” assays exclude NH2-terminal truncated, inactive PTH7–84 fragments that accumulate in chronic renal failure patients (931), but no studies have reported use of these assays in pregnant women. It is expected that these assays would similarly demonstrate low concentrations of PTH during pregnancy.

Despite the preponderance of evidence that PTH becomes suppressed during human pregnancy, some modern medical textbooks still claim incorrectly that pregnancy represents a state of physiological hyperparathyroidism (899).

Although suppression of PTH with modern assays has also been shown in pregnant women from Asia and Africa (11, 33, 813, 826), several cohort and longitudinal studies from these regions have found that PTH did not decline during pregnancy and in some cases it rose above normal (585, 601, 778, 805, 844). The lack of suppression of PTH during pregnancy in women from these regions is likely the consequence of diets that are traditionally low in vitamin D or calcium, and high in phytate (which blocks calcium absorption). Low calcium intake or absorption will provoke compensatory secondary hyperparathyroidism during pregnancy.

C) SUMMARY. During normal human pregnancy, serum or plasma PTH typically declines to low levels early on, before returning to the mid-normal range by term. This suppression may not occur (and secondary hyperparathyroidism may develop) in women who have especially low intakes of calcium or vitamin D, or high intakes of phytate; such diets are more common in certain regions of Asia and Africa. In rodents, the ambient PTH level is influenced by the dietary intake of calcium, with rats typically having increased PTH during pregnancy that rises even higher with a calcium-restricted diet, whereas mice typically have reduced PTH levels on a standard diet that fall even further on a high-calcium diet.

The influence of PTH in regulating mineral metabolism during pregnancy is further discussed in subsequent sections that address the synthesis of calcitriol (sect. IIIA) and the adaptations that occur in the intestines (sect. IIA4) and the kidneys (sect. IIC), and bone (sect. IID). PTH’s role is also illuminated by data on the effects of primary hyperparathyroidism (sect. IIIB), hypoparathyroidism (sect. IIIID), and pseudohypoparathyroidism (sect. IIIE) during pregnancy.

3. Parathyroid hormone-related protein

A) ANIMAL DATA. Parathyroid hormone-related protein (PTHrP) was first cloned in 1987 from human tumors that cause humoral hypercalcemia of malignancy (593, 882, 889). It is a prohormone that is processed into several moieties, each of which has been postulated to have its own receptor and function(s) (682). The NH2-terminal region of PTHrP has partial homology to PTH within its first 13 amino acids, and a similar structure within the first 34 amino acids, which enables it to activate the common PTH/PTHrP receptor and have near-identical functions as PTH (494). The mid-molecular region of PTHrP has been shown to stimulate placental calcium transport in fetuses, but whether it has any role in the mother is unknown (152, 500). The COOH-terminal regions of PTHrP have been shown to inhibit osteoclast-mediated bone resorption both in vitro (280, 281) and in vivo (202), so it is conceivable that this portion of PTHrP could act to prevent excessive resorption of the maternal skeleton during pregnancy. The mid-molecular and COOH-terminal regions of PTHrP have no homology to PTH.

PTHrP conceivably reaches the maternal circulation from several sources, including the parathyroids, placenta (496, 817), mammary tissue (738), and uterus (66). Its expression in rat mammary tissue is increased by prolactin (906), while its expression within the uterine myometrium of the rat increases in response to estradiol (909). Consequently, high prolactin and estradiol levels associated with pregnancy may drive increased expression of PTHrP within these tissues. Although PTHrP is expressed by day 14 of pregnancy in rat mammary glands (738), the level remains quite low compared with the expression achieved in the immediate postpartum (738, 1004) and with suckling (907, 911). Studies in sheep have determined that clearance of PTHrP1–34, PTHrP1–86, or PTHrP1–141 do not change during pregnancy (741, 743).

PTHrP has been assumed to circulate at increased levels in pregnant animals because it does so in humans, but surprisingly few measurements have been made in animals. No significant increase in PTHrP was detected in one study of pregnant versus nonpregnant mice (956). In another study, PTHrP was detected in the circulation of pregnant rats, and the value did not change over the last 5 days of pregnancy, but no measurements were done in nonpregnant rats, so it is unclear if the concentration was increased (364). PTHrP
increased in pregnant goats beginning a day or two before expected delivery and even further during parturition (744, 771) compared with nonpregnant animals. Most of the measurements were with assays designed to detect human PTHrP1–86 (364, 744, 956) rather than species-specific assays, and such assays will not detect the potentially more abundant (and biologically active) PTHrP1–36 (this issue is discussed in more detail in the next section).

b) Human Data. Before discussing data on circulating PTHrP values during human pregnancy, it is important to review common problems of sample collection and preparation, and available assays.

First, PTHrP is rapidly cleaved and degraded in serum. Appropriate samples for PTHrP measurement should be collected as EDTA plasma in a chilled tube that also contains a protease inhibitor (such as aprotinin), kept chilled on ice during transport to the laboratory, and (within 15 min) spun in a refrigerated centrifuge, separated, and frozen. Even with these rigorous steps taken to reduce proteolytic cleavage, PTHrP begins to degrade in EDTA- aprotinin plasma within 15 min of sample collection (423). In contrast to this ideal sample collection and processing, many studies used stored sera that had been allowed to clot at room temperature for 60 min before routine processing (925). Improper sample collection and processing leads to failure to detect the true concentration of PTHrP, and may even result in undetectable values when high values of PTHrP are likely to be present (such as in lactation or humoral hypercalcemia of malignancy). These issues confound individual case reports and small case series, wherein a clinician’s request for a PTHrP level on a patient is likely to lead to routinely collected and processed sera or EDTA-plasma being sent out to a reference laboratory, and an undetectable result obtained despite the likely presence of PTHrP in that patient’s circulation.

Second, the most widely used assays in clinical studies are those that measure PTHrP1–86. Such a length does not exist in humans (or animals) based on the predicted cleavage sites of the translated product (494, 682). There are three predicted full-length forms of human PTHrP that arise through alternative splicing, including PTHrP1–139, PTHrP1–141, or PTHrP1–173 (rodents have only a single full-length form) (682). A PTHrP1–86 assay will detect all three of these forms, but it will not detect PTHrP1–36. This is a significant problem because PTHrP1–36 is expected to derive in vivo from posttranslational processing of all three full-length forms of PTHrP (682), and conceivably may be the most abundant of the biologically active NH₂-terminal forms of PTHrP. The presence of PTHrP1–36 has been confirmed in the media of cultured human and rodent cell lines (682, 853, 1007), but the relative abundance of PTHrP1–36 versus the other circulating forms has not been determined in humans [PTHrP37–74 was shown to be 9-fold higher than PTHrP1–74 in plasma of patients with humoral hypercalcemia of malignancy, but PTHrP1–36 was not assayed in that study (137)]. This problem may be less of a concern in rodents since there is no alternative splicing and only one full-length form. A PTHrP1–34 assay should detect each of PTHrP1–36, PTHrP1–139, PTHrP1–141, and PTHrP1–173, and may be the preferred assay to use but only with optimally collected and processed plasma samples.

As noted earlier, there are predicted mid-molecular and COOH-terminal forms of biologically active PTHrP. In the human these include PTHrP38–94, PTHrP38–101, PTHrP107–139, and possibly PTHrP141–173, each of which may have its own receptor and distinct role (494, 682, 712, 996). The clinically available PTHrP1–34 and PTHrP1–86 assays do not detect these forms. Consequently, no data are available on concentrations of mid-molecular or COOH-terminal PTHrP during pregnancy.

Despite the aforementioned problems with the PTHrP1–86 assay, four longitudinal studies have shown a significant increase in PTHrP across the three trimesters, beginning as early as 3–13 wk of gestation and reaching up to triple the first-trimester value by term (33, 75, 304, 1001). A cross-sectional study found that the PTHrP level in women at term was twice that of nonpregnant controls (333). Other studies have found elevated levels at term based on the expected values in nonpregnant adults, but did not include a nonpregnant control group (111, 278). These results contrast with smaller cross-sectional studies that found PTHrP1–34 (473) or PTHrP1–86 (262, 697) concentrations were no different than in unrelated nonpregnant controls (333). Other studies have found elevated levels at term based on the expected values in nonpregnant adults, but did not include a nonpregnant control group (111, 278). These results contrast with smaller cross-sectional studies that found PTHrP1–34 (473) or PTHrP1–86 (262, 697) concentrations were no different than in unrelated nonpregnant controls or the expected normal range, and two longitudinal studies that found a nonsignificant increase (390) and no change (85) in PTHrP1–86. In the latter study, all PTHrP1–86 values were undetectable, which raises the concern that improper methodology was the cause (sample type, collection, and processing were not specified) (85).

Longitudinal studies are more sensitive and robust in detecting differences in a time course compared with cross-sectional studies. With the assumption that the four longitudinal studies (33, 75, 304, 1001) are correct in demonstrating a gestational increase in PTHrP that reaches peak levels in the third trimester, PTHrP may importantly contribute to the regulation of maternal mineral homeostasis during pregnancy, including an upregulation in calcitriol (see sect. IIA4) and suppression of PTH.

Which tissue sources contribute to the increasing concentrations of PTHrP in the maternal circulation during human pregnancy has not been established. Candidate sites include the placenta, breasts, parathyroids (39, 222, 252, 426, 610), amnion (278, 283), decidua and myometrium (283), umbilical cord (284), and other fetal tissues. The potential importance of placental sources of PTHrP has been illus-
trated by two clinical cases. One woman experienced a late-term hypercalcemic crisis with a high plasma concentration of PTHrP; an urgent C-section was followed within 6 h by symptomatic hypocalcemia and an undetectable PTHrP level (270). A second woman with known hypoparathyroidism was able to gradually eliminate use of calcitriol and halve the dose of calcium taken during her pregnancy. Immediately after delivery, she developed sudden and symptomatic hypocalcemia that resolved after breastfeeding was initiated (890). In both cases, removal of placental PTHrP was the likely factor causing a rapid fall in serum calcium, and in the first case a rapid and marked decline in circulating PTHrP was confirmed.

Other clinical cases have demonstrated that the breasts are also a physiologically relevant source of PTHrP during pregnancy, and can provoke hypercalcemia when PTHrP production is overabundant. Such hypercalcemia has developed in late pregnancy and the puerperium, and even in nonpregnant women who simply have large breasts (603, 800). In one case of marked hypercalcemia during pregnancy, serum PTH was undetectable, PTHrP expression was increased within the mammary tissue, and a bilateral mastectomy was required in the second trimester to correct the hypercalcemia (435, 474). A postpartum PTHrP-mediated hypercalcemic crisis has also occurred when a woman was unable to breastfeed because her newborn baby was in an intensive care unit (800). The hypercalcemia was first noted on the second postpartum day, and persisted into the third day with a plasma PTHrP concentration that was markedly elevated at 28.4 pM (normal <1.1 pM). The delayed time course with elevated PTHrP on the third postpartum day did not implicate the placenta because of PTHrP's short half-life of minutes in the circulation. In another longitudinal study the plasma PTHrP level rose higher by 48 h after delivery rather than declining, which supports sustained PTHrP production by the breasts that rises further with the onset of lactation (1001). Colostrum and milk expressed from pregnant women have also been show to contain PTHrP, with the content in colostrum being no different from breastfeeding women, whereas the PTHrP content rises in milk during each breastfeeding episode (575).

The gradual rise in PTHrP across the trimesters, as demonstrated in four longitudinal studies (33, 75, 304, 1001), parallels a progressive increase in serum calcitriol to peak levels in the third trimester (see sect. II A4). The increase in PTHrP may imply that it is responsible for stimulating renal 1α-hydroxylase (Cyp27b1). However, other data suggest that PTHrP may not be as potent as PTH in stimulating Cyp27b1 in vivo (308, 413, 414, 494, 988). The extent to which PTHrP stimulates the rise in calcitriol during pregnancy remains to be tested in animal models. The high levels of estradiol may be contributing to stimulation of PTHrP production during pregnancy. In vitro studies have demonstrated that estradiol increases PTHrP mRNA expression from cultured human myometrial cells (160), but it did not stimulate mammary epithelial cells to produce PTHrP (808).

There are no data on blood levels or potential roles of mid-molecular and COOH-terminal PTHrP during pregnancy. Fetal-placental calcium transport is regulated by mid-molecular PTHrP within the fetal circulation, but whether maternal PTHrP plays a role in regulating this process is unknown (2, 152, 500).

That the rise in PTHrP during normal pregnancy is physiologically important has been made most evident in hypoparathyroid women, as discussed in sections IID and VD.

C) SUMMARY. PTHrP reaches the maternal circulation during pregnancy, most likely from the placenta and breasts, as well as possibly the uterus and fetal tissues. PTHrP progressively increases in maternal plasma to reach peak levels in the third trimester, and likely contributes to the normal regulation of mineral homeostasis during pregnancy. This includes maintenance of the ionized calcium, stimulation of Cyp27b1 to produce increased levels of calcitriol (see sect. II A4), and suppression of PTH. Several case reports support these findings.

PTHRP's role in regulating mineral metabolism during pregnancy is further addressed in section III, D and F. Its role to regulate placental calcium transport and fetal mineral homeostasis has been addressed in the companion review (492).

4. Calcitriol and calcidiol

A) ANIMAL DATA. Serum calcitriol concentrations increase two- to sevenfold during normal pregnancy in rats, mice, sheep, guinea pigs, and rabbits (90, 92, 358, 477, 478, 502, 512, 718, 735, 775, 1021). The vitamin D binding protein level increased slightly in mice and declined modestly in rats and guinea pigs (104, 478, 958). This confirms that the free concentration of calcitriol must be substantially increased during late pregnancy in these species. Longitudinal studies in rats have shown that the increase in total calcitriol does not occur until the last several days of pregnancy (90, 358, 735), during which time fetal mineral accretion is rapidly occurring. Larger litter sizes or weights correlate with higher maternal calcitriol (90). In guinea pigs, the free level of calcitriol increased before the third trimester and before total calcitriol increased (958).

The rise in serum calcitriol is the result of upregulated synthesis of calcitriol, and not reduced catabolism or metabolic clearance, as confirmed by studies in pregnant rats, sheep, and rabbits (238, 702, 775, 776). Renal Cyp27b1 has been
shown to be upregulated two- to fivefold from in vitro analysis of homogenates obtained from kidneys of pregnant rabbits and guinea pigs (282, 512). Consequently, the maternal kidneys are likely the source of increased production of calcitriol, as they are in the nonpregnant adult.

However, the maternal decidua, placenta, and fetus are also potential sources of calcitriol due to expression of Cyp27b1 in these tissues (334, 346, 896, 977, 978). The placenta has often been assumed to account for the rise in maternal calcitriol during pregnancy, but this appears to be incorrect. The same gene is responsible for renal, decidual, placental, and fetal expression of Cyp27b1 (334). The ability of nonrenal tissues to contribute to the production of calcitriol has been tested by administering tritiated 25-hydroxyvitamin D (calcidiol or 25OHD) immediately after nephrectomy to pregnant and nonpregnant rats. Some tritiated calcitriol appeared in the circulation of pregnant nephrectomized dams (much reduced compared with normal), whereas none appeared in the circulation of nonpregnant nephrectomized rats (345, 978). Additional studies examined 5/6-nephrectomized rats, a model of chronic renal insufficiency, which as virgins have marked secondary hyperparathyroidism and calcitriol concentrations that are reduced 40% compared with intact virgin rats (89). During subsequent pregnancies, calcitriol tripled in the 5/6-nephrectomized rats to the same high level achieved by intact pregnant rats, while PTH decreased by 80% in the 5/6-nephrectomized rats to near-normal values (89).

The authors of the 5/6 nephrectomy study concluded that the rise in calcitriol during pregnancy could only be due to contributions from extra-renal sources of 1α-hydroxylase. However, the result is still compatible with the remnant kidneys, which achieved a calcitriol level 60% of normal in the nonpregnant state, being able to increase calcitriol production in response to the increased stimulation that occurs during pregnancy. The normal peak calcitriol level achieved in 5/6-nephrectomized pregnant rats (89) contrasts with the substantial reduction in calcitriol production during pregnancy in completely nephrectomized rats (345), and supports that the 1/6 remnant kidney was likely responsible for the increased production of calcitriol during pregnancy. Subsequent studies in the 5/6 nephrectomy model have shown that it creates only mild renal insufficiency with creatinine clearance reduced ~50% of normal in nonpregnant or pregnant rats (794).

Taken together, these studies in partly and completely nephrectomized rats showed that extra-renal tissues can contribute some calcitriol to the maternal circulation, but whether this means the placenta, fetus, or other maternal tissues was not ascertained. These extra-renal sources likely do not explain the high calcitriol levels that are normally achieved during pregnancy in rodents. Additional studies in 1α-hydroxylase null Hannover pigs also support that the fetus and placenta likely do not contribute substantial calcitriol to the maternal circulation, because maternal calcitriol levels are very low during pregnancy and similar to nonpregnant values, even when bearing heterozygous fetuses that produce calcitriol (520, 521). Furthermore, in a preliminary study, Cyp27b1 null mice had calcitriol levels near the detection limit during pregnancy, and these values were not significantly different during pregnancy despite the presence of Cyp27b1+/− placenta (330). Additionally, the expression of Cyp27b1 mRNA is 35-fold higher in maternal kidneys during pregnancy compared with placentas obtained from the same mouse (478). This suggests that the relative activity of Cyp27b1 in the placenta is too low to account for the rise in calcitriol in the maternal circulation. However, the expression of Cyp24a1 (24-hydroxylase) in normal mouse placentas was about one-third the level of expression in maternal kidneys; therefore, the multiple placentas in one pregnant rodent may substantially contribute to the conversion of calcitriol and 25OHD into 24-hydroxylated forms (478).

What is driving the increased production of calcitriol during pregnancy? Circulating PTH is increased at this time in some but not all studies of pregnant rats (90, 105, 309, 735), yet there was no correlation between the PTH concentration and the achieved calcitriol level (90). Moreover, PTH fell over 80% in 5/6-nephrectomized rats during which time the calcitriol level tripled (89). PTH is reduced in pregnant mice (296, 330, 477, 478, 992, 994), which achieve up to sevenfold increases in serum calcitriol. These finding suggest that PTH is not responsible for stimulating the Cyp27b1 to increase the concentration of calcitriol in the maternal circulation.

This hypothesis has been confirmed through two independent lines of investigation. First, despite parathyroidectomy in rats, there was a threefold increase in calcitriol during pregnancy to achieve peak levels nonsignificantly lower than in intact rats (658). Second, when the Pth gene was ablated, such Pth null mice have undetectable circulating PTH but achieve a fivefold increase in calcitriol during pregnancy that is modestly but not significantly lower than that achieved by their pregnant WT sisters (478).

Pregnant Pth null and WT mothers had similarly increased expression of Cyp27b1 mRNA in the kidneys compared with their nonpregnant counterparts, whereas 24-hydroxylase (Cyp24a1) mRNA expression was fivefold higher in pregnant Pth null mice compared with their WT sisters (478). These results are consistent with the known role of PTH to suppress Cyp24a1 activity and expression (752, 835, 1023, 1024); therefore, in the absence of PTH, Cyp24a1 expression and activity increase. Parathyroidectomized rats also had consistently higher 24,25-hydroxyvitamin D concentrations throughout pregnancy compared with intact rats (658). Consequently, the modest blunting of
the peak calcitriol level during pregnancy in parathyroidecto-
mized rats and Pth null mice may be the result of in-
creased 24-hydroxylation of calciotriol and 25OHD, and not
reduced synthesis of calciotriol (478, 658). Therefore, al-
though PTH is the dominant stimulator of Cyp27b1 in
nonpregnant rodents, it clearly does not have this role dur-
ing pregnancy.

If not PTH, what stimulates the renal expression and activ-
ity of Cyp27b1 during pregnancy? Among known hor-
mones that could be potential regulators are PTHrP, estradi-
ol, calcitonin, placental lactogen, and prolactin. How-
ever, very few studies have tested the roles of these
hormones. As mentioned, PTHrP mimics the actions of
PTH on the PTH/PTHrP receptor and likely contributes to
the stimulation of Cyp27b1 activity and expression. How-
ever, other investigators have suggested that PTHrP is less
potent than PTH at stimulating Cyp27b1 due to PTHrP’s
somewhat different receptor binding, signaling character-
istics, and crystal structure (308, 494, 988). Estradiol (51),
calcitonin (464), placental lactogen (866), and prolactin
(866, 867) have acutely stimulated renal Cyp27b1 in vitro,
whereas placental lactogen increased calciotriol in hypophy-
sectomized, nonpregnant rats in vivo (894). Calciotriol has
also been shown to have the reciprocal effect of suppressing
calcitonin (647, 840). It is conceivable that a combination
of factors (increasing concentrations of PTHrP, estradiol,
calcitonin, and placental lactogen, for example) contribute
to upregulation of Cyp27b1 during pregnancy, rather than
one factor being primarily responsible. It is also possible
that an unidentified factor stimulates Cyp27b1 during preg-
nancy.

The multiple fetuses in rodent pregnancies represent a
potential drain on maternal stores of 25OHD, the substrate
for calciotriol, since 25OHD readily crosses the placenta
(353) whereas calciotriol does not (665). The concentration
of 25OHD decreased ~50% on day 20 of pregnancy in rats
consuming a vitamin D-replete diet (358, 735), which ap-
ppears to confirm that rodent fetuses are a significant drain
on the maternal supply of 25OHD. However, in guinea pigs
that bore 3–4 pups/litter, there was no decline in 25OHD
during pregnancy (960). Two cross-sectional studies found
that the 25OHD level was 20–30% lower in pregnant versus
nonpregnant sheep (57, 703).

B) HUMAN DATA. Similar to the findings in the animal models,
cross-sectional studies have found a doubling or tripling of
serum calciotriol that begins early in the first trimester and
reaches the highest levels in the third trimester (81, 207,
287, 324, 387, 578, 599, 632, 764, 981, 987). Longitudinal
studies have confirmed a progressive increase in free and
bound calciotriol that is maintained to term (33, 81, 764,
809, 813, 959, 987). Free calciotriol was reported to be in-
creased in the third trimester in one study (81). However,
the more modest (20–40%) increase in vitamin D binding
protein during pregnancy, and the decline in serum albu-
mion, indicate that free calciotriol is likely increased in all
three trimesters (33, 399, 599, 764, 1019).

In a large cross-sectional study, serum calciotriol increased
threefold in women with singleton and twin pregnancies,
and there was no difference between the two groups (646).
There was also no difference in vitamin D binding protein
and albumin levels, which means that singleton and twin
pregnancies likely resulted in identical increases in free cal-
ciotriol (646). A smaller longitudinal study also found no
difference in serum calcitriol between twin and singleton
pregnancies at any time point during pregnancy (746).

PTH is normally the dominant regulator of Cyp27b1 in
adults, and without it (such as in hypoparathyroidism), cal-
cirol levels are quite low (782). A substantial curiosity
about the marked pregnancy-related increase in calcitriol is
that it occurs while PTH is often suppressed to low levels,
which suggests that PTH is not responsible for the upregu-
lation of Cyp27b1.

Estradiol, prolactin, and placental lactogen levels are high
during human pregnancy and may in part stimulate
Cyp27b1, as suggested by data from animals cited above.
The potential role of estradiol is supported by the finding
that estrogen replacement therapy in postmenopausal
women causes an increase in free and total calcitriol levels
in serum (172). Conversely, the elevated prolactin levels of
pregnancy seems unlikely to stimulate Cyp27b1 because
chronic hyperprolactinemia in nonpregnant individuals does
not alter calcitriol (514). However, chronic hyperprolactine-
emia differs in that estradiol levels are low, in contrast to the
high levels during pregnancy. The possibility that high estra-
diol and high prolactin (or placental lactogen) act in concert
during pregnancy to stimulate Cyp27b1 has not been exam-
ined in any human studies. An additional analysis in pregnant
women found that the increased calcitriol did not correlate
with PTH, prolactin, estrone, estradiol, estriol, or human pla-
cental lactogen (746).

That the placenta and other extrarenal sources of Cyp27b1
do not contribute a substantial amount of calcitriol to the
maternal circulation is supported by the finding that an
anephric woman on dialysis had low calcitriol levels before
and during a pregnancy (937). The author is aware of other
unpublished cases in which calcitriol remained low in preg-
nant anephric women.

There is conflicting evidence as to whether serum 25OHD
changes during human pregnancy. In several large studies
there was no significant change (121, 200, 386, 399, 764,
778, 809, 966) or even a slight increase (637). This includes
the placebo arm of a study in which women were vitamin
D-deficient at baseline (mean 25OHD level of 20 nM or 8
ng/ml) (121), and two studies in which women had a mean
25OHD of about 60 nM (24 ng/ml) prior to being randomized to receive up to 4,000 IU of vitamin D per day (399, 966). Conversely, two longitudinal studies found a modest but significant decline in total 25OHD (33, 1019) or calculated free 25OHD values (1019) during pregnancy. These latter studies were smaller and may have been influenced by seasonal and late-pregnancy alterations in sunlight exposure and diet. The bulk of the data indicate that maternal 25OHD is not substantially drained into the fetal circulation, or consumed by conversion into calcitriol.

A large cross-sectional study found that the maternal 25OHD was also stable across all trimesters in both singleton and twin pregnancies; however, the 25OHD level was ~30% lower in women carrying twins at all time points (646). It is unknown whether this difference was due to twin babies draining the maternal supply of 25OHD, or lower sunlight exposure or vitamin D intake in women bearing twins. The lack of a progressive change in 25OHD during pregnancy, especially a progressive difference between mean values in women bearing singletons versus twins, makes it less likely that two babies represent a drain on the maternal supply of 25OHD. A smaller longitudinal study found no difference in 25OHD levels between women bearing twins versus singletons (746), which may be an indication that the larger cross-sectional study was confounded by inherent differences between the two groups of mothers rather than demonstrating an effect of twin pregnancy. There are no data from women bearing triplets.

While the threshold level of 25OHD that indicates optimal vitamin D sufficiency during pregnancy continues to be debated (401, 430, 495, 773), it should be clear that the observed suppression of PTH and doubling or tripling of calcitriol during pregnancy are not attributable to maternal vitamin D deficiency. Moreover, supplementation with the equivalent of 1,000 to as much as 5,000 IU vitamin D daily during pregnancy did not alter maternal serum calcium, albumin-corrected calcium, phosphorus, or PTH, nor did it blunt the rise in calcitriol (239, 399, 778, 966). The increase in free and bound calcitriol, despite a decline in PTH, is evidently a programmed response to pregnancy, with the key stimulators of Cyp27b1 remaining unidentified. In a study in which intakes of 400 IU, 2,000 IU, and 4,000 IU vitamin D were compared, mean calcitriol levels reached a plateau at a 25OHD level of 100 nM regardless of vitamin D intake (399). This plateau may indicate the maximal capacity of renal Cyp27b1 to convert 25OHD into calcitriol, balanced against probable upregulation of Cyp24a1 to catabolize 25OHD and calcitriol. Moreover, the plateau does not necessarily mean that 100 nM is the target level of 25OHD to be achieved in pregnancy, as the authors of that study concluded (399), since no benefit of this high level of 25OHD or calcitriol has been demonstrated.

c) SUMMARY. Total and likely free levels of calcitriol begin to increase in the first trimester and may reach triple or more the nonpregnant value by the third trimester. This increase likely contributes to upregulation of intestinal calcium absorption during pregnancy and, indirectly, to suppression of PTH. Increased production of calcitriol comes almost entirely from maternal kidneys and not the placenta or fetus, but the factors that stimulate renal Cyp27b1 during pregnancy remain to be elucidated. 25OHD levels are stable during pregnancy despite increased conversion to calcitriol and transplacental passage of 25OHD to the baby, and even in the face of a twin pregnancy. Conversely, 25OHD has been shown to decline by 50% in rats that bear large litters, with more modest to no change in animals bearing several pups (pigs) or singletons (sheep).

The influence of calcitriol in regulating mineral metabolism during pregnancy is further discussed in the sections that address adaptations that occur in the intestines (see sect. II) and bone (see sect. II D). Calcitriol’s role is also illuminated by data on the effects of vitamin D deficiency and genetic vitamin D resistance syndromes (see sect. IIIG) during pregnancy.

5. Calcitonin

A) ANIMAL DATA. Serum calcitonin is increased during pregnancy in rats (315), mice (615), monkeys (759), sheep (58, 310, 316), deer (170), and goats (58, 310). In addition to increased production by the C-cells of the thyroid (316), additional sources of expression that may contribute to the circulating level of calcitonin include mammary tissue (450, 938), placenta (450, 496), and pituitary (755, 887). Increased synthesis of calcitonin reasonably explains the higher levels, but clearance of calcitonin has not been measured. Studies in pregnant rhesus monkeys support that synthesis is likely increased, since acute calcium infusions caused a progressively greater calcitonin increment during pregnancy (760). This increase in calcitonin occurs despite high levels of calcitriol, which has been shown to suppress calcitonin (647, 840). Estradiol stimulates calcitonin expression in the rat thyroid (840), so the high levels of estradiol during pregnancy may contribute to increased calcitonin in the circulation.

B) HUMAN DATA. Serum levels of calcitonin generally increase during pregnancy compared with nonpregnant values and may exceed the normal range (33, 221, 255, 388, 503, 797, 839, 981, 985, 990). But the evidence is not entirely consistent, since other longitudinal studies have found no changes (764) or decreased calcitonin in all three trimesters (632). Several longitudinal studies found no increase in pregnancy compared with postpartum values (209, 721, 813). However, since calcitonin has also been shown to be elevated postpartum (see sect. IV A4), the lack of change compared with postpartum does not rule out an increase during pregnancy.
Higher estradiol, estrone, and estriol levels during pregnancy may stimulate calcitonin synthesis. This is supported by the observation that in reproductive-age women, mean plasma calcitonin was three to five times higher in women who were pregnant or taking oral contraceptives, compared with nonpregnant women who did not use oral contraceptives (388). Moreover, treatment of postmenopausal women with either estradiol or a synthetic estrogen (ethinylestradiol) resulted in a significant increase in plasma calcitonin (877).

The thyroid C-cells are often assumed to be the only site of calcitonin synthesis, but the breast and placenta become significant production sites during pregnancy (52, 131). This has been most clearly demonstrated in women who have previously undergone a total thyroidectomy. During subsequent pregnancies the mean serum calcitonin has risen from undetectable to 16 pg/ml, and exceeded the value of 11 pg/ml in nonpregnant women with intact thyroids (131). The potency of the breasts is indicated by the finding of calcitonin in colostrum and milk at 45 times its concentration in serum, with no difference in the values between thyroidectomized and intact women (131).

C) SUMMARY. Calcitonin increases during pregnancy in women and animals, evidently coming from placenta and breasts in addition to the C-cells of the thyroid. Calcitonin’s possible physiological role in protecting the maternal skeleton during pregnancy is discussed in section IIIH.

6. Fibroblast growth factor-23

A) ANIMAL DATA. Fibroblast growth factor-23 (FGF23) is predominantly expressed in osteocytes and osteoblasts (841); a low level of Fgf23 mRNA expression was also found in the murine placenta when WT and Fgf23 null placentas were compared (581). FGF23’s apparent function is to control the availability of phosphorus at mineralizing surfaces of bone, and it does so by regulating the serum phosphorus through actions on the kidneys, intestines, and parathyroids (841). It downregulates the expression of sodium-phosphate cotransporters 2a and 2c (NaPi2a and NaPi2c) in proximal renal tubules (834), thereby causing excretion of phosphorus into the urine. It inhibits Cyp27b1 and increases expression of Cyp24a1, which will respectively decrease synthesis and increase catabolism of calcitriol, thereby leading to reduced calcitriol concentrations in the circulation (834). By lowering serum calcitriol and also by directly reducing the intestinal expression of NaPi2b, FGF23 should also lead to reduced intestinal phosphate absorption (834). FGF23 also inhibits PTH release from the parathyroids (834). High circulating levels of FGF23 cause hypophosphatemia characterized by renal phosphate wasting and low calcitriol, while absence of FGF23 causes hyperphosphatemia with impaired renal phosphate excretion and inappropriately elevated calcitriol (70).

Longitudinal studies in several mouse models have shown that FGF23 increases twofold during pregnancy over nonpregnant values (198, 478). The physiological significance of this increase is unclear given that serum phosphorus shows no change during pregnancy in mice. Moreover, although increased levels of FGF23 have been shown to reduce serum calcitriol in nonpregnant Phex (Hyp) null mice (564) and in Fgf23 overexpressing mice (531), no effect on calcitriol concentrations was seen from increased or decreased FGF23 during pregnancy. Specifically, the serum calcitriol was no different among pregnant Phex null, WT, or Fgf23<sup>-/-</sup> mothers (581, 679).

B) HUMAN DATA. No published data are available on intact FGF23 levels during human pregnancy. One study did report intact FGF23 values obtained within 24 h after delivery (678). The mean values were similar to the levels observed in unrelated adult controls, and about three times the values obtained in cord blood from the mothers’ babies. But FGF23 levels obtained within 24 h after delivery may no longer reflect what was present during late pregnancy.

C) SUMMARY. FGF23 doubles its concentration in the mother’s circulation during murine pregnancy; whether it has a similar increase in women is unknown. Its physiological importance during pregnancy is uncertain given the unchanged serum phosphorus in human and rodent pregnancies, and because markedly increased FGF23 in Phex null mice did not blunt the pregnancy-related rise in calcitriol.

X-linked hypophosphatemic (XLH) rickets is a disorder of FGF23 excess caused by an inactivating mutation in PHEX (the equivalent of the Phex null mice); any known effects of XLH during pregnancy are addressed in section IIIK.

7. Sex steroids and other hormones

A) ANIMAL DATA. Pregnancy induces changes in other hormones that may influence bone metabolism. Estradiol, estrone, progesterone, prolactin, placental lactogen, and placental growth hormone each increases significantly (232, 268, 852, 979). Conversely, IGF-I decreases during late pregnancy in rats, consistent with a suppression in pituitary growth hormone (268, 645). Oxytocin increases fivefold during pregnancy in rats and reaches its highest levels during parturition (1010).

B) HUMAN DATA. During human pregnancy there are similar changes in these hormones that may influence skeletal metabolism and mineral homeostasis. Estradiol increases up to 100-fold, accompanied by lesser increases in estrone and estrone (632, 670, 933), while progesterone increases up to 10-fold (670, 933). Prolactin increases 10-fold or more (33, 207, 632, 670, 695), placental lactogen increases ~10- to 100-fold (33, 649, 695), and these similar hormones will each activate prolactin receptors. Placental growth hormone increases 25-fold while pituitary growth hormone...
becomes suppressed (174). IGF-I may decrease to as low as 50% of basal values during the second trimester before rising two- to threefold above nonpregnant values by term (85, 174, 632, 649). In one study the rise in IGF-I during the third trimester correlated with the increase in bone turnover markers, and the rise in placental lactogen paralleled the increase in IGF-I, leading the authors to infer that placental lactogen was driving the increases in IGF-I and bone turnover (649). Oxytocin shows a slow but modest increase during pregnancy to reach peak levels near term (231).

Prolactin and placental lactogen have been shown to stimulate synthesis of PTHrP in human cultures of extraembryonic membranes (262). Beyond this, no clinical studies have tested whether these pregnancy-related hormones play an important role in regulating maternal bone metabolism.

C) Summary. Numerous hormones show progressive increases or decreases in their circulating concentrations during pregnancy. But the extent to which these hormones may have direct or indirect effects on skeletal and mineral homeostasis during human pregnancy has been largely unexplored. Within animal models, limited data about these hormones are discussed in the relevant sections. Prolactin and placental lactogen have been shown to stimulate intestinal calcium transport (see sect. II B), reduce urinary calcium excretion (see sect. II C), and stimulate both PTHrP and calcitriol (see sect. II, A3 and A4, respectively). Oxytocin and its receptor have been proposed to have possible effects on bone formation and resorption (see sects. IID and IVD).

B. Upregulation of Intestinal Absorption of Calcium and Phosphorus

1. Animal data

The two main routes of calcium absorption are an active, saturable, transcellular pathway that is stimulated by calcitriol and a passive, nonsaturable, paracellular pathway that is also stimulated in part by calcitriol (181, 247). Rapid and active calcium absorption is thought to represent ~20% of net absorption, and it takes place largely in the duodenum of the rodent, while ~80% of calcium absorption occurs through slower, passive mechanisms in the jejunum and ileum, with lesser amounts in the colon (181, 694). Active intestinal calcium absorption is more important when dietary intake of calcium is limited.

Studies using $^{45}$Ca have demonstrated that the fractional absorption of calcium doubles in pregnant rats. Three-day metabolic balance studies found that fractional $^{45}$Ca absorption doubled at days 20–22 of gestation compared with nonpregnant controls (173). At days 13–15 in that study, net $^{45}$Ca absorption was 20% higher and urinary excretion of $^{45}$Ca was double that in nonpregnant controls, confirming that intestinal calcium absorption was also increased at mid-pregnancy (173). When the absorption of $^{45}$Ca in 15 min across an in situ isolated loop of duodenum was measured, the value was increased 30% at day 14 and doubled at day 20 of pregnancy, similar to the results of the metabolic balance studies (735). $^{45}$Ca absorption across everted gut sacs in rats also revealed that calcium absorption at day 20 of pregnancy was double that of nonpregnant controls (360).

An increase in intestinal calcium absorption beginning at mid-pregnancy may enable the pregnant rodent to accrete mineral in advance of the peak demands that occur during late pregnancy and lactation. Balance studies have shown pregnant rats to achieve a net gain in calcium at mid-pregnancy that is sustained to term (339). Consistent with this, an increase in femoral ash weight and calcium content has been found in rats and sheep at mid-pregnancy and term (69, 629), which correlates with an increasing calcium content of the diet (818), while a progressive 10–15% increase in total body mineral content during pregnancy has been observed by DXA in normal Black Swiss mice (296, 478, 827, 994). However, not all studies are consistent with this since no change in femoral or tibial ash weight or calcium content was observed in several studies of rats (362, 598, 628, 972), decreases in lumbar spine BMD by DXA and histomorphometry have been observed at mid-pregnancy and term in rats (408), and BMD of the whole body and lumbar spine decreases by DXA in C57BL/6J mice (477, 580). Some of these differences are likely due to differing calcium contents of the diet, with dietary calcium restriction consistently causing reduced skeletal mineral content by the end of pregnancy in rats (64, 271, 740) and goats (55). The differences between Black Swiss and C57BL/6J mice must be due to genetic differences because both strains were maintained on the same 1% calcium diet in the author’s laboratory. It has been estimated from radioisotope studies that 92% of fetal skeletal calcium content is absorbed from the maternal diet of the rat during pregnancy while consuming a 1% calcium diet (974), but this will likely vary by dietary calcium intake. Clearly if calcium intake is insufficient to meet the needs of the fetuses, then increased skeletal resorption will occur.

In the transcellular pathway, calcium enters enterocytes through apical calcium channels (TRPV6, TRPV5), is shuttled through the cytoplasm while bound to proteins (calbindin-D9k), and is extruded from the cell under the actions of $\text{Ca}^{2+}$-ATPase (PMCA1) and the sodium-calcium exchanger NCX1 (247). Each of these factors is dependent on calcitriol for normal expression in nonpregnant animals (127, 181, 182, 596, 854). Each is upregulated in the intestines of pregnant rats to achieve a net gain in calcium at mid-pregnancy (173). When the absorption of $^{45}$Ca in 15 min across an in situ isolated loop of duodenum was measured, the value was increased 30% at day 14 and doubled at day 20 of pregnancy, similar to the results of the metabolic balance studies (735). $^{45}$Ca absorption across everted gut sacs in rats also revealed that calcium absorption at day 20 of pregnancy was double that of nonpregnant controls (360).
Calcitriol regulates that paracellular or passive route of calcium absorption by stimulating the expression of claudin-2 and claudin-12, which form tight junctions between intestinal epithelial cells (181). These claudins are expressed in the jejunum, ileum, and colon, but most abundantly in the ileum where the bulk of calcium is absorbed (298, 406). Expression of claudin-2 and claudin-12 are reduced in Vdr null mice (298).

A pregnancy-induced increase in fractional calcium absorption occurs despite severe vitamin D deficiency in rats (116, 360), absence of the vitamin D receptor in mice (296), or maternal parathyroidectomy in rats (425). In severely vitamin D-deficient pregnant rats, a doubling to tripling in the efficiency of intestinal calcium, phosphorus, and magnesium absorption was observed that reached levels equivalent to normal (116), which suggests that full upregulation occurs during pregnancy despite the absence of calcitriol. In another study intestinal calcium absorption doubled over baseline in severely vitamin D-deficient rats, but reached two-thirds of the value achieved by pregnant vitamin D-replete rats (360). And so the pregnancy-related increase may not always fully compensate for the absence of calcitriol.

The upregulation in intestinal calcium and phosphorus absorption that occurs in severely vitamin D-deficient rats is physiologically important because by the end of pregnancy, significant increases were seen in femoral ash weight and calcium content (362) and serum calcium and phosphorus (915). Moreover, Vdr null mice (Boston strain) increased their skeletal mineral content by 55% as assessed by DXA, had a marked reduction in secondary hyperparathyroidism, and normalized serum calcium, and increased renal calcium excretion during pregnancy (296). In a separate study of Vdr null mice (Leuven strain), pregnancy resulted in an increase in trabecular BMD of the femur by quantitative computed tomography (QCT) an increase in femoral ash weight, and reduced osteoid and osteoclast parameters as assessed by histomorphometry (786).

In both Vdr null models (i.e., both Boston and Leuven strains), a calcium-enriched diet had been started at the time of mating, and this partly confounded the increase in bone mass and some of the improvements in mineral homeostasis that were observed (296, 786). More recently, a preliminary report in pregnant Cyp27b1 null mice, which lack the ability to make calcitriol, has shown a 45% increase in bone mineral content by DXA, a marked lessening of secondary hyperparathyroidism, and normalization of serum calcium and phosphorus (330). Intestinal calcium absorption has not yet been measured but is likely to be increased, based on these observed changes. The Cyp27b1 null mice had been maintained on the same diet since 3 wk of age, so all of the improvements in skeletal mineral metabolism are attributable to pregnancy.

Not all studies agree with the findings of skeletal improvements during pregnancy in severely vitamin D-deficient rats, Vdr null mice, and Cyp27b1 null mice. Two histomorphometric studies in pregnant vitamin D-deficient rats found significant decreases in mineralized bone, increased unmineralized osteoid, and increased resorptive parameters (598, 628). The calcium content of the diet (0.45%) was the same among the rat studies but the rat strains differed, and that may account for the varying skeletal response to pregnancy.

The increment in calcitriol was prevented in pregnant rats by administering ketoconazole, which blocks the steroid synthesis pathway. This resulted in an 80% reduction in serum calcitriol of pregnant rats and a 50% reduction in intestinal calcium absorption, which suggests that intestinal calcium absorption is partly dependent on calcitriol during pregnancy (92). The serum calcium of the mothers and weight of the pups were unaffected. The finding of reduced intestinal calcium absorption contrasts with negative findings from the same study during lactation (see sect. IVC).

What could explain calcitriol-independent upregulation of intestinal calcium absorption during pregnancy? Vdr null mice show downregulation of intestinal TRPV6 when nonpregnant but a marked upregulation during pregnancy. Prolactin, placental lactogen, and growth hormone have been shown to stimulate intestinal calcium absorption independently of calcitriol, possibly through actions to increase expression of TRPV6, TRPV5, calbindin-D9k, and PMCA1 (12, 171, 586, 690, 894, 901). As noted above, growth hormone is normally suppressed during pregnancy, but placental growth hormone is increased and may stimulate intestinal calcium absorption. Prolactin’s effect to increase intestinal calcium absorption was confirmed in pregnant, severely vitamin D-deficient rats (690). An effect of prolactin and placental lactogen was also observed in everted gut sacs of nonpregnant, hypophysectomized rats, which thereby removed the confounding effects of prolactin release from the pituitary (586, 894). Estradiol also has effects independent of calcitriol to stimulate intestinal calcium absorption and expression of TRPV6 and TRPV5 (192, 949). Calcitonin stimulated intestinal calcium absorption although the
mechanism appeared to be through increased production of calcitriol, rather than being independent of it (436).

The implication of these studies is not to suggest that calcitriol plays no role in upregulating intestinal calcium absorption during pregnancy. Instead, it is quite likely that the two- to fivefold increase in calcitriol contributes to the doubling in the efficiency of intestinal calcium absorption in normal, vitamin D-replete animals. Additional factors (possibly prolactin, placental lactogen, placental growth hormone) likely contribute to the normal upregulation of intestinal calcium absorption during pregnancy. What may be indicated by these findings in severe vitamin D-deficient rats, Vdr null mice, and Cyp27b1 null mice is that in the absence of calcitriol (or the inability to respond to it), these pregnancy-related factors compensate to upregulate intestinal calcium absorption, with the resulting efficiency of calcium absorption reaching the normal peak levels of pregnancy in some rodents, but falling below it in others.

2. Human data

In humans active transport of calcium occurs in the duodenum and proximal jejunum, with passive absorption in the distal jejunum and ileum accounting for the bulk of calcium absorption (181, 247). Active transport is more rapid and becomes especially important when the dietary supply of calcium is low. Stable calcium isotopes ($^{48}$Ca, $^{44}$Ca, $^{42}$Ca) and mineral balance studies have consistently determined that women are in a positive calcium balance during early pregnancy, with fractional absorption of calcium doubling by as early as 12 wk of gestation and maintained to term (207, 324, 372, 467, 469, 764).

The doubling to tripling of serum calcitriol, with increased free calcitriol, has also been assumed to explain the increased efficiency of intestinal calcium absorption during human pregnancy. Compelling animal data described in the preceding section support that this increase in intestinal calcium absorption may not be fully due to calcitriol, and have raised the possibility that prolactin, placental lactogen, growth hormone, and other factors stimulate calcium absorption by enterocytes. However, there are no compelling clinical data because few studies have examined the mechanism of calcium absorption during human pregnancy.

An independent effect of prolactin to stimulate intestinal calcium absorption was not evident when seven hyperprolactinemic subjects were found to have intestinal calcium absorption within the expected normal range (514); however, estradiol should be low in these patients, in contrast to the high levels during pregnancy. Intestinal calcium absorption has not been formally measured during pregnancy in women who are severely vitamin D-deficient or who have genetic disorders of impaired vitamin D physiology. However, the first-trimester rise in intestinal calcium absorption precedes the more marked increase in calcitriol and free calcitriol during the third trimester, which may indicate that the early increase is not wholly driven by calcitriol. Moreover, the extremes of severe vitamin D deficiency or genetic disorders of vitamin D physiology in the mother do not alter serum calcium, ionized calcium, phosphorus, PTH, or skeletal mineral content of the baby, as detailed in the companion review (492). These normal fetal parameters imply that maternal delivery of mineral is normal, or that the placenta is capable of extracting needed mineral regardless of a deficit in the maternal supply.

3. Summary

Intestinal calcium absorption increases twofold beginning in the first trimester of human pregnancy, and a similar doubling of intestinal calcium and phosphorus absorption occurs by mid-pregnancy in rats. The increase in calcium absorption is probably mediated in part by calcitriol-induced increases in TRPV5/6, calbindin-D9k, PMCA1, NCX1, claudin-2, and claudin-12. Many but not all studies in rodents have shown that a normal or near-normal increase in intestinal calcium and phosphorus absorption occurs during pregnancy without requiring calcitriol. Prolactin, placental lactogen, growth hormone, and other factors may contribute to the normal pregnancy-related doubling of fractional calcium absorption. An increase in intestinal calcium absorption early in pregnancy may allow the maternal skeleton to store calcium in advance of peak demands that occur later in pregnancy and during lactation.

Overall, this doubling in the fractional absorption of calcium from the intestines appears to be the major maternal adaptation to meet the fetal requirement for calcium in humans and rodents. In rats it accounts for more than 90% of the mineral present in the fetus at birth.

C. Altered Renal Mineral Handling

1. Animal data

Glomerular filtration rate and creatinine clearance increase during rodent pregnancy (65). In 3-day metabolic studies in rats, a twofold increase in urinary calcium excretion has been observed by mid-pregnancy that rises even higher by late pregnancy (173). This hypercalciuria develops concurrently with the significant increase in intestinal calcium absorption (173), confirming that hypercalciuria is a consequence of the increased renal filtered load, or absorptive hypercalciuria. Such 24-h renal calcium excretion studies have not been done in pregnant mice. However, in longitudinal studies carried out in the author’s laboratory, the calcium-to-creatinine ratio in a spot urine is normal or significantly increased during pregnancy when the samples are obtained from nonfasted mice (296, 477, 994), whereas the result is reduced below the nonpregnant level when the mice...
are fasted overnight (478, 580). This supports that absorptive hypercalciuria also occurs in pregnant mice. Additional evidence in support of this comes from pregnant Vdr null mice, which had significantly increased urine calcium/creatinine ratios, concurrent with their increase in intestinal calcium absorption (296).

Prolactin and placental lactogen reduce urinary calcium excretion in nonpregnant rabbits (135, 136). Whether these hormones are helping the kidneys conserve calcium during pregnancy has not been tested. An increase in urine calcium excretion during pregnancy suggests that the kidneys are not conserving calcium; the suppressed PTH levels may be a contributing factor. This is supported by the observation that urinary calcium excretion was no different in pregnant Pth nulls and their WT sisters, despite the lower filtered load of calcium in Pth nulls (478). An additional consideration is that intestinal calcium absorption increases before the fetal demand for calcium; consequently, the excess absorbed calcium must be excreted.

2. Human data

As in rodents, pregnancy causes increased creatinine clearance and glomerular filtration rate (303). As early as the 12th week of gestation, the 24-h urine calcium excretion increases significantly, with mean values reaching the upper limit of normal, and values in the hypercalciuric range often observed (18, 207, 221, 324, 707, 764, 809). Fasting urine calcium concentrations are normal or low, confirming that the increase in 24-h urine calcium is due to absorptive hypercalciuria, i.e., that increased intestinal calcium absorption leads to an increased renal filtered load and excretion of calcium (304, 324, 468). Since the fetal demand for calcium does not occur until the third trimester, but intestinal calcium absorption increases in the first trimester, this explains why absorptive hypercalciuria is an obligatory, physiological consequence of pregnancy. It is one of the factors that contributes to an increased risk of kidney stones during pregnancy (815).

Many recent clinical studies have eschewed the use of 24-h urine collections during pregnancy and have relied solely on calcium/creatinine values in spot urines to assess renal calcium excretion. When second morning void spot urines were obtained in longitudinal studies from women who were not required to fast, the Ca/Cr ratios during pregnancy reached double the value of those obtained prior to pregnancy (85, 632), and the mean values were at the upper end of the normal range in one of the studies (85). Conversely, fasting Ca/Cr ratios were no different between singleton and twin pregnancies, and the mean values showed no change between 10 and 38 wk of pregnancy, consistent with failure to detect absorptive hypercalciuria on fasted urine specimens (646).

The Ca/Cr ratio in a random spot urine has been used in several recent randomized trials that studied the apparent safety of high-dose vitamin D supplementation during pregnancy. No adverse increase in Ca/Cr ratio was reported when 5,000 IU vitamin D per day was compared with placebo (778), when 4,000 IU daily and 400 IU daily were compared (399), or when 4,000 IU daily and 2,000 IU daily were compared (966). These findings have been interpreted to mean that high-dose vitamin D supplementation does not cause hypercalciuria during pregnancy (399, 778, 966). However, since it is absorptive hypercalciuria that occurs during pregnancy, and random spot urines are neither sensitive enough nor timed appropriately to detect absorptive hypercalciuria, the available data have not adequately excluded whether hypercalciuria worsens during pregnancy when high-dose vitamin D is taken. Twenty-four hour urine collections are needed.

In this context, it is relevant to mention that hypocalciuria during pregnancy has been associated with preeclampsia and pregnancy-induced hypertension (42, 293, 524, 707, 810). In turn, the hypocalciuria has been reported in association with low serum calcitriol (293, 524, 707, 810), whereas PTH, calcitonin, and ionized calcium are normal (42, 293, 707, 810). The calcitriol levels are reduced to values seen in nonpregnant women. The possibility that low maternal 25OHD or low vitamin D intake could explain the increased risk of preeclampsia (and, thereby, low calcitriol) has been investigated with a similar number of studies supporting (46, 95, 370, 768) and refuting (294, 726, 810, 823) an association.

The low calcitriol, hypocalciuria, and reduced creatinine clearance that occur in preeclampsia and PIH are likely secondary to disturbed renal function, rather than being causes of the hypertension (293). Consistent with this, in a longitudinal study, calcitriol levels were normal earlier in pregnancy and became low only after hypertension and proteinuria developed (357). Another study found that serum levels of the fat-soluble vitamins A, D, and E were each lower in preeclamptic women compared with normotensive pregnant and nonpregnant controls (485), which raises the possibility that confounding from overweight, obesity, and poor nutrition may explain why the levels of several fat-soluble vitamins were all lowered in preeclamptic women. A placental abnormality has been hypothesized to explain lower calcitriol levels in preeclampsia, with conflicting results obtained showing decreased (248) and increased (286) placental expression of Cyp27b1. However, since the placenta contributes very little calcitriol to the circulation of pregnant women (see sect. II A4), it is unlikely that loss of placental Cyp27b1 leads to reduced serum calcitriol in preeclamptic women. Alternatively, abnormal placental function could indirectly reduce maternal calcitriol concentrations if placental hormones (such as placental lactogen) are important stimulators of Cyp27b1 in the maternal kidneys.
However, plasma concentrations and placental expression of placental lactogen are not reduced in preeclamptic women (73, 74, 561, 595, 623, 675, 1027).

Numerous clinical trials have been carried out to assess the efficacy of calcium supplementation at preventing preeclampsia or pregnancy-induced hypertension, and a net benefit has been seen for women with the lowest intakes of calcium, whereas no effect is evident for women with adequate calcium intake (398). In multiple clinical trials, no effect of high-dose vitamin D supplementation has been seen on the incidence of preeclampsia in pregnant women (183, 184, 399, 710, 966).

3. Summary

Increased intestinal calcium absorption during pregnancy leads to increased urine calcium excretion, with 24-h urine values near or above the upper limit of normal. This is absorptive hypercalcuria, which will not be present on Ca/Cr ratios in fasting urine specimens, and not well discriminated from Ca/Cr ratios obtained from random spot urine samples. The pregnancy-related suppression of PTH increases further by the second postpartum day, while in control experiments, removal of fetuses at C-section revealed that they accounted for <2% of the maternal mineral content at term (827). This increase in whole body bone mineral content is not attributable to calcium in the fetuses since the maternal value increases further by the second postpartum day, while in control experiments, removal of fetuses at C-section revealed that they accounted for <2% of the maternal mineral content at term (827). This increase in whole body bone mineral content is consistent with the previously noted up-regulation of intestinal calcium absorption and positive calcium balance. It is noteworthy, in these studies at least, that the maternal skeleton continues to accrete mineral during the last 5 days of gestation despite calcium being transferred to the fetuses at a peak rate.

Apart from serial assessment of bone mineral content by DXA at multiple time points during pregnancy in Black Swiss mice, most studies in rodents have simply compared end-of-pregnancy BMC or BMD to pre-pregnancy in the same mice, or to age-matched virgin mice, without determining whether any changes in bone mass occurred earlier in pregnancy. This approach has shown that bone mass or mineral content at the end of pregnancy varies significantly by skeletal site and genetic background in mice. Outbred Black Swiss mice consistently show a 10–20% increase in whole body bone mineral content at the end of pregnancy, the lumbar spine remains unchanged, and the hindlimb may gain 10–15% (296, 478, 827, 994). Conversely, inbred C57BL/6J mice show a 5% decline in whole body bone mineral content, a 15% drop in lumbar spine bone mineral content, and a 10–20% gain in the hindlimb (477, 580). CD-1 mice show no significant changes in mineral content during pregnancy at any skeletal site (35, 956). Rats showed no change in whole body BMD and nonsignificant declines in BMD of femora and tibiae at the end of pregnancy (816), whereas another study found a modest but significant decline in lumbar spine BMD (920), and a third study found a gain in femoral and vertebral BMC and BMD (888). Another study found that femoral calcium content at the end of pregnancy was linearly related to the calcium content of the diet (818). Overall, apart from the serial
and reduced trabecular bone volumes of the tibiae (107), all in Sprague-Dawley rats with no correlation to the dietary calcium content. A microCT study found that C57BL/6 mice consuming 2% calcium increased cortical area and trabecular volume of the femora at day 16 of pregnancy compared with day 9 (489).

The available data discussed in this section may seem inconsistent and contradictory. For example, within some of these studies there is histomorphometric evidence of markedly increased bone turnover in late pregnancy but little or no change in bone mass. A problem contributing to the inconsistency is the comparison of a single time point (end of pregnancy) to prepregnancy or age-matched virgins, without any assessment at intermediate time points. If a net increase in bone mass and mineralization occurs earlier in pregnancy (as suggested by calcium balance studies, up-regulated intestinal calcium absorption, and serial DXA studies in Black Swiss mice), and is then followed by up-regulated bone resorption with net bone loss during the latter third of pregnancy (corresponding to when fetal demand for mineral is at its peak), then that can certainly explain why bone mass may appear relatively unchanged at the end of pregnancy despite objective evidence of significantly increased bone resorption. Furthermore, even if there was no increase in bone mass earlier in pregnancy, if bone resorption increases only in late pregnancy, that may be too short of a duration to detect a loss in bone volumes by histomorphometry. There are likely site-specific differences as well, with trabecular-rich vertebrae possibly differing in their response to pregnancy compared with more cortical-rich sites such as the tibiae and femora. Increased weight-bearing during pregnancy may contribute to a gain in bone mass of the limbs, as shown in some of the studies.

As noted earlier, when a low calcium diet is consumed, the skeleton will be markedly resorbed, leading to consistently reduced bone mass by the end of pregnancy in rats (64, 271, 740) and goats (55). This resorption is mediated in part by compensatory secondary hyperparathyroidism, which is more marked on a calcium-restricted diet (see sect. II A2). Conversely, a high-calcium diet (1.6 or 1.2% calcium) leads to a higher femoral calcium content at the end of pregnancy in rats compared with consuming 0.5% calcium (719, 818), which is compatible with the high-calcium diet facilitating reduced resorption of the maternal skeleton.

Does the lack of change or small net change in bone mass or volumes at the end of pregnancy have any effect on bone strength? No differences have been found in the biomechanical properties of the trabecular bone in the vertebrae (crush test) of Sprague-Dawley rats (947), or the cortical bone of the femora (3-point bend test and rotational stress) of C57BL/6 mice and Sprague-Dawley rats (489, 548, 947). However, the material properties of rat femora were deduced to show reduced shear stress and increased stiffness in the femora of pregnant rats and compared with age-matched virgins. The results are inconsistent across studies and not clearly explainable by variations in the calcium content of the diet or the strain of rat. One reported marked increases in bone resorption (osteoclast number, resorptive surfaces) and bone formation (osteoblast number, osteoblast surface, osteoid volume) during pregnancy in Cobs rats consuming a 0.45% calcium diet (598). Another found that osteoclast parameters increased three- to sixfold while bone formation parameters were unchanged in Sprague-Dawley rats on a 1.2% calcium diet (920). A third study used a 1.1% calcium diet in Sprague-Dawley rats and found divergent effects with reduced bone formation parameters in the vertebrae but increased bone formation parameters within cortical bone of the femora; bone resorption parameters were not assessed (947). Pregnant beagles consuming a 2% calcium diet also showed significantly increased bone formation parameters in the ilium, whereas bone resorption parameters were not assessed (300). In all four studies bone turnover was found to be increased on the basis of at least one parameter (i.e., either bone resorption or formation), but the failure to assess bone resorptive parameters in two of the studies makes it difficult to be certain whether net bone formation or resorption is occurring in these pregnant animals.

Histomorphometry and DXA studies have not been carried out in pregnant sheep; however, calcium balance studies inferred that a 20% decrease in skeletal calcium content occurs by the end of pregnancy (108), consistent with net bone resorption. A calcium-restricted diet in ewes leads to increased resorption and reduced ash weight, particularly within the vertebrae and the pelvis by the end of pregnancy, with relative sparing of the long bones (69). A high calcium diet prevents these changes.

In most histomorphometry, microCT, and electron microscopy studies, despite this apparent increase in bone turnover, there was no net change in femoral or tibial bone mass at the end of pregnancy in Cobs, Holtzman, Lister, and Sprague-Dawley rats on a 0.45% calcium diet (362, 598, 628, 972) or in beagles on a 2% calcium diet (300). Exceptions include reduced bone volumes and trabecular thickness in the lumbar spine at the end of pregnancy in Sprague-Dawley rats consuming 1.2% calcium (920), and the opposite result of increased trabecular number, thickness, and bone volume of the vertebrae, and increased calcein labeling and bone formation rates throughout the vertebrae, in Wistar rats on a 0.88% calcium diet (822). Other studies have found increases in cortical volumes of the femora (106, 548, 947), increased cortical porosity in the femora (548), and reduced trabecular bone volumes of the tibiae (107), all in Sprague-Dawley rats with no correlation to the dietary calcium content. A microCT study found that C57BL/6 mice consuming 2% calcium increased cortical area and trabecular volume of the femora at day 16 of pregnancy compared with day 9 (489).
when subjected to rotational stress (548). Two studies from the same investigator provided contradictory results in that load to failure in the three-point bend test was increased (216) and reduced (217) at the end of pregnancy in Wistar rats compared with age-matched virgins. The work to failure did not differ between pregnant and age-matched virgins in both studies (216, 217), suggesting that there was no real change in bone strength.

Although there may be no net change or modest changes in bone mass by the end of pregnancy when a calcium-replete diet is consumed by rodents, there is other evidence that the macro structure of bone may be altered. In Sprague-Dawley rats, an increase in femoral cortical bone volumes, radii, and cortical thickness have been found at the end of pregnancy, which appear to result from increased periosteal bone formation during pregnancy (106, 216, 548, 947). These changes are conceivably a compensatory response to the increased weight bearing during pregnancy and the resorption of trabecular bone. The change in bone volumes and radii may become more marked during lactation (see sect. IV).

Bone turnover during pregnancy may be impacted by diverse hormones, including the previously noted increases in estradiol, PTHrP, prolactin, placental lactogen and growth hormone, and oxytocin. That prolactin or placental lactogen may influence bone metabolism directly has been suggested by the finding that osteoblasts express prolactin receptors (188, 888), and prolactin receptor deficient mice exhibit decreased bone formation (188). Furthermore, when the prolactin increase during pregnancy was reduced by treating Sprague-Dawley rats with bromocriptine, it blunted the pregnancy-related gain in femoral and vertebral bone mineral content (888).

The oxytocin receptor is expressed by osteoclasts and osteoblasts (195), which may enable oxytocin to regulate bone metabolism during pregnancy. In support of this, oxytocin and oxytocin receptor-null mice of both sexes have reduced bone formation and an osteoporotic phenotype (895). Oxytocin also stimulates osteoblast differentiation and function, whereas it stimulates osteoclast formation but inhibits osteoclast function and skeletal resorption (565, 895). An in vivo role for oxytocin has not been examined in these mice.

2. Human data

Intestinal calcium absorption increases in the first trimester, months before the fetal demand for mineral in the third trimester. Metabolic studies have shown that women are in a positive calcium balance by mid-pregnancy (372), so skeletal mineral content may become increased at that time-point to explain where that calcium is stored. However, mid-pregnancy remains a relative “black box” due to the absence of data on bone mass, structure, or mineralization at this time point.

Some maternal bone resorption during late pregnancy may contribute mineral to the fetus. This has shown by study of women living near the Techa River in Russia, who inadvertently ingested $^{90}$Sr from water contaminated by nuclear waste during the 1950s. Years after the exposure ended, $^{90}$Sr could be detected in the skeletons of their fetuses, which could only have come from resorption of $^{90}$Sr that had been previously fixed in the maternal skeleton (921, 922). But women who ingested $^{90}$Sr during pregnancy, especially during the third trimester, gave birth to babies with 10-fold higher $^{90}$Sr content, consistent with dietary absorption of mineral being responsible for much more of the mineral content of the fetal skeleton (819, 921, 922).

The sole study reporting iliac crest histomorphometry during human pregnancy was carried out in 15 women who planned to have elective first trimester abortions, 13 women scheduled to have C-sections in the third trimester, and 40 nonpregnant women (some living and some cadaveric specimens) (732). At the first trimester time point, bone resorption indexes were increased, bone formation indexes were decreased, and trabecular bone volume was significantly lower, compared with nonpregnant women (732). Conversely, the third-trimester biopsies were no different from the nonpregnant values for bone mass and indexes of bone resorption and formation (732). Taken at face value, these results suggest that early pregnancy is a bone-resorptive state, which induces bone loss that is later recovered by the end of pregnancy. That interpretation does not fit easily with the fact that little transfer of calcium is occurring from mother to offspring during the first trimester; consequently, skeletal resorption should not be increased at this time point, especially given that intestinal calcium absorption is already upregulated. Instead, increased bone resorption is more likely to occur in the third trimester when the fetal demand for mineral is at its peak. The observed differences may be the result of small sample sizes with confounding among the groups. Age matching was poor and may have contributed to apparent differences, with mean ages of 22.4 in the first trimester group, 25.1 in the late pregnancy group, and 32.6 in the nonpregnant group. More histomorphometric data from pregnant women are needed.

Cross-sectional and serial measurement of bone turnover markers have been carried out in pregnant women. These indexes are generally considered more accurate at detecting changes in bone resorption compared with bone formation and are subject to diurnal variation as well as differences between fasted and postprandial values (142, 235, 570). Pregnant women were often compared with nonpregnant controls or normal ranges, rather than prepregnancy measurements from the same women. Additional potential confounders include hemodilution affecting serum measurements (confirmed to affect osteocalcin and CTX, Ref. 461),

Some maternal bone resorption during late pregnancy may contribute mineral to the fetus. This has shown by study of women living near the Techa River in Russia, who inadvertently ingested $^{90}$Sr from water contaminated by nuclear waste during the 1950s. Years after the exposure ended, $^{90}$Sr could be detected in the skeletons of their fetuses, which could only have come from resorption of $^{90}$Sr that had been previously fixed in the maternal skeleton (921, 922). But women who ingested $^{90}$Sr during pregnancy, especially during the third trimester, gave birth to babies with 10-fold higher $^{90}$Sr content, consistent with dietary absorption of mineral being responsible for much more of the mineral content of the fetal skeleton (819, 921, 922).

The sole study reporting iliac crest histomorphometry during human pregnancy was carried out in 15 women who planned to have elective first trimester abortions, 13 women scheduled to have C-sections in the third trimester, and 40 nonpregnant women (some living and some cadaveric specimens) (732). At the first trimester time point, bone resorption indexes were increased, bone formation indexes were decreased, and trabecular bone volume was significantly lower, compared with nonpregnant women (732). Conversely, the third-trimester biopsies were no different from the nonpregnant values for bone mass and indexes of bone resorption and formation (732). Taken at face value, these results suggest that early pregnancy is a bone-resorptive state, which induces bone loss that is later recovered by the end of pregnancy. That interpretation does not fit easily with the fact that little transfer of calcium is occurring from mother to offspring during the first trimester; consequently, skeletal resorption should not be increased at this time point, especially given that intestinal calcium absorption is already upregulated. Instead, increased bone resorption is more likely to occur in the third trimester when the fetal demand for mineral is at its peak. The observed differences may be the result of small sample sizes with confounding among the groups. Age matching was poor and may have contributed to apparent differences, with mean ages of 22.4 in the first trimester group, 25.1 in the late pregnancy group, and 32.6 in the nonpregnant group. More histomorphometric data from pregnant women are needed.

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As for animal data, studies in rats treated with bromocriptine or prolactin receptor-null mice which decreased the prolactin increase during pregnancy have shown that bone mass and bone indexes are increased, bone formation indexes are decreased, and trabecular bone volume is significantly lower, compared with nonpregnant rats (732). The increased bone resorption is more likely to occur in the third trimester when the fetal demand for mineral is at its peak. The observed differences may be the result of small sample sizes with confounding among the groups. Age matching was poor and may have contributed to apparent differences, with mean ages of 22.4 in the first trimester group, 25.1 in the late pregnancy group, and 32.6 in the nonpregnant group. More histomorphometric data from pregnant women are needed.

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increased GFR and altered creatinine excretion affecting urinary measurements, possible contributions from placenta and other tissues, increased degradation or clearance by the placenta (confirmed to affect osteocalcin, Ref. 769), and lack of fasted or diurnally timed specimens.

Given these caveats, several markers of bone resorption [urine N-telopeptide (NTX) or C-telopeptide (CTX), serum CTX, tartrate-resistant alkaline phosphatase, deoxypyridinoline/creatinine, pyridinoline/creatinine, and hydroxyproline/creatinine] have been found to be low in the first trimester but to increase steadily thereafter, rising to as much as twice normal in the third trimester (85, 206, 207, 304, 461, 632, 695, 764, 1002). Correcting for the decline in serum albumin obliterated the apparent fall in serum CTX earlier in pregnancy (461). Osteocalcin has been the most widely used marker of bone formation, and its values have been low to undetectable in the first trimester, after which it may stay low or rise to normal values by term (33, 329, 458, 632, 695, 764, 769, 813, 1002). Its apparent decline early in pregnancy was attributable to hemodilution of pregnancy in one study (461), whereas a surge in osteocalcin after delivery of the placenta is compatible with loss of placental degradation or clearance (769). Procollagen I N-telopeptides (P1NP) and carboxyterminal propeptides, and bone specific alkaline phosphatase, are also low in the first trimester, and have either remained low (304), or risen to normal or above by term (85, 206, 207, 461, 632). Total alkaline phosphatase increases mainly due to the placental fraction and is not a useful indicator of the relative changes in bone formation during pregnancy (33, 85, 221, 304, 769).

The overall pattern of results suggests that bone turnover may be relatively normal earlier in pregnancy but that it increases during the third trimester to create a net resorptive state. Such a pattern would be consistent with the expected late-pregnancy demand for mineral and, thereby, a concurrent need to resorb from the maternal skeleton, especially if intestinal absorption of mineral is not sufficient to meet the combined needs of mother and fetus. Consistent with this, a randomized trial found that consuming a mean 2,300 mg of calcium daily resulted in a 15% lower NTX level during the second and third trimesters compared with women consuming 1,100 mg calcium daily (274). Twin pregnancy resulted in slightly higher urinary NTX and CTX, and a slightly increased bone-specific alkaline phosphatase at term; these findings are compatible with further increased resorption to meet the needs of two fetuses (646).

Cross-sectional and serial studies of areal bone mineral density have been carried out in pregnant women. Most studies have been done before and after a planned pregnancy to avoid fetal radiation exposure, so very limited information is available about changes in maternal BMD during pregnancy. The readings may also be affected by altered body composition, weight, and skeletal volumes during pregnancy. An early study used X-ray spectrophotometry of the radius and femur during the first or second trimester and found that trabecular but not cortical bone density had decreased by the time of a postpartum measurement (526). Several prospective studies used single- or dual-photon absorptiometry (SPA or DPA) of the forearm serially during and after pregnancy (18, 185, 468), or of the femur before and after a planned pregnancy, and found no significant changes in cortical or trabecular bone density (858). Another study compared preconception SPA and DPA measurements to those taken 6 wk postpartum and found a 2.4% decrease in the femoral neck, a 2.2% decrease in radial shaft, a 3.4% increase in the tibiae, and no change in lumbar BMD (256).

More modern studies have used DXA at two time-points, 1–8 mo before planned pregnancy and 1–6 wk after delivery (85, 462, 633, 649, 705, 764, 943). These generally small studies found 0% to at most a 5% decrease in lumbar spine bone density between the two measurements, and little to no change at other skeletal sites. However, the largest study involved 92 women who had DXA of hip, spine, and radius done at baseline (up to 8 mo prior to planned pregnancy); DXA of the forearm repeated during each trimester; and DXA of hip, spine, and radius repeated 15 days postpartum. Seventy-three women completed the postpartum visit and were compared with 57 nonpregnant women who had DXA measurements done at similar intervals. The findings included that BMD decreased during pregnancy by 4% at the ultradistal radius but increased by 0.5% at the proximal one-third of the forearm (633). At the total forearm a 1% lower value in pregnant women versus nonpregnant controls became discernible with the third trimester measurement, coinciding with the peak interval of placental-fetal calcium transfer. When the prepregnancy to postpregnancy readings were compared, BMD was reduced by 1.8% at the lumbar spine, 3.2% at the total hip, and 2.4% at the whole body (633). Overall, this study found statistically significant but small declines in BMD at several skeletal sites. For perspective, it is important to realize that such changes would not be distinguishable from error of measurement in an individual subject, but were resolvable in this study due to the large cohort. Whether all of these small changes in BMD are attributable to pregnancy is also unclear, because all women went on to breastfeed before the day 15 ± 7 postpartum measurement, and bone loss occurs rapidly during lactation (see sect. IV.E). In contrast, radial BMD was measured in a longitudinal study of 22 women and a cross-sectional study of 75 women, and no change was seen across the three trimesters in either study (609).

Peripheral ultrasound has been used during pregnancy. Cross-sectional (307, 698) and longitudinal studies (234, 307, 378, 504, 722, 918, 1002) have found lower apparent BMD in the os calcis or phalanges at the end of pregnancy, but no effect was seen on the mid-tibial shaft (1015). How
relevant peripheral ultrasound at the heel or a finger during pregnancy is for revealing changes in bone mass or mineralization at the spine or hip is uncertain. Notably, although bone mass declines significantly during lactation, with the most marked changes in the lumbar spine (see sect. IVE), ultrasound of the os calcis and mid-tibial shaft showed no changes (533, 917, 1015).

Overall, available data suggest that bone turnover increases during pregnancy, especially during the third trimester. There are real but small declines in BMD throughout the skeleton that require a sufficiently large cohort to be detected with confidence.

Are there any long-term impacts of skeletal changes induced by pregnancy? This has been examined through numerous epidemiological studies that have ascertained whether parity confers any risk of lower BMD, an osteoporotic level of BMD, or fragility fractures. The majority of such studies have found either a neutral effect of parity (16, 38, 63, 71, 83, 144, 158, 162, 203, 212, 356, 368, 374, 383, 443–445, 457, 463, 482, 506, 507, 509, 525, 541, 547, 619, 636, 648, 683, 704, 715, 761, 770, 828, 833, 845, 846, 861, 864, 865, 935, 951, 969) or that parity confers greater BMD (27, 215, 244, 290, 292, 336, 341, 365, 522, 597, 611, 642, 661, 700, 804, 864, 879, 881, 934). Additional studies have reported that parity decreases the risk of hip fractures (384, 397, 421, 460, 625, 689, 869, 997). One of the studies reporting a protective effect of parity was particularly strong because it involved 1,852 twins and their female relatives, including 83 identical twins who were discordant for ever being pregnant (700).

For balance, a few studies have found that parity is associated with reduced BMD (17, 80, 375, 472, 559, 686, 699) or increased vertebral (97) or hip fracture risk (299). However, these reports are substantially outnumbered by the aforementioned studies that found a neutral or protective effect of parity. There are also divergent effects seen among the different studies. For example, one study reported that parity reduces femoral neck BMD but had no effect on lumbar spine (416). Another found that parity reduces total hip and femoral neck BMD in postmenopausal women, but not in premenopausal women who are closer in time to the event and therefore less likely to show confounding (1020). Several studies also found that adolescent pregnancy was associated with decreased bone density (292, 861, 864), even though parity was not a significant factor in the overall cohort. However, an NHANES study of 819 women ages 20–25 found that adolescent pregnancy did not reduce peak bone mass as previously feared, since BMD was no different regardless of whether these women had experienced an adolescent pregnancy, an adult pregnancy, or no prior pregnancies (169).

These epidemiological studies have a number of limitations. It is difficult to separate the effects of parity from those of lactation. Decades have passed in some cases between the reproductive years and the time of the first BMD measurement or fracture, such that confounding factors and events in the elapsed time may have influenced the outcome. Such confounding may be less likely in the NHANES study involving women ages 20–25 since the pregnancies were only a few years earlier, wherein no detrimental effect of parity was seen. Overall, it seems most likely that parity has no long-term adverse effect on BMD or fracture risk for most women. It is not recognized as a significant factor for estimating 10-yr risk of fracture in FRAX (929). However, there may be a subset of women in whom parity does adversely affect bone mass. These may include women in whom significant nutritional deficiencies lead to increased skeletal resorption during pregnancy to meet the fetal mineral requirements, and some of these women may present with fractures from pregnancy-associated osteoporosis (see sect. IIIA).
have a protective effect against low BMD and future risk of fractures.

III. DISORDERS OF BONE AND MINERAL METABOLISM DURING PREGNANCY

A. Osteoporosis in Pregnancy

1. Animal data

Fragility fractures do not normally occur as a result of pregnancy in animal models. This is consistent with upregulation of intestinal calcium absorption usually being sufficient to meet the mineral requirements of the fetuses, and the finding that bone strength is unchanged at term. However, it is clear that the maternal skeleton will be resorbed when the maternal diet is inadequate. A low-calcium diet provokes marked secondary hyperparathyroidism during pregnancy (321), but even then, spontaneous fractures have not been noted until lactation (350).

2. Human data

Osteoporotic fractures during pregnancy are very uncommon, but they do occur. They have probably been occurring in association with reproduction for the past five millennia, because vertebral compression fractures and low BMD have been found in Egyptian mummies and other archaeological skeletons of women who were 16–30 yr of age when they died (883).

Osteoporosis presenting in pregnancy has been reviewed in depth recently (501), and will be covered more briefly here. There are two main presentations: vertebral fractures and so-called transient osteoporosis of the hip.

A) VERTEBRAL AND OTHER FRACTURES IN PREGNANCY. Fragility fractures of the spine (and less commonly other skeletal sites) have occurred during the third trimester, and in the puerperium of women who did not breastfeed. In both situations these fractures may be considered to have been provoked by pregnancy (fractures during lactation are considered separately in sect. VA). In most affected women there is no preceding bone mineral density (BMD) reading because of no prior indication for it to have been done. Serum chemistries and calciotropic hormone levels are usually unremarkable. However, bone loss is likely a factor in pregnancy-related vertebral fractures, because the BMD is usually low at presentation (475, 713, 849, 1005), and it spontaneously improves afterward (259, 433, 713). In the few cases where bone biopsies were done, mild osteoporosis was observed (849, 850, 1005).

Insufficient calcium absorption (from dietary calcium deficiency, lactose intolerance, celiac disease, other malabsorptive disorders, and vitamin D deficiency) is likely to induce resorption of the maternal skeleton during the third trimester. A recent report described a woman with a calcium intake of 229 mg daily, who developed multiple vertebral compression fractures during pregnancy (501). Since that amount was insufficient for her own nonpregnant needs, the normal upregulation of intestinal calcium absorption during pregnancy would not have benefited her. Significant skeletal resorption was an inevitable consequence of her pregnancy.

Another potential cause of physiological bone loss during pregnancy is greater than normal release of PTHrP from the placenta and breasts. The gradual rise in plasma PTHrP (sect. IIA3) likely contributes to upregulation of calcitriol synthesis, intestinal calcium absorption, and bone turnover in all pregnant women. However, in individual reported cases, high circulating levels of PTHrP have arisen from the breasts or placenta and led to pseudohyperparathyroidism, which is characterized by increased bone resorption and severe hypercalcemia (see sect. IIIF) (270, 435, 474, 549). High circulating levels of PTHrP that contributed to bone resorption have also been noted in women who presented with fragility fractures weeks after delivery (29, 751); such high levels may have begun in pregnancy and contributed to bone loss before delivery.

Additional factors that contribute to fracture risk during pregnancy include increased weight-bearing (average weight gain of 12 kg), lordotic posture, reduced bone mass or strength that precedes pregnancy, and conditions that cause bone loss during pregnancy. Published cases have revealed such diverse conditions as anorexia, longstanding hypomenorrhea or relative estradiol deficiency, premature ovarian failure, petite stature or body frame, mild osteogenesis imperfecta, inactivating mutations in LRP5, dietary calcium deficiency, renal calcium leak (hypercalciuria), bedrest and inactivity, and pharmacotherapy before or during pregnancy that may induce bone loss [heparin, oral glucocorticoids, gonadotropin releasing hormone (GnRH) analogs, depot medroxyprogesterone acetate, and certain anticonvulsants] (501). A maternal family history of severe osteoporosis is likely to be present in women with pregnancy-associated fragility fractures (259), which implies that genetic causes of low bone mass or skeletal fragility may be present.

Pregnancy-associated osteoporosis typically occurs in a first pregnancy, higher parity does not increase the risk, and fractures usually do not recur in subsequent pregnancies (243, 259, 475, 666, 713). These observations imply that in many cases reversible factors (such as nutritional deficiencies) may have been corrected after the first pregnancy. Permanent disorders such as osteogenesis imperfecta or LRP5 mutations should be anticipated to maintain an in-
creased risk of vertebral and other fractures in subsequent pregnancies.

Many uncontrolled treatments [nasal calcitonin (263, 687, 885), bisphosphonates (43, 143, 178, 263, 438, 475, 671, 677, 685, 799, 885, 897), strontium ranelate (897, 1016), and teriparatide (98, 178, 377, 527, 544, 885)] have been reported in various case series. Most of the authors of these reports evidently did not appreciate that substantial spontaneous recovery of bone mass occurs even in women who fractured, with BMD increases in the range of 20–70% in individual cases. For example, a 50% increase in BMD demonstrated by QCT in a woman who presented with a compression fracture 1 wk postpartum (556). Therefore, the attributable effect of pharmacological therapy may have been greatly overestimated. It may be reasonable to wait 12–18 mo for spontaneous recovery to occur before evaluating whether the fracture risk remains high enough to warrant pharmacological therapy (501).

b) Transient Osteoporosis of the Hip. Transient osteoporosis of the hip in pregnancy (also known as bone marrow edema syndrome) is a rare condition of skeletal fragility. It appears to be a form of chronic regional pain syndrome 1 or reflex sympathetic dystrophy that most often involves the hips (112, 147, 335, 351, 571). When it occurs in association with pregnancy, its onset is usually during the third trimester or the puerperium with unilateral hip pain, limp, or a hip fracture; it can also be bilateral at presentation (112, 272, 301, 335, 573, 688). The femoral head and neck are osteopenic and radiolucent on plain radiographs (147, 218, 571), while DXA suggests that the hip BMD is quite low (301). The lumbar spine BMD is usually normal but if it is also low, it is unusually substantially higher than the femoral neck BMD (31). Magnetic resonance imaging (MRI) studies have revealed that the radiolucent femoral head and neck are edematous, which suggests that the low DXA values in the hip may be an artifact of that increased water content (31, 523, 688, 892). Nevertheless, that bone marrow edema is still an indication of fragility in the affected femora, and prophylactic arthroplasties have been carried out in women in whom the fracture risk was considered to be high. In women who do not fracture, the MRI and DXA abnormalities typically resolve rapidly within several months to a year, leading to a 20–40% increase in femoral neck BMD as the edema subsides (31, 43, 128, 147, 301, 335, 573, 892). Whether that is a real increase in mineralization or removal of an artifact from bone marrow edema remains uncertain.

The etiology of this condition is uncertain. It occurs equally in men and women, and its occurrence during pregnancy may simply be by chance. It might also be triggered by pregnancy-related factors. Femoral venous stasis caused by pressure from the pregnant uterus, relative immobilization from bed rest, and fetal pressure on

the obturator nerve are among the theories proposed to explain its occurrence in pregnant women (112, 147, 218, 335, 499, 571). It is considered separately here because, unlike the vertebral and other fractures that sometimes occur during pregnancy, it does not seem to result from systemic bone resorption. It also has different epidemiological characteristics, including that it can occur in any pregnancy, and it has recurred in the other hip either during a subsequent pregnancy or when not pregnant.

On the other hand, some patients who clearly had transient osteoporosis of the hip also had very low spine BMD, vertebral fractures, severe vitamin D deficiency, and prolonged pregnancy/lactation cycles that may have caused generalized bone loss (43, 48, 98, 691). Therefore, the two conditions can coincide, despite evidence that they appear to have a different pathogenesis and outcome.

3. Summary

Vertebral and (less commonly) other fractures may occur during pregnancy in women with preexisting disorders of skeletal fragility, and in women who experience greater than expected skeletal resorption during gestation. Nutritional deficiencies and malabsorptive disorders are particularly likely to cause problems because insufficient mineral delivery to the fetus will prompt compensatory resorption of the maternal skeleton. Transient osteoporosis of the hip may be a distinct disorder that does not occur from generalized bone resorption, but instead results in focal edema and fragility of the femoral head and neck.

B. Primary Hyperparathyroidism

1. Animal data

Several mouse models for primary hyperparathyroidism, such as mutations in Men1, have been created, but as yet none has been studied during pregnancy. Maternal hypercalcemia induced by calcium infusions causes increased fetal calcium and suppressed fetal PTH concentrations in ewes, confirming that maternal hypercalcemia adversely affects fetal and neonatal parathyroid function (456). Heterozygous inactivating mutations in the calcium sensing receptor (CaSR) create a condition similar to primary hyperparathyroidism, and relevant data from pregnant Casr+/− mice are discussed in section III C. A postpartum surge in serum calcium occurs in normal rodents, and the peak serum calcium should be even higher in rodents with underlying primary hyperparathyroidism.

2. Human data

The exact prevalence of primary hyperparathyroidism during pregnancy is uncertain. A recent retrospective series
found that it occurred in 0.03% of reproductive age women who underwent routine screening of serum calcium (391). Several hundred cases have been described in English-language medical journals (354, 391, 466), while the author’s advice has been sought for several cases each year by clinicians around the globe. Primary hyperparathyroidism is 10–20 times more likely to occur in women over age 45 than in women of child-bearing age (373, 980), but in two case series, ~1% of all parathyroidectomies were done in pregnant women (466, 490). The pathology is similar to nonpregnant cases: a predominance of single adenomas followed by four-gland hyperplasia (267, 466).

The dilutional fall in serum albumin and calcium (see sect. II A1) and suppression of PTH (see sect. II A2) during normal pregnancy may mask the hypercalcemia and delay the diagnosis. An elevation in ionized or albumin-corrected calcium, combined with a nonsuppressed PTH level, confirms the presence of primary hyperparathyroidism during pregnancy.

The physiology of normal pregnancy can worsen hypercalcemia caused by primary hyperparathyroidism since both conditions cause increased intestinal calcium absorption, hypercalciuria, and some degree of increased bone resorption. This may explain the perceived increased risks of severe hypercalcemia, pancreatitis, and kidney stones when primary hyperparathyroidism occurs during pregnancy. Conversely, rapid placental calcium transfer and uptake of calcium by the fetal skeleton during the third trimester may help protect against severe hypercalcemia in the mothers. Loss of outflow to the placenta likely explains why hypercalcemic crises have occurred after delivery of the placenta (612, 662, 701, 795). Physical inactivity and bedrest will add an additional component of skeletal resorption. Consistent with the animal models, maternal hypercalcemia will increase the flow of calcium across the placenta, thereby suppressing the developing fetal parathyroids. The increased fetal serum calcium may in turn cause increased renal water excretion, thereby explaining association of maternal primary hyperparathyroidism with polyydramnios (825).

Historically, primary hyperparathyroidism had been considered to be an adverse condition that should be dealt with during pregnancy. Older cases series suggested that significant maternal morbidity and mortality occurred in up to 67% of mothers, while up to 80% of fetuses and neonates experienced morbidity or mortality (803). Hypercalcemia causes nonspecific, constitutional symptoms that are difficult to distinguish from normal symptoms encountered during pregnancy, including nausea, hyperemesis, constipation, fatigue, weakness, and mental symptoms (508). More marked hypercalcemia during pregnancy has been associated with hyperemesis, weight loss, seizures, and pre-eclampsia (418, 466, 490). Additional maternal morbidity includes nephrocalcinosis, nephrolithiasis, urinary tract infections, acute pancreatitis, and increased bone loss (508, 824). Fractures are uncommon but have occurred with more severe primary hyperparathyroidism and parathyroid carcinoma (381, 651). In up to 15% of cases women presented with pancreatitis during the second or third trimester (153, 186, 205, 429, 508, 511). Hypercalcemic crises have occurred during the third trimester (153, 186, 662, 795), which coincides with peak release of PTHrP by the placenta and breasts. There are risks to the fetus and neonate that must be considered, including miscarriage, stillbirth, and hypoparathyroidism, and these are discussed in more detail in the companion review (492).

This historic view has led to a general preference for parathyroidectomy to be done during the second trimester to prevent fetal and neonatal morbidity and mortality, and a postpartum parathyroid crisis in the mother. There are no randomized trials comparing surgical, medical, and observational approaches, while consensus guidelines for the management of primary hyperparathyroidism have not addressed pregnancy (82). A retrospective review found significantly fewer neonatal deaths and complications in surgically treated mothers compared with the medically managed group (466). The second trimester is considered to have a lower risk of anesthetic or surgical complications, precipitated delivery, and neonatal death, compared with surgery during the third trimester (153, 236, 508, 824), although individual case reports have supported the apparent safety of third trimester surgery (354, 662, 711, 802).

A more modern view is that the maternal and fetal risks of primary hyperparathyroidism may have been exaggerated by reporting bias of more severe cases in the older literature, with the often milder cases seen today not necessarily requiring intervention during pregnancy. However, the most recent consensus guidelines for management of asymptomatic primary hyperparathyroidism recommend surgery for all individuals under age 50, which should encompass all pregnant women (82). From that perspective the only question is whether surgery should be done during the second trimester or postpartum.

A recent retrospective analysis of a database registry found 1,057 reproductive-aged women with primary hyperparathyroidism, of whom ~60% had been pregnant before the diagnosis and ~15% had been pregnant after the diagnosis (3). Compared with 3,171 age-matched women, there was no apparent difference in the incidence of spontaneous abortions, but the rate of C-sections was twice that of controls in women who had a pregnancy after the diagnosis was known (3). That study is limited because no data were available on the mothers during the pregnancies or on neonatal complications. Another retrospective study reported on 74 women with primary hyperparathyroidism who had had 134 pregnancies and who were compared with 175
normocalcemic women who had experienced 431 pregnancies over the same interval (391). There was no difference in the rate of spontaneous abortions or pregnancy-related complications between women with and without primary hyperparathyroidism during pregnancy, but neonatal complications were not reported (391). Additional cases suggest that the rate of still birth, neonatal death, and neonatal tetany are far less with modern compared with older case series (824). But even in modern cases fetal death still occurs, such as in 30/62 medically managed cases that ended in a late spontaneous abortion (the risk correlated with the level of maternal serum calcium), whereas no complications were seen in 15 cases operated upon during the second trimester (667). Neonatal hypocalcemia and tetany still occur after supposed mild primary hyperparathyroidism in the mothers (716) and the hypoparathyroidism can be prolonged and permanent (125, 576, 824). A twin pregnancy illustrated the variability of responses to maternal primary hyperparathyroidism, with one neonate having hypocalcemic seizures and the other staying normocalcemic (616).

3. Summary

Key points about primary hyperparathyroidism during pregnancy are that maternal and fetal complications appear to be less likely with the milder forms of the condition that are usually encountered today; however, the risks are not absent. If surgery is to be carried out it should be safer to do this in the second trimester. In cases that are only observed, the clinician should be aware of the potential for a hypercalcemic crisis to occur during the third trimester and even more abruptly after delivery. The baby must be watched for neonatal hypocalcemia that can have a delayed onset, a prolonged course, and even be permanent (492).

C. Familial Hypocalciuric Hypercalcemia

1. Animal data

Casr<sup>+/−</sup> mice have an inactivating mutation in one allele of the gene encoding the CaSR and are the murine equivalent of familial hypocalciuric hypercalcemia (FHH). They have classical findings of chronic hypercalcemia, relative hypocalciuria, and increased PTH (394); the condition develops in utero with hypercalcemia (498). Pregnancies are uneventful, but maternal hypercalcemia leads to suppressed PTH in the fetuses compared with offspring of the same genotype borne by their WT sisters (498). Homozygous offspring provide a model for neonatal severe hypercalcemia in humans, and die within 3 wk after birth unless rescued by surgical parathyroidectomy or genetic ablation of Pth or Gcm2 (394, 491, 498, 562, 932).

2. Human data

FHH is caused by autosomal dominant, inactivating mutations in CASR that lead to chronic hypercalcemia, hypocalciuria, and nonsuppressed PTH (122). It is asymptomatic because its origin in utero causes all tissues to be fully adapted to a higher level of calcium, while the relative hypocalciuria protects against nephrocalcinosis and nephrolithiasis. The hypercalcemia with elevated PTH persists during pregnancy as expected (727); however, increased intestinal calcium absorption during pregnancy causes absorptive hypercalciuria and not the expected diagnostic finding of hypocalciuria (638, 968). Consequently, the diagnostic criteria for FHH can be confounded during pregnancy by results that suggest primary hyperparathyroidism may be present. Pregnancies in affected women are uneventful; in fact, many documented cases involve the diagnosis being made in the mothers only after their babies presented with neonatal hypocalcemia or seizures (727, 913, 914). This neonatal hypoparathyroidism originates in utero with increased flux of calcium across the placenta causing suppression of the fetal parathyroids (as in the animal model), and that suppression can last for days to months after birth. Suppression has even occurred in a baby carrying the inactivating CASR mutation, who presented with neonatal hypocalcemia, but later went on to develop the expected hypercalcemia with high PTH (912). Babies who are homozygous or compound heterozygous for inactivating CASR mutations (297, 579), and occasionally babies with only a single known mutation that may exert a dominant-negative effect (45, 673), develop life-threatening neonatal severe hypercalcemia that can be rescued by three-and-a-half gland parathyroidectomy.

It is important to consider the possibility of FHH whenever a woman presents with hypercalcemia during pregnancy, and to be wary that the urine calcium excretion will not be reduced as expected. Surgery is not indicated for FHH, and if the serum calcium were to be reduced to “normal,” it would cause symptomatic hypocalcemia. Unfortunately, at least one woman with FHH underwent a three-and-a-half gland parathyroidectomy during the second trimester when she presented with marked hypercalcemia and hypercalciuria, and she was only recognized to have FHH when hypercalcemia persisted and her baby was affected too (968).

3. Summary

Key points about FHH in pregnancy are that it is uneventful in the mother, it may be confused with primary hyperparathyroidism because the normal physiological changes of pregnancy lead to hypercalcemia, and it is not necessarily benign for the offspring who are at risk for developing neonatal hypocalcemia.

D. Hypoparathyroidism

1. Animal data

The role that PTH might play in regulating maternal mineral and bone homeostasis during pregnancy has been in-
vestigated by studies in parathyroidectomized rats and Pth null mice.

When hypocalcemic, parathyroidectomized Sprague-Dawley rats maintained on a 1.2% calcium diet became pregnant, the serum calcium increased to near-normal, serum phosphorus reduced to normal, and intestinal calcium absorption more than doubled compared with nonpregnant, parathyroidectomized rats (425). In fact, the increased rate of intestinal calcium absorption during pregnancy was not significantly different between intact and parathyroidectomized rats (425). Similarly, studies in pregnant Pth null mice consuming a 1% calcium diet found that serum calcium increased to normal, serum phosphorus normalized, serum calciotin increased over fivefold, and bone mineral content increased by 15% over prepregnancy values (478). The mean peak serum calciotin concentration was nonsignificantly lower than in pregnant WT mice, while increased renal expression of Cyp24a1 in Pth nulls suggested that increased catabolism of calciotin likely explained any reduction in its peak level (478). In these studies in rats and mice, the sole intervention was that the animals became pregnant; there was no change in diet to explain the observed biochemical and physiological changes. These findings indicate that PTH is not essential for regulating mineral homeostasis during pregnancy, and that in particular, other factors stimulate synthesis of calciotin and upregulation of intestinal calcium absorption in the absence of PTH.

On the other hand, in a series of related studies from one laboratory, parathyroidectomized Wistar rats on a 0.9% calcium diet developed worsening hypocalcemia and hyperphosphatemia between 16.5 and 21.5 days of gestation, and up to 40% experienced tetany or sudden death either during late pregnancy or while giving birth (163, 313, 314, 328). Serum calciotin and intestinal expression of calcitriol-D levels in parathyroidectomized rats were ~50% of the values in intact rats at day 21.5 of pregnancy (313), but no earlier measurements were done. Therefore, whether the calciotin level or calcitriol-D expression had increased from earlier in pregnancy in parathyroidectomized or intact rats remains unknown.

Parathyroidectomized Sprague-Dawley rats maintained on a 0.75% calcium diet also became hypocalcemic in late gestation, but serum calciotin more than tripled over the last several days of pregnancy, and achieved a mean value that was modestly but not significantly lower than the calciotin level of intact pregnant rats (658). Moreover, 24,24-dihydroxyvitamin D, a product of the Cyp24a1 enzyme, showed consistently higher levels in parathyroidectomized rats throughout pregnancy (658). This is consistent with the known effect of PTH to suppress Cyp24a1 activity and expression (752, 835, 1023, 1024); consequently, loss of PTH causes increased Cyp24a1-mediated catabolism of 25-hydroxyvitamin D and calciotin.

A set of studies done in 1936 remain illuminating. When parathyroidectomized rats from an unspecified strain consumed a 1.2% calcium diet with low phosphorus content, the serum calcium at term was normal or elevated (94). On the other hand, when a 0.45% calcium diet was provided, the serum calcium in pregnant parathyroidectomized rats was ~50% of the value of intact rats on the same diet (94).

Collectively these studies support that PTH-independent upregulation of calcitriol synthesis and intestinal calcium absorption occur during pregnancy, but they differ in whether the parathyroidectomized rodent’s ability to maintain her own serum calcium is improved or worsened during pregnancy. Since late pregnancy is the interval when rapid fetal accretion of calcium is occurring, it is clear that flux of calcium to the fetuses provokes some parathyroidectomized rats to become hypocalcemic, whereas others normalize serum calcium.

Why do some PTH-depleted rodents become hypocalcemic while others become normocalcemic during pregnancy? Genetic differences may account for Sprague-Dawley rats and Black Swiss mice upregulating calcitriol synthesis and intestinal calcium absorption through PTH-independent mechanisms during pregnancy, whereas Wistar rats may not do this. However, another relevant factor is the calcium content of the diet, with parathyroidectomized rats more likely to have hypocalcemia during pregnancy when consuming a moderately restricted (0.45%) calcium diet. Despite increased intestinal calcium absorption, intact rats consuming a 0.45% calcium diet have secondary hyperparathyroidism with increased skeletal resorption during the last several days of pregnancy (see sect. II, A2 and D), but parathyroidectomized rats lack the ability to upregulate PTH-mediated skeletal resorption. Instead, parathyroidectomized rats must rely on dietary calcium intake alone to meet the fetal demand for mineral. A 0.45% calcium diet is clearly insufficient despite any upregulation of intestinal calcium absorption and calciotin that may occur in the absence of PTH. A previously cited study showed that a 1.0% calcium diet enables 92% of the calcium in the fetuses to come from the maternal diet during pregnancy in intact rats (974), but on a 0.45% calcium diet, more of the calcium in the fetuses must have been resorbed from the maternal skeleton.

A series of studies in an unspecified rat strain, likely consuming a 0.45% calcium diet, found that thyroparathyroidectomy reduced the number of implantations, embryos, and live pups. The mothers also had a lower unadjusted serum calcium at day 14 and 20 of pregnancy compared with sham-operated rats (49, 50). Sequential treatments with PTH, calciotin, and thyroid hormone revealed that these reductions in offspring numbers were due to the hypothyroidism, whereas the hypoparathyroidism itself had no effect (49, 50).
2. Human data

Hypoparathyroidism is usually known to be present prior to pregnancy. However, in specific cases, asymptomatic women have not been recognized until the newborn presented with severe secondary hyperparathyroidism, hypercalcemia, increased bone resorption, and fractures (242, 912). If the mother remains hypocalcemic during pregnancy, maternal hypoparathyroidism can have a serious adverse impact on the fetus and neonate (492).

The animal data discussed above indicate that hypoparathyroidism can improve or worsen during pregnancy, and the same divergent outcomes are true for hypoparathyroidism in women.

Multiple case reports described that hypoparathyroidism improves during pregnancy, with subjective evidence of fewer hypocalcemic symptoms or objective evidence of reduced maternal requirement for calcitriol, 1α-hydroxyvitamin D, vitamin D, and supplemental calcium (88, 120, 213, 285, 340, 343, 747, 777, 785, 890). In the most well-documented of these cases, calcitriol had to be progressively decreased and then stopped due to recurrent hypercalcemia, and the patient was then managed solely on 1.2 g calcium daily during the third trimester (890). The author is aware of other unpublished cases in which hypoparathyroidism improved during pregnancy and calcitriol was either significantly decreased or stopped. A report of 10 cases of hypoparathyroidism found that the ionized calcium was normal during pregnancy with 2 g supplemental calcium and either vitamin D$_2$ or D$_3$ but no calcitriol, and that the only change that occurred was a marked decrease in the albumin-bound fraction of serum calcium (340). These improvements in hypoparathyroidism during pregnancy imply that upregulation of calcitriol and/or intestinal calcium absorption occurs, which is consistent with the rodent models. However, in at least one case where subjective improvement in maternal symptoms was reported but no objective monitoring of serum calcium had been done, a fetus had secondary hypoparathyroidism, which implied that maternal serum calcium was not optimal (120).

Some reports have found no change in the serum calcium during pregnancy while on a stable dose of calcium with vitamin D or calcitriol (213, 264, 995), with two of these reporting a small increase in serum calcium in the last few days before delivery (264, 995). Since the serum calcium was not adjusted for the serum albumin in these cases (213, 264, 995), the lack of fall in unadjusted serum calcium during pregnancy may indicate that the ionized calcium had increased. Two reports found that the ionized calcium did not change throughout pregnancy (30, 302), but in one of these cases it abruptly fell and became symptomatic during labor (302).

In contrast to these reports of improved or stable calcium metabolism, other reports have found that hypoparathyroidism significantly worsens during pregnancy: serum calcium falls, hypocalcemic symptoms may or may not increase, and the doses of supplemental calcium and vitamin D, 1α-hydroxyvitamin D, or calcitriol have been increased (96, 141, 148, 411, 434, 510, 517, 600, 669, 790, 796, 821, 837, 884, 936). In one woman the endogenous calcitriol level was low at mid-pregnancy, but measurements in the third trimester were not reported (510). In most cases calcitriol levels cannot be interpreted due to ongoing treatment with exogenous calcitriol or 1α-hydroxyvitamin D (endogenous and exogenous calcitriol are indistinguishable).

Among some of the reports suggesting that hypoparathyroidism may worsen during pregnancy, it appears that the normal dilutional fall in serum calcium during pregnancy prompted an increase in doses of calcium, vitamin D, or vitamin D analogs. For example, in one case in which a significant fall in serum calcium had been inferred, and increases in both calcium and calcitriol intakes were done, the albumin had fallen from 4.2 to 2.0 g/dl (837). With the use of the data in that report, it is evident that the albumin-corrected serum calcium was 9.5 mg/dl (normal) when pregnancy was diagnosed and 10.2 mg/dl (normal) 2 wk before delivery, with an increase and not a decline evident. Treatment of the albumin-related fall in serum calcium resulted in a woman being made iatrogenically hypercalcemic (2.80–4.0 mM) for the duration of pregnancy (600), while another woman became hypercalcemic in late gestation (411). This iatrogenic hypercalcemia must be avoided since maternal hypercalcemia suppresses the fetal parathyroids and increases the risk of spontaneous abortion (492). In another case the physiological, absorptive hypercalcemia of pregnancy was misinterpreted as evidence of worsening hypoparathyroidism, and resulted not only in increases to the calcium and calcitriol doses, but the addition of a thiazide diuretic to reduce renal calcium excretion (thiazides are category C medications for pregnancy) (517). Clear worsening of hypoparathyroidism during pregnancy occurred in a woman taking high-dose prednisone and azathioprine due to a kidney transplant (736), and it is likely that the effect of high-dose prednisone to reduce intestinal calcium absorption was a significant factor in the apparent worsening during pregnancy.

Why do hypoparathyroid women exhibit such markedly different responses to pregnancy, with evidence of clear improvement in some cases and clear worsening in others? This may be explained in part by variations in the adaptive responses or achieved concentrations of other hormones that are thought to stimulate intestinal calcium absorption or calcitriol synthesis. For example, high estradiol concentrations of pregnancy may cause more suppression of bone turnover in some women, and more potent stimulation of Cyp27b1 in other women. Calcium intake is likely an im-
important factor, as it is in the rodent models, with the ideal minimum intake considered to be 1.2 g daily based on the Institute of Medicine guidelines (774). In half of the reports in which hypoparathyroidism appeared to worsen during pregnancy, either no supplemental calcium or at most 300 mg daily was being taken, and the dietary intake of calcium was unknown (96, 411, 434, 510, 600, 669, 790, 936). Lack of adequate intake of calcium in the first half of pregnancy likely prevents the net positive calcium balance being achieved that most women experience during pregnancy, and increases the likelihood of problems in the third trimester. There may be variations in the degree to which PTHrP release from breasts and placenta, or increases in other pregnancy hormones (placental lactogen, oxytocin, etc.), contribute to regulating maternal mineral homeostasis during pregnancy. A more recent series of 10 cases from one group of investigators suggests the same variability in response to the challenge of pregnancy (369).

To avoid confusion, the ionized calcium or albumin-corrected calcium must be followed during pregnancy. Adjustments will need to be made to calcitriol and calcium intake as determined by the individual woman’s response to pregnancy, with some women requiring decreases in both supplements, others requiring progressive increases, and still others requiring no changes. PTH itself is not normally replaced because of its expense and short half-life, but one case demonstrated that a continuous infusion of teriparatide can normalize calcium homeostasis during pregnancy (427).

The treatment target in nonpregnant adults is to maintain the albumin-adjusted calcium at or just below the lower end of normal, to minimize symptoms while protecting the kidneys from an increased filtered load of calcium and consequent nephrocalcinosis. However, for the 9 mo of pregnancy, the target should be to maintain the ionized or albumin-corrected calcium within the normal range, thereby minimizing the risk of fetal and neonatal complications from maternal hypocalcemia, including premature labor and severe secondary hyperparathyroidism (492, 528). Conversely, hypercalcemia must also be avoided because of the adverse effects that it has on fetal development (492).

3. Summary

Available animal and human data indicate that two polar opposite outcomes can occur with hypoparathyroidism during pregnancy: it can become objectively improved (normalization of serum calcium and phosphorus with reduced need for supplemental calcium and calcitriol) or worsen (more marked hypocalcemia requiring significant increases in supplemental calcium and calcitriol). It may also exhibit no significant changes during pregnancy. Furthermore, reliance on the unadjusted serum calcium has led to unneeded interventions in some cases where clinicians did not appreciate that the serum calcium normally falls during pregnancy.

The variability in responses to pregnancy may be related to the calcium content of the diet, contributions of PTHrP and other pregnancy-related hormones to regulating maternal mineral homeostasis, and genetic or ethnic differences. Significant maternal hypocalcemia must be avoided during pregnancy because it increases the risk of fetal mortality (spontaneous abortion and still birth) and severe secondary hyperparathyroidism with fractures in the fetus and neonate. Iatrogenic hypercalcemia should also be avoided. The albumin-corrected or ionized calcium should be monitored for certainty about when a change in therapy is needed.

E. Pseudohypoparathyroidism

1. Animal data

A mouse model of pseudohypoparathyroidism has not been studied during pregnancy (322).

2. Human data

Pseudohypoparathyroidism results from genetically inherited, post-receptor defects in Gsα that create end-organ resistance to PTH (61). Characteristic findings include hypocalcemia, hyperphosphatemia, blunted phosphaturic response to PTH, and high concentrations of PTH. Type I demonstrates blunting of PTH-induced urinary excretion of both phosphate and cAMP, while type II has blunting of PTH-induced urinary excretion of phosphate only.

Type I pseudohypoparathyroidism appeared to improve during pregnancy in two women who maintained 1 g supplemental calcium intake daily during four pregnancies, including that hypocalcemic symptoms abated, normocalcemia was achieved, PTH levels decreased to near-normal, calcitriol levels increased three- to fourfold, urinary excretion of calcium after a 1 g calcium load normalized (an indicator of normal intestinal calcium absorption), and supplemental vitamin D or vitamin D analogs were no longer required (110). In both women the hypocalcemic symptoms promptly returned within 3 wk after delivery (they did not breastfeed) (110). These reports suggest that PTH-independent increases in intestinal calcium absorption and calcitriol synthesis occur during pregnancy in pseudohypoparathyroidism type I, and that the effects are sustained for some time after delivery.

On the other hand, four case reports involving seven pregnancies in women with types I and II pseudohypoparathyroidism found that the dose of calcium, calcitriol, or 1α-calcidiol had to be increased to maintain a normal serum calcium during pregnancy (668, 676, 793, 814). In the first report involving two pregnancies in type I pseudohypopara-
thyroidism complicated by severe nausea and vomiting, a woman experienced an asymptomatic drop in the unadjusted serum calcium during a first pregnancy that prompted an increase in both calcium and calcitriol, while during her second pregnancy she proved intolerant of more than 500 mg calcium per day and had a modest decline in the ionized calcium during the third trimester (668). In the second report a woman who was diagnosed to have type II pseudohypoparathyroidism years after her two pregnancies, recalled that worsening of hypocalcemic-like symptoms had occurred during the second half of each pregnancy, and a single low value of serum calcium from the second pregnancy (left untreated) was found in an old document to corroborate her statement (793). She had not taken any calcium supplements during those pregnancies. The third report involved two pregnancies in the same woman with type I pseudohypoparathyroidism who took a fixed dose of 1α-calcidiol but no supplemental calcium throughout each pregnancy, and the albumin-adjusted serum calcium declined in the latter half of both (814). In the second pregnancy serum calcitriol doubled during the first half, before declining to the prepregnancy value in the second half, accompanied by the fall in albumin-adjusted calcium and a rise in PTH (814). In a fourth case, the protein-adjusted serum calcium was lowered during a pregnancy of a woman with type I pseudohypoparathyroidism, the dose of calcitriol remained unchanged, but the dose of calcium was increased from 250 mg in the first trimester to a median 1 g during the third trimester (676). There is a fifth report involving a woman with type I pseudohypoparathyroidism who presented at the 24th week of pregnancy and was placed on calcium and calcitriol, but the lack of baseline data means that it is unknown whether the condition was altered at all by pregnancy (843).

An older case predating the availability of calcitriol involved a woman who presented in the second trimester, had normal serum calcium and phosphorus, and was prospectively given 4 g calcium daily. Her unadjusted serum calcium remained normal, but the supplemental calcium was increased to 5 g daily, and 1,000 IU vitamin D daily was added when she reported intermittent mild paresthesias (323).

As with hypoparathyroidism, PTH-independent upregulation of calcitriol synthesis or intestinal calcium absorption could explain why pseudohypoparathyroidism may improve during pregnancy. Calcitriol doubled during the second and third trimester for two women in which pseudohypoparathyroidism improved and supplemental calcitriol was stopped (110). In one woman whose serum calcium was stable in the first two trimesters before hypocalcemia occurred in the third trimester, this was paralleled by increases in endogenous calcitriol during the first two trimesters and a decline to prepregnancy values in the third trimester (814). The change in serum calcitriol among these women suggests that whether or not the expected pregnancy-related increase in serum calcitriol occurs during the third trimester predicts whether pseudohypoparathyroidism objectively improves or worsens during pregnancy.

In addition to the possibility that hormones of pregnancy stimulate Cyp27b1 or intestinal calcium absorption directly, it is possible that the hormonal changes during pregnancy (such as higher estradiol levels) could improve post-receptor signaling. The level of calcium intake is likely an important variable. In the four pregnancies in which pseudohypoparathyroidism objectively improved, 1 g supplemental calcium was taken throughout (110), whereas in four of the pregnancies where it worsened, the women took no supplemental calcium (793, 814), and in the remaining three pregnancies the worsening occurred when the supplemental intake was 250 or 500 mg daily (668, 676). In none of these pregnancies was the dietary intake of calcium reported. As noted in sections I and II, the normal physiology of pregnancy means that recommended dietary intake of calcium (1.25 g daily) (430), combined with doubling of efficiency of intestinal calcium absorption, should be more than sufficient to meet the combined needs of mother and fetus during the third trimester. Women normally achieve a positive calcium balance in the first half of pregnancy which likely facilitates meeting the fetal demand for calcium in the third trimester. But if calcium intake is below 1.25 g daily in normal women, then secondary hyperparathyroidism must be invoked to provide additional mineral, so it should not be surprising that pseudohypoparathyroidism will worsen if calcium intake is inadequate during pregnancy.

Placental production of calcitriol is unlikely to improve pseudohypoparathyroidism, given the evidence cited in section II A4 that the placenta contributes little to the rise in calcitriol during human pregnancy. This was further confirmed by analysis of placentas from four pseudohypoparathyroid women, which revealed that calcitriol production was no different than in placentas from normal women (1017).

3. Summary

As with hypoparathyroidism, it seems that pseudohypoparathyroidism can have polar opposite outcomes during pregnancy: objectively improved or objectively worsened. In the absence of dietary intake data, a minimum supplemental calcium intake of 1 g taken throughout pregnancy is likely necessary for the physiological adaptations of pregnancy to be able to meet the fetal demand for mineral. Additional variability may be due to relative contributions of pregnancy-related hormones to regulating maternal mineral homeostasis, and genetic or ethnic differences that influence the pregnancy-related adaptations and the phenotype of pseudohypoparathyroidism. PTHrP may work on bone but would not be expected to contribute to the increase in renal Cyp27b1 activity because of the absence of...
G\textsubscript{}\alpha activity in the proximal tubule of women with pseudohypoparathyroidism.

The goal of management during pregnancy should be to maintain a normal ionized or albumin-corrected serum calcium in the mother, thereby minimizing the risk of fetal and neonatal complications from maternal hypocalcemia or hypercalcemia (492). This means expectant management with appropriate increases or decreases in calcium and calcitriol as required during each pregnancy.

**F. Pseudohyperparathyroidism**

1. Animal data

Animal models have confirmed the production of PTHrP by mammary tissue and placenta, but the extent to which these sites contribute to mineral homeostasis during pregnancy has not been determined. Conversely, it is clear from animal models that PTHrP derived from mammary tissue is an important regulator during lactation, as discussed in sections IV, A3, B, D–F, and VD.

2. Human data

As noted in section IIA3, the breasts and placenta are sources of PTHrP in the maternal circulation during pregnancy. The achieved release of PTHrP appears to be relatively autonomous, independent of serum calcium but correlating with the amount of mammary tissue.

Pseudohyperparathyroidism is PTHrP-mediated hypercalcemia. Its occurrence during pregnancy confirms the potential physiological importance of the breasts and placenta in contributing to the regulation of maternal mineral homeostasis.

Separating out the potential effects of breasts and placenta as sources of excess PTHrP has been possible based on individual case reports. PTHrP-mediated hypercalcemia has occurred in women with massive mammary hyperplasia while pregnant or nonpregnant, and in pregnant women with normal-sized breasts (435, 474, 549, 603, 639, 800). In several cases hypercalcemia became evident during pregnancy, serum PTH was undetectable, PTHrP was either increased in plasma or high levels of PTHrP expression were found in the breasts, and the hypercalcemia resolved only after bilateral mastectomy or weaning (435, 474, 549). In another case a woman with normal-sized breasts was unable to breastfeed because her neonate was critically ill, and she went into a postpartum hypercalcemic crisis (800). The cord blood serum calcium was higher than normal, confirming that mother and baby had been hypercalcemic prior to delivery (800). Furthermore, the mother was hypercalcemic 3 days after delivery with a PTHrP level of 28.4 pM. Since PTHrP has a half-life of minutes in the circulation, similar to PTH, this confirms that the breasts must have been the sustained source of PTHrP, and not (as the authors of that report inferred) the placenta. Another pregnant woman presented with marked hypercalcemia (serum calcium 4.00 mM) associated with high calcium intake and elevated PTHrP of 34.9 pM (639). The hypercalcemia resolved promptly, and she did not breastfeed, but the elevated PTHrP did not become undetectable until 3 mo postpartum.

Pseudohyperparathyroidism also occurs during lactation, and the ability of breast-derived PTHrP to normalize mineral homeostasis in lactating women with hypoparathyroidism is further validation of the importance of the breasts as a physiologically relevant source of PTHrP (see sections IVA3 and V, D and F).

But pseudohyperparathyroidism has also occurred from excess PTHrP that clearly originated from the placenta. This has been demonstrated in a woman with normal-sized breasts who developed severe hypercalcemia (21 mg/dl or 5.25 mM), undetectable PTH, and a serum PTHrP of 21 pM in the third trimester (270). Six hours after an urgent C-section, she was profoundly hypocalcemic with undetectable PTHrP and elevated PTH (270). The rapid reversal in status is most consistent with placental-derived PTHrP causing hypercalcemia.

3. Summary

Hypercalcemia can occur during pregnancy as a result of excess physiological release of PTHrP from the breasts and placenta; this differs from pathological release that occurs in hypercalcemia of malignancy (see sect. III). When the placenta is the cause, the hypercalcemia should be corrected within hours of delivery, whereas production by the breasts is more likely to lead to sustained hypercalcemia after delivery.

**G. Vitamin D Deficiency and Genetic Disorders of Vitamin D Physiology**

1. Animal data

Calcitriol regulates the active, saturable, transcellular mechanism of intestinal calcium delivery, as well as the passive, paracellular mechanism of absorption (181). After pups are weaned and in adult animals, severe vitamin D deficiency and genetic absence of either calcitriol or VDR lead to the common phenotype of reduced intestinal calcium absorption, hypocalcemia, hypophosphatemia, and rickets. Intestinal mineral delivery appears to be the main route through which calcitriol has direct and indirect actions on mineral and bone metabolism. Several lines of evidence have confirmed this, including that the abnormal mineral homeostasis and rachitic phenotype of Vdr null
mice is rescued by expressing \( Vdr \) in intestinal cells (555, 1000), whereas selective ablation of \( Vdr \) from intestinal cells (555) or dietary calcium restriction create the rachitic phenotype (555). Moreover, ablatting \( Vdr \) from chondrocytes, osteoblasts, or osteocytes, or \( Cyp27b1 \) from chondrocytes, do not cause rickets either (555, 606, 644, 1006). Also, a high calcium diet bypasses the need for calcitriol and intestinal expression of its receptor, and normalizes serum chemistries, skeletal morphology, and skeletal mineral content in severely vitamin D-deficient, \( Cyp27b1 \) null, and \( Vdr \) null models (28, 102, 103, 224, 241, 273, 553, 554, 693, 874, 875, 948, 1011). However, remaining evidence for a direct role of calcitriol or VDR in regulating bone cells is that \( Cyp27b1/Vdr \) null double mutants have a skeletal phenotype that is not fully prevented by a high-calcium diet (692).

What is the impact of disordered vitamin D physiology on rodent pregnancy? In several models the mothers conceive less frequently and bear fewer pups in each liter. This includes severely vitamin D-deficient rats (359, 361–363), \( Cyp27b1 \) null mice that cannot make calcitriol (330), and \( Vdr \) null mice that lack the receptor for calcitriol (296, 502), but not \( Cyp27b1 \) null (Hannover) pigs which have either singleton or twin pregnancies (520, 521). The low conception rate and smaller litter sizes of rodents are rescued by administering a high-calcium diet (223, 330, 446, 502, 553, 693, 886), which indicates that calcium and not calcitriol is the missing component responsible for these fertility issues. Normal pregnancies still occur in \( Vdr \) null mice maintained on a normal-calcium diet (502), but the increased frequency of pregnancies on the high calcium diet facilitates their study during reproductive cycles.

What is the role of calcitriol in regulating intestinal calcium absorption and mineral metabolism about during pregnancy? This has been studied in severely vitamin D-deficient rats (117, 358, 362, 363), \( Cyp27b1 \) null pigs (520, 521), \( Cyp27b1 \) null mice (330), and \( Vdr \) null mice (296, 1026). In these models occasional sudden deaths occur during late pregnancy, suggesting that rapid placental calcium transfer can overwhelm the ability of the mother to maintain her own ionized calcium level. The pregnancies are otherwise uneventful and result in fetuses that have normal ionized calcium, phosphorus, PTH, weight, skeletal development, and mineralization (see companion review for details, Ref. 492). A notable abnormality is that offspring of \( Vdr \) null females were globally but proportionately smaller than offspring of their WT and \( Vdr^{+/-} \) sisters, a difference that was not seen between offspring of vitamin D-deficient and replete rats. This may indicate that maternal absence of VDR affects offspring growth independent of the fetal genotypes and that it is exerted by the receptor but not calcitriol.

More recently, severe vitamin D deficiency has been studied in mice, which had identical litter sizes but bore pups of modestly greater weights and slightly increased lengths, compared with vitamin D-sufficient mothers (563). The diet was deficient in vitamin D but not enriched in calcium. No mention was made as to whether maternal vitamin D deficiency led to a reduced conception rate, and no measurements of bone mass or mineralization were performed. The placentas from vitamin D-deficient pregnancies were of identical weight but had smaller diameters, and histological evidence of reduced vascular diameters within the labyrinthine placenta, compared with pregnancies from vitamin D-sufficient mothers (563).

As noted earlier, severely vitamin D-deficient rats, \( Vdr \) null mice, and \( Cyp27b1 \) null mice achieve significant gains in bone mineral content during pregnancy, with reduced secondary hyperparathyroidism and increased mineralization of osteoid (296, 362, 489, 786). An increase in intestinal calcium absorption during pregnancy has been confirmed in severely vitamin D-deficient rats (116, 360) and \( Vdr \) null mice (296), and inferred to be present but not yet studied in \( Cyp27b1 \) null mice (330). During pregnancy serum calcium and phosphorus increased in severely vitamin D-deficient rats (915), \( Vdr \) null mice (Boston and Leuven strains) normalized serum calcium and increased renal calcium excretion (296, 786, 1026), and \( Cyp27b1 \) null mice also normalized serum calcium and phosphorus (330). In all of these studies the main intervention was that the animals became pregnant, thus confirming that pregnancy invokes improvements in intestinal calcium absorption and mineral homeostasis that are independent of vitamin D, calcitriol, and VDR.

Nonskeletal outcomes of pregnancy have received little attention in these animal studies, but the recent study of vitamin D-deficient mice found higher systolic and diastolic blood pressure, and upregulation of renal expression of renin and angiotensin II receptor mRNA, compared with vitamin D-sufficient mice (563). These findings support the possibility that vitamin D deficiency may increase the risk of pregnancy-induced hypertension.

2. Human data

As in rodents, calcitriol-dependent active absorption of calcium represents ~20% of net calcium absorption, with the rest occurring by passive, nonsaturatable mechanisms. Active absorption is considered to be more important when dietary intake of calcium is limited. Multiple dual or single isotope studies carried out in adults and adolescents have examined the level of 25OHD at which intestinal calcium absorption is maximized or reaches a plateau, and have established that a plateau occurs below 20 nM 25OHD, with very little increase (if any) in intestinal calcium absorption above that value (7, 24, 26, 306, 650, 774). Secondary hyperparathyroidism occurs at lower levels of 25OHD and maintains high levels of calcitriol despite the reduced availability of substrate, which may be why vitamin D deficiency does not
impair intestinal calcium absorption until the 25OHD level is below 20 nM (650).

What evidence is there that maternal vitamin D deficiency, or genetic disorders causing loss of calcitriol or VDR, affect maternal mineral metabolism and obstetrical or fetal outcomes?

Unfortunately, no study has measured intestinal calcium absorption during pregnancy in vitamin D-deficient compared with vitamin D-sufficient women, so it remains unknown whether or not intestinal calcium absorption doubles in pregnant, vitamin D-deficient women.

The most robust clinical data generally come from large, randomized, blinded clinical trials, which control for confounding through the randomization of subjects into two or more treatment groups. Several modestly sized clinical trials have examined the effects of vitamin D supplementation on obstetrical and fetal/neonatal outcomes, and the maternal results will be discussed in the following paragraphs. The fetal and neonatal outcomes of these studies have been described in the companion review (492); in brief, no consistent change in cord blood calcium, phosphorus, PTH, birth weight, or anthropometric measurements were observed when babies of vitamin D-supplemented mothers were compared with babies of placebo-treated mothers. Several studies did note a reduction in the incidence of neonatal hypocalcemia after 48 h when the babies of placebo-treated mothers had cord blood 25OHD levels below 20 nM (492). This is consistent with the aforementioned adult data that intestinal calcium absorption is reduced when the 25OHD level is below 20 nM. However, calcium absorption across the fetal intestines is a trivial circuit not relevant to fetal mineral homeostasis, whereas the main route of calcium delivery is transportation across the placenta through mechanisms that do not require calcitriol (492).

Because the potential adverse effects of vitamin D deficiency during pregnancy have garnered much recent attention, the individual studies will be described in sufficient detail to understand the doses of vitamin D tested, the sizes of the studies, the achieved increment in 25OHD between groups, and any obstetrical (maternal) benefit observed. The companion review should be consulted for details of any fetal or neonatal benefit.

Brooke et al. (121) studied 126 severely vitamin D-deficient Asian women living in England, beginning at 28–32 wk of gestation. At term the mean serum 25OHD was 16 nM (6.4 ng/ml) in the placebo group and an exuberant 168 nM (67 ng/ml) in women who received 1,000 IU vitamin D daily (the dose was likely higher given the 25OHD increment achieved). By comparing these two extremes of severe vitamin D deficiency versus repletion to a high 25OHD level, this study was well poised to demonstrate any obvious maternal benefit of vitamin D repletion. A greater weight gain and a small increase in the unadjusted serum calcium were observed in the vitamin D-treated group, but no other obstetrical benefit was seen (121).

Cockburn et al. (191) randomized 164 women to 400 IU vitamin D versus placebo beginning at the 12th week of pregnancy, and achieved maternal 25OHD of 42.8 vs. 32.5 nM (17 vs. 13 ng/ml) at term, but no obstetrical benefit was described (191). Mallet et al. (588) randomized 77 women to receive 1,000 IU vitamin D3 daily during the third trimester, or 200,000 IU vitamin D3 at the start of the third trimester, or no supplementation (588). The respective maternal 25OHD levels were 25.3, 26.0, and 9.4 nM (10, 10.4, and 3.8 ng/ml) with no effect on maternal serum calcium or calcitriol, and no obstetrical benefit reported.

Delvin et al. (239) randomized 40 women in France to 1,000 IU vitamin D3 daily versus placebo beginning at 6 mo of pregnancy. Maternal 25OHD increased to 56 nM (22 ng/ml) compared with 27.5 nM (11 ng/ml) in the placebo group, but there was no difference in ionized calcium, serum calcium, calcitriol, or PTH, and no reported obstetrical benefit.

Marya and co-workers (604, 605) performed two studies in vitamin D-deficient Asian women in India. In each of these, severe vitamin D deficiency was presumed to be present on the basis of estimated dietary intakes of vitamin D at <35 IU/day, but 25OHD was not measured and sunlight exposure leading to vitamin D formation was not considered. The first study involved 20 women who received 800,000 IU vitamin D3 in the seventh and eighth months of pregnancy, 25 women who received 1,200 IU vitamin D3 per day during the third trimester, and 75 women who received nothing (605). No significant effect on serum calcium or phosphorus was noted, but the alkaline phosphatase was reduced in vitamin D-treated subjects. The second study gave 100 women 600,000 IU of vitamin D2 in the seventh and eighth months of pregnancy, and compared the results to 100 women who received nothing (604). The unadjusted serum calcium and phosphorus were slightly but statistically significantly higher in the women who received vitamin D, and the alkaline phosphatase was lower, but there were no significant differences in reported constitutional symptoms.

In the studies cited thus far, there are concerns as to whether or not subjects were properly blinded and randomized, the sample sizes were small, and obstetrical data were not fully reported. In contrast, the following studies were carried out within the past decade, used modern randomization methods and clinical trial protocols, and reported more obstetrical outcomes.
Yu et al. (1013) randomized 180 women in London to receive unblinded treatment beginning at week 27 of pregnancy: 800 IU vitamin D$_2$ per day, a single dose of 200,000 IU vitamin D$_3$, or no treatment (1013). Mean 25OHD increased to 42 and 34 nM (16.8 and 13.6 ng/ml) in the respective treatment groups at term compared with 27 nM (10.8 ng/ml) in the untreated women. There was no effect on preterm delivery and no other obstetrical outcomes.

Roth et al. (778) randomized 160 women in Bangladesh (NCT01126528) to receive 35,000 IU vitamin D$_3$ per week or placebo beginning at 26–29 wk. Maternal 25OHD was 134 nM (53.6 ng/ml) in the supplemented women versus 38 nM (15.2 ng/ml) in the placebo group. There was no effect on maternal serum calcium, but a borderline significant increase in albumin-adjusted calcium and decrease in PTH were found (779). There was no effect on mode of delivery, C-section rates, adverse events, live versus stillbirths, or gestational age at delivery.

Hashemipour et al. (367) randomized 160 women in Iran to receive 50,000 IU vitamin D per week or 400 IU daily beginning at 26–28 wk. Maternal 25OHD was 120 nM (48 ng/ml) versus 40 nM (16 ng/ml), and the unadjusted serum calcium was modestly higher in the vitamin D-treated women. There was no difference in gestational age of delivery; no other obstetrical outcomes were reported.

Grant et al. (342) randomized 260 women in New Zealand (ACTRN12610000483055) to placebo, 1,000 IU vitamin D daily, or 2,000 IU vitamin D daily. Maternal 25OHD levels at term were 50, 98, and 103 nM (20, 39, and 41 ng/ml), respectively. There was no difference in serum calcium or gestational age of delivery; no other obstetrical data were reported (342).

Kalra et al. (455) randomized 97 women in India to one dose of 60,000 IU vitamin D in the second trimester or 120,000 IU vitamin D in each of the second and third trimesters; 43 “usual care” women were the controls. Maternal 25OHD levels at term were 26.2, 58.7, and 39.2 nM (10.5, 23.5, and 15.7 ng/ml), respectively (455). There were no differences in obstetrical outcomes, including gestational age at delivery, intrauterine death, pregnancy-induced hypertension, cephalopelvic disproportion, nonprogression of labor, cesarean section, and placenta previa (455).

Hollis and Wagner (399) reported on 350 women (NCT00292591) randomized at 12–16 wk of gestation to receive 400, 2,000, or 4,000 IU vitamin D$_3$ per day. Maternal 25OHD levels at term were 79, 98, and 111 nM (31.6, 39.2, and 44.4 ng/ml), respectively. There was no significant difference in serum calcium across the treatment groups, but there was a borderline-significant reduction in PTH when the high-dose and low-dose groups were compared. There were no differences in any obstetrical outcomes, including mode of delivery, gestational age at delivery, preterm birth, preterm labor, preeclampsia, and infection (399, 963).

A second study by Hollis and Wagner and co-workers (966) reported on 160 women (NCT00412087) randomized at 12–16 wk to receive 2,000 or 4,000 IU vitamin D$_3$ daily. Achieved maternal 25OHD was 90.5 nM (36.2 ng/ml) and 94.8 nM (37.9 ng/ml), respectively. There was no effect on any obstetrical outcome, including mode of delivery, preterm labor, preterm delivery, hypertension, infection, gestational diabetes, or combinations of these outcomes (966).

Hollis and Wagner have subsequently carried out several post-hoc analyses of these two trials, including analyses in which selective data from both studies were pooled, out of which some borderline significant results have been obtained (399, 401, 963, 964, 967). However, these analyses suffer from lack of adjustment for multiple comparisons, arbitrary grouping of outcomes, and exclusion of some ethnicities from the analysis. For example, the combination of infection/preterm labor/preterm birth/gestational diabetes/preeclampsia/hypertension/HELLP had a P value of 0.03 (not adjusted for multiple comparisons), whereas excluding preterm labor (which is linked to preterm birth and likely means women are counted twice) resulted in a P value of 0.06, including all ethnicities resulted in a P value of 0.17, while multiple other subgroupings and the individual outcomes were not statistically significant (401, 963).

Dawodu et al. (229) studied Arab women who had mean 25OHD concentrations of 20.5 nM (8.2 ng/ml) at baseline and were randomized to 400, 2,000, or 4,000 IU vitamin D/day. Achieved 25OHD levels at delivery were 40 nM (19.3 ng/ml), 65 nM (25.9 ng/ml), and 90 nM (35.9 ng/ml), respectively (229). There were no differences in maternal serum calcium or urine calcium/creatinine between groups at any time point during pregnancy, PTH was reduced in the high-dose group but correlated poorly with 25OHD, no effect was seen on gestational age at delivery or the babies’ anthropometric parameters at birth, and no other obstetrical outcomes were reported.

Most recently, Cooper and Harvey and co-workers (199, 200) provided preliminary results of the MAVIDOS study from the United Kingdom. The 900 women who completed the study randomly received either 1,000 IU vitamin D or placebo beginning at 14 wk of pregnancy. Baseline 25OHD was ~45 nM (18 ng/ml), did not change in placebo-treated women, and rose to 68 nM (27 ng/ml) near term in the vitamin D-supplemented women. During the oral and poster presentation of results (199, 200), the study was revealed to be negative in its primary outcome (neonatal bone area, bone mineral content, and bone mineral density within the first 14 days after birth) and secondary outcome
(anthropometric and body composition parameters within 48 h of birth). No obstetrical benefit was reported. A post-hoc analysis, which was not prespecified in the clinical trial registrations (ISRCTN 82927713 and EUDRACT 2007-001716-23), suggested a possible benefit on bone mineral content of winter-born babies. If the apparent benefit on winter-born babies is real and not a chance result, it may not be an indication of an effect on the fetus but instead a postnatal effect, since (as noted earlier) the neonatal skeleton should gain 100 mg calcium per day over the first 14 days after birth. A measurement at 14 days does not indicate bone mineral content at term.

Overall, these studies have largely examined women who, prior to the intervention, had 25OHD values well above the 20 nM threshold that has been shown to result in normal intestinal calcium absorption. Only Brooke’s study (and possibly Marya’s two studies, although 25OHD levels were not measured) compared true vitamin D deficiency to a vitamin D-replete state, and an increase in maternal serum calcium was observed. No obstetrical outcome differences were reported, but it is unclear that the authors of that report were looking for any. It may take a study of that extreme to determine if there are differences in obstetrical outcomes, but ethical considerations may prevent it being carried out in the modern day. Among the modern studies, Dawodu’s began with vitamin D-deficient women but at the end of pregnancy the mean 25OHD level was in the insufficient range at 40 nM (229). In that arm of the study, 79% of women had a 25OHD value <50 nM, so it contained many vitamin D-deficient women, but still no significant maternal or fetal/neonatal differences were seen apart from an increase in serum 25OHD.

Pregnancies have been unremarkable in women with genetic absence of Cyp27b1 (vitamin D-dependent rickets type 1, VDDR-I) or relatively inactive VDRs (VDDR-II) (266, 589, 602). In one case of VDDR-II, the pregnancy was unremarkable while the woman was maintained on her prepregnancy doses of calcium (800 mg) and high-dose calcitriol (602). The clinicians did increase the dose of calcitriol during that pregnancy “because of the knowledge that the circulating 1,25-(OH)2D concentration normally rises during pregnancy,” but not because of any change in the albumin-adjusted serum calcium (602). In multiple cases of VDDR-I, the dose of calcitriol was unchanged in one-third of pregnancies and increased 1.5- to 2-fold in others (266). In both disorders, calcium and calcitriol or 1α-cholecalciferol should be adjusted as needed to maintain a normal ionized or albumin-corrected serum calcium.

More recently, hereditary absence of Cyp24a1 has been shown to lead to maternal and fetal hypercalcemia (249, 820), likely because reduced catabolism of calcitriol leads to relatively unopposed actions of the active hormone to stimulate intestinal calcium absorption and potentially induce osteoclast-mediated bone resorption.

On the basis of associational studies, vitamin D deficiency has been suggested to have nonskeletal effects during pregnancy, such as increasing the risk of preterm delivery, C-sections, low birth weight, preeclampsia/pregnancy-induced hypertension, and vaginal infections. One can find approximately as many studies suggesting these associations (46, 95, 370, 622, 768, 832) as there are studies indicating no association (47, 276, 294, 618, 726, 810, 823). Each of these studies has low power and are confounded by factors that may lead to lower 25OHD levels and the outcome in question, including race/ethnicity, maternal overweight/obesity, lower socioeconomic status, poor nutrition, etc. For example, maternal overweight/obesity is a significant risk factor for preterm delivery, C-sections, low birth weight, preeclampsia/pregnancy-induced hypertension, vaginal infections, and other adverse obstetrical outcomes (542). But maternal overweight/obesity also causes low 25OHD by binding the fat-soluble vitamin D into the maternal fat stores, and by additional associations with reduced vitamin D intake, less time spent outdoors, etc. Consequently, the studies reporting associations between vitamin D intake or 25OHD levels and obstetrical outcomes may really be demonstrating residual confounding from the link between obesity and these outcomes, and also need to consider that the results of the existing randomized trials did not find these outcomes in their primary analyses (183, 184, 367, 399, 455, 710, 779, 966, 1013). Larger randomized trials that compare truly vitamin D-deficient to sufficient mothers are needed to properly test the hypothesis, and eliminate the confounding caused by maternal overweight and obesity.

3. Summary

Several animal models (severe vitamin D deficiency and absence of VDR or Cyp27b1) have demonstrated improved to near-normalized mineral homeostasis and intestinal calcium absorption during pregnancy, suggesting that calcitriol is not required for the adaptations that are invoked during pregnancy, or that other physiological mechanisms are able to compensate for its absence. Clinical data are not as extensive, but the results of available clinical trials do not show a clear benefit of high-dose vitamin D supplementation on maternal mineral or skeletal homeostasis, or obstetrical and fetal outcomes. Most of the clinical trials did not enroll women who were vitamin D deficient, so the ability to detect any benefit was likely lost.

H. Calcitonin Deficiency

1. Animal data

Early attempts to create experimental models of calcitonin deficiency did not appreciate that there are extrathyroidal
sites of calcitonin synthesis during pregnancy, including the mammary glands and placenta. In pregnant goats or rats, calcitonin deficiency was created by a complete thyroparathyroidectomy followed by parathyroid gland autotransplantation and treatment with exogenous thyroid hormone (55, 58, 163, 551, 900). Serum calcitonin, TSH, PTH, and ionized calcium were not measured, so it is uncertain whether a calcitonin-deficient, euthyroid, euparathyroid state was achieved. In three studies the animals were killed the day before expected delivery (55, 58, 163), whereas two other studies reporting a pregnancy-related effect must be excluded because the animals were killed after 3 wk of lactation (551, 900) (see sect. VH). Among the three pregnancy studies, the ash weight and calcium content were 5–10% lower in thyroidectomized compared with sham-operated animals at the end of pregnancy; these differences were statistically significant in goat metacarpals and metatarsals (55, 58), but not in rat femora (163). The spine is the potential site of greatest bone loss during pregnancy, but it was not examined in these studies.

In contrast to these surgical models, Ctcgrp null mice represent true calcitonin deficiency because the gene encoding calcitonin and calcitonin gene-related peptide has been ablated, but they are also confounded by loss of CGRP-α (394). At the end of pregnancy there were no differences from WT sisters in BMC of the whole body, lumbar spine, or hindlimb, suggesting that loss of calcitonin does not impair the ability of the mother to gain bone mass during pregnancy (992, 994). These mice received standard 1% calcium intake and have not been studied on a calcium-restricted diet.

Overall, the surgical models suggested that an intact thyroid gland may protect the maternal skeleton from loss of bone mineral during pregnancy and that this effect is attributable to calcitonin, assuming that parathyroid and thyroid function remained normal. However, Ctcgrp null mice did not display excess bone loss during pregnancy.

2. Human data

Increased serum calcitonin has been proposed to protect the skeletons of women from excessive resorption during pregnancy, but this concept has not been directly tested. Totally thyroidectomized women have placentas and breasts as sources of calcitonin production during pregnancy, so they are not truly calcitonin deficient. No women with genetic loss of calcitonin or its receptor have been identified or studied during pregnancy.

Nevertheless, several studies have looked at the long-term effects of total thyroidectomy on bone density and risk of fractures in both men and women, with some studies suggesting an increased risk and others finding none (326, 338, 422, 630, 659). Studies from the last decade are confounded by the common practice to treat with suppressive doses of thyroid hormone to reduce the risk of cancer recurrence, but even in those studies bone loss has not been consistently found (476, 543). None of these studies looked at the effects of pregnancy, during which the women may not have been calcitonin deficient anyway.

It is notable that with modern calcitonin assays, the basal calcitonin level is unchanged before and after total thyroidectomy in patients without medullary thyroid cancer; it is only the calcium-stimulated or pentagastrin-stimulated calcitonin that is lost (513). This suggests that extrathyroidal calcitonin sources are functional in nonpregnant, nonlactating women.

3. Summary

Thyroidectomy to create partial calcitonin deficiency during pregnancy led to bone loss at term compared with sham-operated animals; however, no effect was seen in pregnant mice lacking the calcitonin gene. There are no comparable human data for the surgical or genetic models of calcitonin deficiency during pregnancy.

I. Low or High Calcium Intake

1. Animal data

As noted earlier (sect. II, A1, A2, and D), a calcium-restricted diet provokes marked hypocalcemia and secondary hyperparathyroidism in pregnant rats; it also leads to compensatory secondary hyperparathyroidism and skeletal resorption in the fetuses (492). In pregnant ewes it causes hypocalcemia and bone resorption, but the birth weights of offspring are unaffected (detailed analysis of the fetal bones was not done) (69). Conversely, high calcium intake prevents hypocalcemia and secondary hyperparathyroidism, and enables most of the calcium transported to the fetuses to come from the maternal diet rather than her skeleton. It also reduces the likelihood of net loss of calcium from the skeleton and may enable a net gain. Rats consuming a 1.6% and 1.4% calcium diet had a higher femoral calcium content at the end of pregnancy compared with rats that consumed 0.5% calcium (719, 818).

2. Human data

Pregnancy is normally a state of absorptive hypercalciuria with suppressed PTH, which implies that for most women calcium is absorbed in excess of maternal and fetal requirements. But as noted earlier (sect. II, A1, A2, and D), PTH does not fall and may rise above normal in women with low dietary intakes of calcium or high intake of phytate (which blocks calcium absorption). However, in some women with chronically low calcium intakes, the efficiency of intestinal calcium absorption may be greater than normal, as suggested by a study in which most women with a dietary
intake of less than 420 mg were in a positive calcium balance in all three trimesters (830). A lower calcium intake is more likely to induce bone loss during pregnancy (10) and has been associated with increased risk of osteoporosis developing during pregnancy (501). Whether the mother obtains the needed mineral from her diet or skeleton may be of no consequence to the fetus, although a randomized trial found that 2 g of supplemental calcium versus placebo improved the BMC of babies whose mothers were in the lowest quintile of dietary calcium intake (<600 mg/day) (488). Significant maternal hypocalcemia impairs the delivery of calcium to the fetuses, who may develop secondary hyperparathyroidism, skeletal demineralization, and fractures, as has occurred in cases of poorly treated maternal hypoparathyroidism during pregnancy (492). The lowest quintile of maternal calcium intake is also associated with increased risk of pre eclampsia, whereas calcium supplementation reduces that risk (see sect. II C).

In contrast to low calcium intake, excessively high intake will create similar effects as primary hyperparathyroidism in pregnancy: increased net absorption of calcium, maternal hypercalcemia, increased maternal to fetal transfer of calcium, and suppression of the fetal parathyroids (100, 483, 767). Neonatal hypoparathyroidism has resulted from women consuming 3–6 g elemental calcium daily to treat nausea of pregnancy (100, 767).

3. Summary

Extremes of low and high calcium intake should both be avoided in pregnancy due to adverse effects that each can have on the mother and the fetus. Low calcium intake causes secondary hyperparathyroidism in mother and fetus and resorption of the maternal and fetal skeletons with consequent fragility. Conversely, high calcium intake leads to increased flow of calcium across the placenta, suppression of the fetal parathyroids, and a predisposition to neonatal hypoparathyroidism with hypocalcemia.

J. Hypercalcemia of Malignancy

1. Animal data

No animal models have been studied during pregnancy.

2. Human data

There are over a dozen published cases of hypercalcemia of malignancy during pregnancy, which is usually due to PTHrP overproduction (4, 211, 325, 424, 428, 608, 614, 634, 898, 945). This is usually but not always a terminal condition, so the first decision is whether to terminate the pregnancy to enable chemotherapy to be administered, defer such treatment until the baby is born, or (as has also been reported, Ref. 614) to proceed with chemotherapy during pregnancy regardless of its potential teratogenic effects. In about half of case reports, the baby’s outcome is not mentioned, but the cord blood calcium is likely to be high and followed by neonatal hypocalcemia and tetany.

Treatment options include debulking or curative surgery, hydration, diuresis, and possibly calcitonin or bisphosphonates. There are potential teratogenic effects of calcitonin and bisphosphonates (492), but they have been used during pregnancy to treat this condition.

3. Summary

Hypercalcemia of malignancy causes severe and potentially terminal maternal hypercalcemia; if the baby survives, it is at high risk for neonatal hypocalcemia.

K. Fibroblast Growth Factor 23-Related Disorders

1. Animal data

Pregnancies are unremarkable in Phex/Hyp+/- mice, a model of XLH that leads to FGF23 excess (240, 581, 679). Despite very high levels of FGF23 in pregnant Phex+/- females (581, 679), which normally downregulate calcitriol synthesis and increase its catabolism, maternal serum calcitriol increases to the expected high levels of pregnancy (581, 679), and likely contributes to pregnancy-related increases in intestinal calcium and phosphorus absorption. Despite the maternal hypophosphatemia, the fetuses display normal serum mineral and calcitriol hormone concentrations, lengths, placental phosphorus transport, skeletal development, and mineralization (581).

Since phosphorus is actively transported across the placenta, maternal hypophosphatemia can be expected to increase the flow of phosphorus and, thereby, potentially affect fetal mineralization. Hyperphosphatemic disorders due to absence of FGF23 have not been studied during pregnancy because Fgf23 null mice die by several weeks of age (848), whereas Klotho null mice have marked hypogonadism and die by 9–10 wk of age (516). Experimental renal failure in rats has shown that maternal hypophosphatemia does not impair skeletal development or mineralization (765, 766); however, there is a modest reduction in fetal weight and length that has been attributed to decreased food intake in uremic mothers (664, 765).

2. Human data

Isolated case reports have confirmed that hypophosphatemia persists during pregnancy in women with XLH (447, 745), but the lack of adverse outcomes from untreated pregnancies has made it uncertain whether the hypophosphatemia needs to be treated. Nevertheless, the general
principle is to maintain calcitriol and phosphorus supplementation throughout pregnancy to ensure adequate delivery of phosphorus to the fetus (538).

There are no published reports on pregnancies in women with hyperphosphatemic disorders caused by deficiency of FGF23 or its co-receptor. Large case series of pregnancies in women on dialysis have shown increased obstetrical risks of gestational hypertension, preeclampsia, eclampsia, and maternal mortality, and increased adverse fetal outcomes including premature birth, intrauterine growth restriction, low birth weight, stillbirth, and neonatal mortality (139, 594, 656). These outcomes may relate more to the maternal renal failure than the hyperphosphatemia per se. No data have been reported about cord blood chemistries or skeletal development and mineralization in the fetuses or neonates of these hyperphosphatemic mothers.

3. Summary

Hypophosphatemic and hyperphosphatemic disorders in the pregnant mother have the potential to adversely affect the fetus. There are insufficient human data to know for certain, but the limited animal data are reassuring that fetal mineral and bone metabolism may be unaffected by extremes of low and high serum phosphorus in the mother.

IV. SKELETAL AND MINERAL PHYSIOLOGY DURING LACTATION AND POST-WEANING RECOVERY

In their widely quoted seminal text The Parathyroid Glands and Metabolic Bone Disease (15), Albright and Reifenstein used data from several sources (77, 407, 836) to estimate that maternal losses of calcium are fourfold higher during 9 mo of lactation than in pregnancy, or ~80 g in milk versus 20 g in the fetal skeleton. This means an output of more than 300 mg of calcium in milk each day. However, the authors misread source data that showed closer to 30 g of calcium in a term fetus (77), used an overestimate of milk production (407), and lacked any skeletal calcium content data at 9 mo. Modern studies, referred to earlier in the overall introduction, have shown that the average full-term fetus has 30 g of calcium and that another 30 g (~100 mg/day) is added by 9 mo. Consequently, the calcium required by the neonate during 9 mo of lactation equals that required by a fetus during all of pregnancy and does not exceed it. However, as explained in the following paragraphs, maternal losses of calcium during 9 mo of lactation are about double that of pregnancy.

Other older studies similarly overestimated average daily loss of calcium into milk due to overgenerous estimates of milk volumes. In a large study of 200 women who expressed milk manually for 24 h, the volume ranged from 0.8 to 1.8 liters for a mean daily loss of 400 mg calcium (582, 584). Individual women were identified who had sustained production of up to 3 liters of milk per day during 6–12 mo of lactation (419, 583, 838); analysis of their milk revealed daily calcium losses of 500–1,200 mg (419, 838). However, manual expression of milk can yield much greater volumes than a suckling baby demands, thereby overestimating milk production. Further bias was introduced by studying women known to produce large volumes of milk, some of whom were employed as wet nurses.

As noted in the overall introduction, more recent studies have relied on weighing the baby before and after feeds, whereas analyses of milk calcium content at different stages of lactation revealed similar results to older studies. An average daily output of 780 ml milk (20, 138, 376, 653) with an average calcium content of 260 mg/l during the first 6 mo of lactation (41) results in a more realistic average calcium loss of 200–210 mg/day (774), from which the baby is expected to accrete ~100 mg daily.

These are average amounts, and it is recognized that individual daily losses can vary considerably. Losses have been much higher in the more extreme examples cited above, and in women nursing twins who generally produce about double the amount of milk of women who nurse singletons (233, 792). The composition of milk also varies within and between feedings (536, 654), between an individual’s breasts (654), and between mothers and different ethnic groups (536). The calcium content of milk is higher at 3 mo than at 6 mo postpartum (459, 536, 856, 957), but the volume of milk per feed is higher at 6 mo (856). Consequently, daily maternal loss of calcium could be greater at 6 mo and beyond, compared with the first 3 mo of lactation (856). However, the number of feeds is usually fewer after 6 mo, thereby leading to an overall decline in daily calcium losses.

Maternal milk output also increases in proportion to litter number and weight in lactating animals, thereby maintaining a similar weight of pups regardless of their number. In mice, for example, the weight of individual pups at 12 days of age shows <10% variation from a litter of 4 versus a litter of 12. But the combined weight of a litter of 12 is three times that of a litter of 4, confirming that a larger litter means a greater maternal output of milk and calcium (87). A similar constancy of neonatal pup weight has been seen in pigs whose litter sizes range from 5 to 12 at 8 wk of age (87). Ewes suckling twins produce about double the amount of milk of ewes nursing singletons (970).

To meet the mineral requirements of lactation, maternal adaptations might include increased intestinal absorption of calcium, renal conservation of calcium, and increased skeletal resorption of calcium. Studies reviewed in this section reveal that the main adaptation is a temporary demineralization of the skeleton, which appears to meet the cal-
Calcium requirements of milk production. Two processes, osteoclast-mediated bone resorption and osteocytic osteolysis, are responsible for mobilizing skeletal calcium. Conservation of calcium by the kidneys is also evident (in contrast to pregnancy), but intestinal calcium absorption falls to normal in lactating women from the elevated values of pregnancy (it remains elevated in lactating rodents). PTH and calcitriol do not appear to be the main regulators of these processes; instead, secretion of PTHrP by breast tissue and systemically low estradiol levels are two of the key factors that stimulate bone resorption, while PTHrP also stimulates renal calcium conservation. So it is evident that the maternal adaptations during lactation differ from those that are invoked during pregnancy, which in turn means different consequences for normal women, and for women who have disorders of bone and mineral metabolism.

A. Changes in Mineral Ions and Calciotropic Hormones

Progressive changes in serum calcium, phosphorus, and calciotropic hormone levels during human lactation are schematically depicted in Figure 2. Important differences among human, rat, and mouse lactation are listed in Table 2.

1. Calcium and phosphorus

A) Animal data. The serum calcium of lactating rats has been quite variable in published reports, and not clearly explained by the calcium content of the diet: hypercalcemia on 1.5 or 1.2% calcium (86, 318); normocalcemia on 1.6% (568), 1% (991), 0.5% (114), 0.4% calcium (91, 318, 569), and an unspecified diet (337); low blood calcium on 1.2, 0.9, 0.8, and 0.4% calcium (76, 90, 320, 321, 568, 718, 920, 927, 989); and marked hypocalcemia on 0.1, 0.04, and 0.01% calcium (32, 320, 321, 568, 991). The ionized calcium has been low on a 1.6% calcium diet (568), high on a 1.2% calcium diet (318), low on a 0.8% calcium diet (90, 568, 569), and low on a 0.4% calcium diet (90, 568, 569), and low on a 0.1% calcium diet (32, 568).

FIGURE 2. Schematic depiction of longitudinal changes in calcium, phosphorus, and calciotropic hormone levels during lactation and post-weaning skeletal recovery in women. Normal adult values are indicated by the shaded areas. PTH does not decline in women with low calcium or high phytate intakes and may even rise above normal. Calcidiol (25OHD) values are not depicted; most longitudinal studies indicate that the levels are unchanged by lactation, but may vary due to seasonal variation in sunlight exposure and changes in vitamin D intake. PTHrP and prolactin surge with each suckling episode, and this is represented by upward spikes. FGF23 values cannot be plotted due to lack of data. Very limited data suggest that calcitriol and PTH may increase during post-weaning, and the lines are dashed to reflect the uncertainty. The data from which this summary figure has been derived are cited in section 1A.
Differences in rat strains, litter sizes, and whether the samples were drawn fasted or not likely contribute to the variation in results. Greater demands for milk production (such as larger litter number and combined weight of all pups) provoke a lower maternal serum calcium (90, 320, 567, 708). Abrupt weaning causes the serum calcium to rebound into the hypercalcemic range for several days (76, 86, 396, 718). This is likely due to sudden loss of the outflow of calcium into milk while increased bone resorption and intestinal calcium absorption persist (see sect. IV, B and E).

An increase in serum calcium after abrupt weaning occurred even when dietary intake of calcium was negligible on a 0.02% calcium diet (396), which confirms that increased skeletal resorption is a driver of the post-weaning rebound in calcium.

Serum calcium declined a greater amount during lactation in vitamin D-deficient rats (91), indicating that vitamin D deficiency impairs the ability of the mother to maintain her serum calcium during lactation. This contrasts with pregnancy in which the same vitamin D-deficient rats achieved a significant increase in serum calcium (915). In the first 3 days after weaning, serum calcium rebounded twofold higher to near-normal values in severely vitamin D-deficient rats before declining significantly to the prepregnancy value (915). This is also consistent with persistent upregulation of intestinal calcium absorption and bone resorption for some days after the outflow into milk has ceased.

In multiple studies from the author’s laboratory wherein WT Black Swiss or C57BL/6 mice consumed a standard 1% calcium diet, there was no change in the ionized calcium or the serum calcium during lactation regardless of whether the samples were drawn nonfasting or fasting (296, 330, 477, 478, 580, 992, 994). The ionized calcium was also measured every other day in the same mice from the time of mating, and no change occurred during pregnancy or lactation (827, 992). These findings suggest that a normal mouse may be more capable of maintaining her serum calcium in the normal range than a rat during reproductive cycles. However, CD1 mice maintained on a 1% calcium diet showed a modest but statistically significant decline in serum calcium during lactation (34). Consequently, there may be strain differences in the ability of mice to maintain normal serum calcium during lactation. More marked hypocalcemia was provoked in CD1 mice on a 0.01% calcium diet (37).

In lactating ewes serum calcium rose after delivery and declined as lactation progressed, with a low-calcium diet causing a steeper decline in serum calcium (69). Deer maintained an increased albumin-corrected serum calcium during lactation (170). Serum calcium remained normal in lactating goats (55).

Serum phosphorus has also shown widely variable responses in lactating rats, with increases above normal (320, 568, 718), normal values (76, 114, 337, 568), and significant decreases (86, 319). Phosphorus rose during the first several days after abrupt weaning, simultaneous with the spike in serum calcium (86, 718). It either remained normal or declined modestly in lactating mice (477, 478, 580), declined in lactating goats (55), and increased above normal in deer (170).

Serum magnesium is unaltered or modestly reduced in lactating rats (86, 132), and unchanged in mice (580) and goats (55). Magnesium also increases abruptly after forced weaning in rats (86).

### Table 2. Key differences in mineral physiology of human and rodent lactation

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<tr>
<th>Human</th>
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<td>Serum calcium</td>
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<td>Albumin-corrected calcium</td>
<td>Normal to slightly increased</td>
<td>Low</td>
</tr>
<tr>
<td>Ionized calcium</td>
<td>Normal to slightly increased</td>
<td>Low</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Normal to increased</td>
<td>Variable</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Normal to increased</td>
<td>Normal</td>
</tr>
<tr>
<td>PTH</td>
<td>Low to low-normal*</td>
<td>Increased</td>
</tr>
<tr>
<td>Calcitriol</td>
<td>Normal</td>
<td>Increased</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>PTHrP</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>FGF23</td>
<td>No data</td>
<td>No data</td>
</tr>
<tr>
<td>Intestinal calcium absorption</td>
<td>Normal</td>
<td>Increased</td>
</tr>
<tr>
<td>Urinary calcium excretion</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Trabecular bone losses</td>
<td>5–10%</td>
<td>25–35%</td>
</tr>
</tbody>
</table>

*Women with low calcium or high phytate intakes have normal or increased PTH.
Numerous studies have shown that serum phosphorus increases during lactation, with mean values exceeding the upper limit of normal in some studies (33, 156, 157, 164, 207, 221, 294, 348, 349, 385, 453, 454, 468, 470, 497, 558, 632, 721, 757, 805, 871). Phosphorus rose higher in women nursing twins than mothers nursing singletons in one study (347) but not in another report (646). In studies in which serum calcium and ionized calcium were modestly but significantly increased, mean serum calcium remained within the normal range. However, substantial hypercalcemia can occur during lactation and resolve with weaning (see sect. V, D and F) (549).

In contrast to the studies in rats, longitudinal studies of Black Swiss and C57BL/6 mice consuming a 1% calcium diet have found that PTH is suppressed during lactation from prepregnancy values, and similar to low values of pregnancy (330, 477, 478, 994). A 2% calcium diet suppresses PTH further (330, 478), whereas fasting overnight leads to nonsuppressed PTH (580). Conversely in lactating CD1 mice consuming a 1% calcium diet, the serum PTH was no different than in virgin mice, whereas a 0.01% calcium diet caused a marked rise in PTH (34).

These findings suggest that PTH is needed to support the delivery of calcium during lactation, such as by stimulating bone resorption, Cyp27b1 expression and activity, and renal calcium conservation. Low dietary calcium intake and larger litter sizes provoke higher PTH to stimulate these processes, whereas high calcium intake and smaller litter sizes prevent the need for PTH to increase. Rats may be more dependent on PTH than mice, given that lactating rats have been consistently found to have elevated PTH despite even high calcium intake, while lactating mice may have suppressed PTH unless fasted. The role that PTH plays in regulating mineral homeostasis during lactation, as revealed through studies of parathyroidectomized rats and Pth null mice, is discussed in more detail in sections IV, C–E, and VD.

In contrast to these studies, others have reported increased PTH during lactation in women from regions of Africa and Asia in which low calcium, high phytate, and low vitamin D intakes are more prevalent (168, 228, 439, 601, 805). Such a difference has also been seen when African American and Caucasian women, all from North America, have been compared (156).

The influence of PTH in regulating mineral metabolism during lactation is further discussed in subsequent sections that
address the synthesis of calcitriol (sect. IIA4) and the adaptations that occur in the intestines (sect. IVB), kidneys (sect. IVC) and bone (sect. IVD). PTH’s role is also illuminated by data on the effects of hyperparathyroidism (sect. VB) and hypoparathyroidism (sect. VD) in breastfeeding women. 

PTH rises to normal (206, 558) or above normal (207, 470, 530) in breastfeeding women. In contrast, hypoparathyroidism (sect. VD) and parathyroidectomy (sect. IVB) do not result in higher plasma PTH. 

C) SUMMARY. PTH is generally suppressed in lactating mice unless the samples are drawn fasting, whereas PTH is almost invariably elevated in lactating rats regardless of the calcium content of the diet. In largely Caucasian women from North America and Europe who are consuming adequate calcium, PTH is typically suppressed during lactation, so the original hypothesis of hyperparathyroidism in lactation has not been substantiated. However, PTH is elevated in women from certain regions of Asia and Africa, as well as in African American women from North America, so lactation does represent a state of physiological secondary hyperparathyroidism in some women. A rise in PTH during lactation is attributable to lower calcium and higher phytate intakes, as well as probable ethnic differences.

3. PTHrP 

A) ANIMAL DATA. Few measurements of plasma PTHrP have been made in mice due to the lack of rodent-specific assays and the large sample sizes required for the human assays. Nevertheless, PTHrP is increased in the circulation of lactating mice (37, 530, 953, 956, 989, 994). The source is largely mammary tissue, in which PTHrP mRNA and protein become substantially upregulated during lactation (738, 992, 994). The fall in progesterone and estradiol after delivery, and surge in prolactin, are thought to accelerate PTHrP production (737, 906, 916, 940, 956). Suckling induces expression of PTHrP mRNA and protein within rat mammary tissue (906, 1004; this effect is partly mediated by prolactin and blocked by bromocriptine (906), whereas oxytocin has no effect (906). In the goat, prolactin also stimulates PTHrP, while treatment with bromocriptine reduces the PTHrP content of milk (744). Studies of cultured rat mammary epithelial cells found that PTHrP production was increased by low progesterone but not responsive to prolactin or estradiol (940). The PTHrP content of milk is higher at the end of lactation in rats, by which time prolactin has been normal for days, which suggests that prolactin is not required for sustained PTHrP synthesis and secretion during lactation (129).

Loss of calcitonin in Ctcgrp null mice resulted in upregulation of PTHrP mRNA and protein expression within mammary tissue (992, 994), but whether this was a direct effect of loss of calcitonin signaling within mammary tissue, or secondary to other systemic changes in those mice (see sect. VH), is unknown. Serotonin increases during lactation in mice (101) and may stimulate mammary gland PTHrP, since increased dietary intake of a serotonin precursor raised PTHrP mRNA and protein expression in the mammary glands, and also increased plasma PTHrP significantly (530). Conversely, deletion of tryptophan hydroxylase-1 in mice results in global serotonin deficiency, and reduced PTHrP expression in mammary tissue during lactation (380). This was reversed in a dose-dependent fashion by a serotonin receptor agonist and worsened by a serotonin receptor antagonist (380).

Local factors are important for stimulating PTHrP, since milking one goat mammary gland increases the PTHrP concentration in milk from that gland but not the contralateral gland (916).

Higher PTHrP concentrations in the venous outflow of goat mammary glands was the first confirmation that PTHrP reaches the maternal circulation from this source (744). A suckling-induced phosphaturia and increase in nephrogenous cAMP, which persists in parathyroidectomized, lactating rats, appears to be a physiological response to systemic release of PTHrP (1004). Similarly in cows, a milking-induced phosphaturia blocked by a PTH/PTHrP receptor antagonist implicated PTHrP as the culprit (56). Definitive confirmation that PTHrP reaches the maternal circulation from mammary tissue came from a mouse model in which the Pthrp gene was deleted from mammary tissue at the onset of lactation, and this reduced the plasma PTHrP concentration (953). Moreover, in these mice lacking mammary-derived PTHrP, bone resorption was decreased and less bone was lost by the end of lactation (953). Conversely, Ctcgrp null mice had increased mammary expression of PTHrP and lost twice as much bone mineral content during lactation as their WT sisters (994).

Collectively, these findings confirm that through its expression of PTHrP, mammary tissue plays a central role in regulating bone resorption during lactation (see sects. IV, E and F, and V, D and F). In addition to affecting skeletal metabolism, mammary-derived PTHrP also appears to locally regulate the fluid and mineral content of milk, as discussed in section IVB.

B) HUMAN DATA. PTHrP was originally cloned from small amounts expressed by tumors causing humoral hypercalcemia of malignancy, and shown to circulate in picomolar amounts in the blood of affected patients. But it later became apparent that 1,000–10,000 times the concentration of the elusive protein was readily available in milk, whether produced by breastfeeding women or obtained from the corner store (134, 208, 473, 681, 742, 812). Milk was tested in part because it had been known for some time that hypoparathyroid and parathyroid women normalize mineral homeostasis or even become hypercalcemic while...
breastfeeding, which suggested the presence of a PTH-like factor during lactation (see sect. VD).

In section IIA3b, the inherent problems with measuring PTHrP in serum as opposed to plasma, and limitations of the available assays, apply to published studies of lactating women. Despite these problems, and with few exceptions (473), most studies have found the circulating PTHrP1–86 or PTHrP1–34 level to be significantly increased in lactating women vs. nonpregnant women or bottle-feeding controls (251, 349, 497, 558, 863). An assay directed against a mid-region sequence (PTHrP63–77) also found PTHrP to be elevated in the blood of lactating women (130).

Circulating PTHrP increases after suckling (251, 557, 558); together with its very high content in milk, this confirms that the breasts must be the source. PTHrP may not be elevated in the first 3 days postpartum before lactation is fully established (151). PTHrP immunoreactivity rises steadily in breast milk over the first few days postpartum, and subsequently declines as lactation wanes (129, 208, 251). PTHrP concentrations correlate positively with ionized calcium and negatively with PTH of lactating women (251, 497). Increased plasma PTHrP correlates with a greater loss of bone mineral density during lactation (863). Furthermore, there have been several cases of excess production of PTHrP by the breasts during lactation that have caused hypercalcemia (pseudohyperparathyroidism) (see sect. VF).

PTHrP declines significantly during the post-weaning interval (558). Exactly when it disappears from the maternal circulation have not been established, but the variable response of hypoparathyroid women to weaning indicates that it may be gone by weaning or persist for up to many months (see sect. VD).

c) SUMMARY. Lactating mammary tissue upregulates expression of PTHrP, some of which is secreted into the maternal circulation to alter mineral and skeletal homeostasis. In a real sense the breasts become accessory parathyroids by producing substantial amounts of PTHrP. As discussed in sections IV, B–F, release of PTHrP into the maternal circulation has systemic effects to alter milk composition, intestinal mineral absorption, renal mineral handling, and skeletal resorption. The most convincing evidence comes from hypoparathyroid or hypoparathyroid women who release PTHrP from the breasts and normalize mineral homeostasis during lactation (see sect. VD) (148–150, 213, 785) and pseudohyperparathyroid women who become hypercalcemic while breastfeeding (see sect. VF).

4. Calcitriol

a) ANIMAL DATA. Serum calcitriol increases twofold or more in lactating rats (90, 92, 93, 114, 718, 1021), and higher levels are achieved with larger litters or more intense lactation (90, 567). The fall in serum and ionized calcium and rise in PTH, observed in most studies of lactating rats, likely contribute to the increase in calcitriol. This increase is also influenced by the diet, rising higher with a 0.1% calcium diet and being prevented by a 1.6% calcium diet (568). In that study PTH was no different between lactating and nonlactating rats despite the three different diets, indicating that the changes in calcitriol were independent of changes in PTH (568). Another set of analyses also indicated that PTH is not a significant predictor of the calcitriol level in lactating rats (90, 567). But PTH must account for some of the increase in calcitriol during lactation since two studies found that serum calcitriol in lactating parathyroidectomized rats was only half the concentration of lactating intact rats, although the achieved level was at least double that of nonlactating rats (569, 718). In normal rats calcitriol falls below normal by 2 days after weaning, simultaneous with a rise in calcium and phosphorus, before increasing to normal values by 7 days after weaning (718).

25OHD is likely not altered during lactation, as suggested by studies of rats on three different calcium diets (568). But in two other studies from one lab the 25OHD level in lactating rats was significantly lower than in control rats (114, 115).

b) HUMAN DATA. Free and total calcitriol falls to normal during postpartum (206, 439, 578, 746, 809, 987) and remains normal throughout lactation and postweaning (156, 157, 206, 207, 348, 385, 470, 505, 632, 764, 805, 871, 987). One study found that extended lactation (>6 mo) was associated with higher calcitriol (348), another report found that calcitriol increased during the postweaning interval of bone recovery (871), and a third report found that calcitriol was 10% higher during lactation and that this persisted during the post-weaning interval (454). Women nursing twins had higher calcitriol levels than women nursing singletons in one study (347) but not in another (646).

25OHD levels do not appear to be altered by up to 12 mo of breastfeeding in most studies (33, 156, 157, 165–167, 470, 505, 558, 764, 780, 805, 862, 871). This is consistent with milk normally containing very low amounts of vitamin D and 25OHD (see sects. IVB and VG).

The decline to normal concentrations of calcitriol may result from pregnancy-related factors being lost at parturition, such as high levels of estradiol and placental lactogen. PTHrP reaches peak levels during lactation and likely stimulates Cyp27b1, but there is some evidence that it may be less potent than PTH in stimulating Cyp27b1 (308, 413, 414, 494, 988).

c) SUMMARY. Calcitriol remains elevated in lactating rats and mice, whereas in breastfeeding women the level promptly declines to nonpregnant values. These differences between
rodents and women are consistent with indications that rodents require increases in both intestinal calcium absorption and skeletal resorption to meet the mineral requirements of lactation, whereas women require only increases in skeletal resorption (see sect. IV, C and E).

5. Calcitonin

A) ANIMAL DATA. Similar to serum calcium and PTH, serum calcitonin levels in lactating rats have been quite variable, including low values on a 1.4 and 1.5% calcium (86, 627); high levels on 1.2% and 0.9% calcium (76, 926) and an unspecified diet (201); and normal values on an unspecified diet (337). Calcitonin levels are higher in fed versus fasting lactating rats (201). In ewes, calcitonin drops to nonpregnant levels at delivery, rises early in lactation, and declines to normal as lactation progresses (312). Deer show increased calcitonin during lactation which rises further during post-weaning before it declines (170). No measurements have been published in lactating mice.

Calcitonin has been shown to inhibit lactation by acting on suckling-induced prolactin release by pituitary lactotrophs (177, 680, 756, 919). Calcitonin is also expressed by pituitary gonadotrophs (755), wherein its targeted overexpression leads to hypoprolactinemia (1014). This leads to consideration of a physiological negative-feedback loop during lactation in which calcitonin inhibits the release of prolactin by the pituitary, thereby reducing milk production, releasing the inhibition of ovarian function, and inhibiting PTHrP release by mammary tissue.

Calcitonin surges within 24 h after abrupt weaning in rats, at a time when osteoclasts are undergoing widespread apoptosis (627), so calcitonin may be one of the signals that contributes to the sudden downregulation of osteoclast-mediated bone resorption. Alternatively, it may simply be responding to the post-weaning spike in serum calcium.

B) HUMAN DATA. Few studies have measured calcitonin in lactating women. Available data show high levels throughout lactation (221, 878) but also normal values between 6 wk and 12 mo postpartum (347, 385, 505, 632, 764). There was no difference in serum calcium among these studies that could explain the variable calcitonin values. Women nursing twins had higher serum calcitonin than women nursing singletons, but mean values were normal (347).

The breast is an important extrathyroidal source of calcitonin. It is secreted into breast milk at 45 times its concentration in blood, such that during lactation, totally thyroidectomized women and women with intact thyroids have the same mean calcitonin concentration (131).

The effect of calcitonin to inhibit prolactin in rodents, and similar findings in human studies (155, 431), led to a common warning in prescribing information that calcitonin should not be used in breastfeeding women because of its potential to inhibit lactation.

C) SUMMARY. Calcitonin may be normal or increased in lactating rodents and breastfeeding women. Although largely considered a vestigial hormone, there is convincing evidence from animal models that calcitonin protects against excess skeletal resorption during lactation (sects. IV E and VH). It surges to high levels at weaning, which may point to a functional role in inducing osteoclast apoptosis, or it may simply be responding to the post-weaning spike in serum calcium. Calcitonin can also inhibit prolactin and lactation in women and animal models when administered at pharmacological doses.

6. FGF23

A) ANIMAL DATA. There are no published data of circulating FGF23 concentrations in lactating animals. The physiological importance of FGF23 is uncertain given the variable changes in serum phosphorus during lactation in rodents. The effect of excess FGF23 on serum minerals and milk composition is discussed in sections IV B and VJ.

B) HUMAN DATA. No measurements of FGF23 in breastfeeding women have been reported. The relative hyperphosphatemia of lactation could conceivably cause a compensatory increase in FGF23. The influence of excess FGF23 on milk composition is discussed in sections IV B and VJ.

C) SUMMARY. There are insufficient data to know whether serum FGF23 concentrations are altered in lactating rodents or humans.

7. Sex steroids and other hormones

A) ANIMAL DATA. Lactation induces a surge in prolactin and a fall in estradiol and progesterone in the rat, mouse, and ewe; the fall in progesterone and estradiol are triggers of lactogenesis (655).

In mice, serum estradiol is usually near the detection limit of available assays. Although it has been shown to be low in lactating mice in one study (34), in most studies serum estradiol is not significantly different from prepregnancy, pregnancy, or virgin measurements (627, 956, 992, 1026). It is unclear whether the lack of suppressed levels during lactation is because estradiol is not low or because the assays are not sensitive enough to detect differences. Moreover, lactating mice go through estrus cycles and can become pregnant while lactating, so estradiol levels may not be sufficiently low in lactating mice to cause bone resorption.

Prolactin levels surge during early lactation as it exerts its role in initiating milk production; dopaminergic agents in-
hibit prolactin and stop milk production. Prolactin and prolactin receptor null mice cannot be studied because they are infertile, but loss of one allele of the prolactin receptor causes impaired lactation (465). Prolactin has been proposed to directly stimulate osteoblasts and osteoclasts during lactation, but inhibiting prolactin with bromocriptine achieved inconsistent results (888). Moreover, inhibiting prolactin does not necessarily test a direct role of prolactin on bone, since loss of prolactin will inhibit milk production, reduce ovarian suppression, and reduce PTHrP production by mammary tissue.

Oxytocin is required to cause contraction of myoepithelial cells within mammary tissue, thereby causing milk ejection. Oxytocin has also been proposed to regulate osteoblasts and osteoclasts during lactation (565, 895), but this has not been studied. Oxytocin null mice are unable to lactate because lack of milk ejection causes apoptosis of mammary cells (663).

IGF-I did not change in lactating mice compared with age-matched virgins, but approximately doubled by the third week of post-lactation recovery (101). As noted above, serotonin increased during lactation and may stimulate PTHrP production; there was a further increase during post-weaning recovery (101).

**B) HUMAN DATA.** Human lactation is also characterized by increased prolactin, and reduced estradiol and progesterone. Delivery of the placenta is responsible for the fall in progesterone and estradiol, which in turn trigger lactogenesis (617, 655). Failure to drain the breasts of milk (via suckling or pumping) leads to milk stasis, apoptosis of mammary cells, and cessation of lactation.

Estradiol is suppressed to menopausal levels during early lactation (157, 409, 505, 695, 764), especially when basal levels of prolactin are elevated (409, 695). Low estradiol may persist throughout lactation or recover to normal values after several months. It likely contributes to bone resorption during lactation, as discussed in section IV.E. Prolactin declines as the postpartum days pass but continues to spike upwards with each suckling episode (409, 505, 655, 695). Any effect of low estradiol may be lessened during prolonged lactation in women whose menses resume.

Oxytocin spikes in the maternal circulation within a few minutes of the baby being put to the breast (230, 655).

**C) SUMMARY.** Continuously low estradiol, and intermittently high prolactin and oxytocin levels, characterize the hormonal milieu of lactation. Low estradiol contributes to skeletal resorption, but the extent to which prolactin and oxytocin have direct or indirect effects to regulate skeletal metabolism during lactation is uncertain. Lactation also causes changes in luteinizing and follicle stimulating hormone, progesterone, testosterone, inhibins, and activins. Whether these play direct or indirect roles in regulating maternal skeletal metabolism during lactation is uncertain.

**B. Calcium Pumping and Secretion in Mammary Tissue**

**1. Animal data**

The process by which calcium enters mammary epithelial cells and is eventually secreted into milk is incompletely understood (Figure 3). Calcium appears to enter the basolateral membranes of mammary epithelial cells via stretch-activated and other calcium channels. A low concentration of calcium is maintained within the cytoplasm by PMCA1 actively pumping calcium into the Golgi apparatus, wherein calcium becomes bound to proteins (casein and α-lactalbumin) or complexed to phosphate and citrate. Calcium is also bound to carrier proteins such as calbindin-D9k within the cytoplasm (442) and transported to the apical membrane. Calcium is extruded from the Golgi apparatus into milk through transepithelial secretion (617, 652), a process that accounts for ~30% of calcium transport into milk. About 70% of calcium entry into milk results from plasma membrane calcium ATPase isoform 2 (PMCA2) pumping calcium across the apical membranes directly into milk (753, 954).

The calcium and fluid content of milk are tightly regulated. Identified regulatory factors include suckling, the calcium receptor, PTHrP, prolactin, calcitriol, and others (442, 655, 952, 953). Lactating mammary epithelial cells also express numerous genes and proteins that are involved in calcium homeostasis elsewhere in the organism, including the calcium receptor, PTHrP, calcitonin, the calcitonin receptor, Cyp27b1, VDR, and calbindin-D9k; however, TRPV5 and TRPV6 are not expressed (938, 952, 1026).

PTHrP plays a key role in the embryonic and adolescent development of the mammary glands (99, 258, 389, 999), so its later expression by lactating mammary tissue and breast cancers is understandable. The intense expression of PTHrP in lactating mammary tissue, and its secretion into milk at high concentrations, have prompted investigations to determine whether PTHrP regulates the calcium content of milk. Early studies showed that the PTHrP concentration in milk was positively correlated with calcium content of milk from cows (539, 681) and rats (989), but not in all studies from rats (738, 1004). One study found that with time since suckling, PTHrP content of milk decreased while the calcium content increased (1004). Treatment of lactating goats with bromocriptine reduced the milk content of both PTHrP and calcium (744). PTHrP also has vasodilatory effects on mammary vessels, through which it may regulate mammary blood flow (907, 908). PTHrP’s expression in mammary tissue is increased by low estradiol, low
progesterone, low dietary calcium, systemic hypocalcemia, high prolactin, and suckling (737, 906, 910, 916, 940, 952, 956, 1003, 1004). Each of these stimulatory factors supports a role for PTHrP in regulating mammary gland and mineral physiology during lactation. On the other hand, an early study failed to detect a change in milk calcium content when lactating mice were passively immunized with anti-PTHrP antibody (620).

The role of PTHrP has been further explored through the use of gene deletion models in mice. Selective deletion of Pthrp from mammary epithelial cells at the onset of lactation resulted in reduced milk calcium content (953). However, this may have been an indirect effect because the supply of calcium to mammary tissue was also reduced, as indicated by reduced bone turnover markers and less bone resorbed by the end of lactation (953). Conversely, significant upregulation of PTHrP mRNA and protein occurred within mammary epithelial cells of Ctcgrp null mice (which lack calcitonin), and the milk calcium content was significantly doubled at day 14 of lactation (994), and nonsignificantly increased at day 7 of lactation (992). Again, this may have been an indirect effect because in these mice the supply of calcium to mammary tissue was significantly increased, as shown by increased bone turnover markers and a doubling of net bone lost by the end of lactation (992, 994).

Further studies that examined the effect of calcimimetic agents, and selective deletion of the calcium receptor, have clarified the role of PTHrP within mammary tissue. It is the calcium receptor, expressed by mammary epithelial cells, which appears to directly regulate production of PTHrP, and the fluid and calcium content of milk (36, 591, 952). While a low calcium diet increases mammary production of PTHrP, this effect is blocked by a calcimimetic drug, which activates the calcium receptor to downregulate PTHrP (952). Ablation of the calcium receptor globally, or selectively within mammary epithelial cells, upregulates mammary gland PTHrP expression and reduces calcium transport into milk (36, 591). Moreover, selective ablation of the calcium receptor from mammary epithelial cells raises the plasma PTHrP level, induces hypercalcemia, and causes increased bone resorption and net loss of bone by the end of lactation (591). But despite systemic hypercalcemia and increased bone resorption, the milk calcium content remains low because of loss of the calcium receptor’s actions to promote calcium entry into milk (591).

The calcium receptor also upregulates expression of PMCA2, expressed on the apical plasma membranes of mammary epithelial cells, to stimulate calcium secretion into milk (952, 954). The importance of PMCA2 has been revealed by a spontaneous loss of function mutation of
*Pmca2* in deafwaddler mice, which produce milk of low calcium content (753, 954). Deletion of the calcium receptor from mammary epithelial cells did not affect expression of PMCA2, thereby confirming that it is loss of the calcium receptor function that specifically leads to the low calcium content of milk (591), perhaps by reduced activity but not expression of PMCA2.

Studies in goats have revealed that the calcium receptor and PTHrP may play opposing roles with respect to mammary epithelial cell survival. Activation of the calcium receptor by calcium or a calcimimetic drug inhibited proliferation and induced apoptosis of mammary epithelial cells, whereas overexpression of PTHrP had the opposite effect to inhibit apoptosis and promote proliferation (552).

Putting these observations together, it appears that mammary epithelial cells utilize the calcium receptor to sense the availability of calcium for milk production. The calcium receptor will promote calcium secretion into milk when the supply is adequate, and stimulate production of PTHrP when the calcium supply is insufficient (Figure 4). In this model, PTHrP’s role is to maintain systemic delivery of calcium to the mammary epithelial cells by resorbing calcium from the skeleton and reabsorbing calcium from the kidney tubules. Thereby, PTHrP plays an indirect but key role in determining the calcium content of milk.

This model implies a negative feedback loop that will enable milk production to decrease or cease if the serum calcium becomes dangerously low for the mother (955). However, it is not a fail-safe loop because hypocalcemia leading to tetany and death can occur during lactation in rats and mice that nurse large litters, and in dairy cows (milk fever). Blocking the effect of PTHrP to resorb bone through use of a bisphosphonate or osteoprotegerin (OPG) treatment in lactating mice did not cause a compensatory increase in PTHrP (37, 956), but this might be explained in part because the serum calcium did not decrease. In a separate study, a fall in serum calcium and an increase in calcitriol were observed in bisphosphonate-treated rats which may imply increased PTHrP activity (115), but PTHrP was not

![Figure 4](http://physrev.physiology.org/)

**FIGURE 4.** The role of PTHrP and calcium receptor (CaSR) within the lactating breast. The calcium receptor (represented schematically) is expressed by lactating mammary epithelial cells. It monitors the systemic concentration of calcium to control PTHrP synthesis and, thereby, the supply of calcium to the breast. An increase in serum calcium or administration of a calcimimetic inhibits PTHrP expression (A), whereas a decrease in serum calcium or ablation of the calcium receptor from mammary epithelial cells stimulates PTHrP expression (B). The calcium receptor also directly regulates the calcium and fluid composition of milk independent of PTHrP. Administration of a calcimimetic stimulates calcium and water transport into the breast, while ablation of the calcium receptor results in low milk calcium despite increased PTHrP and systemic hypercalcemia. PTHrP produced by mammary epithelial cells enters the maternal circulation to stimulate maternal bone resorption and renal calcium conservation. It also enters milk at 1,000- to 10,000-fold higher concentrations, from where it may influence neonatal accrual of calcium.
measured. Moreover, several additional physiological observations suggest that the mammary gland calcium receptor has quite limited ability to prevent systemic hypocalcemia. In severely vitamin D-deficient rats, which have a serum calcium 50% of normal, the calcium content of milk was unchanged compared with normocalcemic, vitamin D-sufficient mothers (91). In cows, the calcium content of milk is unaffected by low, normal, or high dietary calcium content, or low to high milk output (204).

Notably, the effect of the calcium receptor to inhibit mammary gland production of PTHrP is reversed in certain malignant breast cancers, such that altered G protein coupling causes the calcium receptor to stimulate rather than inhibit the production of PTHrP (592). This may explain why a positive feedback loop results when certain breast cancers produce PTHrP to resorb bone, and the released calcium acts through the calcium receptor to stimulate more PTHrP rather than inhibiting it. Altered G protein coupling is also a potential explanation for why lactation may lead to PTHrP-mediated hypercalcemia in some women (see sect. VF).

Other work has established that serotonin stimulates not only rat mammary gland expression of PTHrP, but milk PTHrP content, plasma PTHrP, systemic bone resorption, and milk calcium content (530). These findings confirm an important physiological role for serotonin in regulating mammary gland physiology during lactation, but also imply that increased expression of PTHrP causes increased milk calcium content. However, expression of PMCA2 more than doubled in mammary tissue in these studies (530), and that can be expected to increase milk calcium independent of PTHrP. It is unclear whether the increase in PMCA2 resulted from increased serotonin signaling or the increase in PTHrP expression.

Although Cyp27b1 and VDR are expressed in mammary epithelial cells and increase their expression 20- and 50-fold, respectively, during late pregnancy and lactation (1026), their relative roles are uncertain and the data are conflicting. In Vdr null mice, the milk calcium content was no different than in WT, although the mice were studied on normal range, and this may explain why PTH is often elevated in normal lactating rats. In Ctcgrp null mice that had elevated mammary expression of PTHrP and increased milk calcium content, serum PTH was also increased above normal and may have contributed to the milk calcium level (992, 994). Milk calcium content was normal in parathyroidectomized rats, but the study was confounded by the serum calcium remaining in the normal range on a 1.2% calcium diet (319).

2. Human data

Fewer studies of calcitropic factors affecting milk content have been done in women. The PTHrP concentration of milk (245, 812, 941) and in the maternal circulation (245) correlates positively with total milk calcium content. A randomized trial found no effect of calcium supplementation on milk PTHrP content, but there may be a diurnal variation with more PTHrP in milk obtained in the morning compared with the afternoon (208). Diets that are high (206, 452, 484, 723) or low (728–731) in calcium, or high (60) or low (731) in vitamin D, do not affect milk calcium content.

The vitamin D content of milk is normally quite low, and this is why breastfeeding is a risk factor for vitamin D deficiency and rickets in neonates and infants. It is a curiosity as to why the vitamin D content is so low. It is not possible for humans to develop vitamin D toxicity from sunlight exposure due to limited substrate produced in skin each day, but it is possible to become vitamin D toxic from overingestion of vitamin D from supplements and vitamin D-fortified foods. Therefore, a teleological argument is that neonatal physiology was designed to obtain sufficient vitamin D from sunlight exposure, with milk content of vitamin D kept low to prevent excess intake by the baby, and also to prevent excess loss of 25OHD from the mother.
Two cases of women with hypophosphatemia due to XLH found that the phosphorus content of milk was reduced to about half the normal value (447, 745). The reduced serum phosphorus in these women may explain the low phosphorus content of milk, without requiring direct role of FGF23 to regulate the phosphorus content of milk.

3. Summary

The calcium receptor appears to directly control the calcium content of milk by inhibiting PTHrP and stimulating PMCA2. There is also evidence that PMCA2 may be locally regulated by PTHrP, serotonin, and calcitriol. PTHrP is expressed by mammary epithelial cells wherein it plays a supportive role in regulating the calcium content of milk, largely by stimulating systemic bone resorption and renal tubular conservation of calcium. The calcium receptor may inhibit PTHrP release to prevent maternal hypocalcemia, but this effect must be modest since lactation-induced hypocalcemia occurs in rodents and cows, and extremes of low and high dietary calcium intake do not alter milk calcium content in women. XLH in women results in milk with low phosphorus content.

C. Intestinal Mineral Absorption

1. Animal data

Lactating rodents maintain an increased rate of intestinal calcium absorption accompanied by high serum calcitriol, compared with nonpregnant values (93, 114, 360, 718). Duodenal expression of calbindin-D9k, PMCA1, and VDR are higher in lactating compared with virgin rats (949, 1021). Lactating mice similarly maintain increased circulating levels of calcitriol and presumably have increased intestinal calcium absorption as well. These findings indicate that increased intestinal calcium absorption and increased skeletal resorption are both invoked to meet the calcium demands imposed by a rodent’s relatively large litters, and a short (3 wk) duration of lactation.

On the other hand, although reducing either the dietary intake of calcium or skeletal resorption during lactation will cause maternal hypocalcemia, neither manipulation reduces the calcium content of milk. It is only when both are reduced, such as a calcium-restricted diet in mice also treated with OPG to block bone resorption, that more profound maternal hypocalcemia and reduced milk calcium content result (see also sect. IVE). These findings indicate that the rodent balances input from both skeletal resorption and increased intestinal absorption of calcium, and that either route of delivery can make up for the other during lactation. It is only when both are impaired that systemic hypocalcemia and reduced milk calcium content will result.

As with studies in pregnancy, factors other than calcitriol may stimulate intestinal calcium absorption during lactation, since severely vitamin D-deficient rats increase duodenal calcium absorption to a value equal to that of vitamin D-sufficient rats; they also raise their serum calcium significantly but not to normal (93, 360). When these severely vitamin D-deficient rats were also parathyroidectomized and transferred to a high-calcium diet, an increased rate of intestinal calcium absorption was maintained despite absence of both calcitriol and PTH, and it was no different from the rate achieved in intact, vitamin D-sufficient rats (93). Moreover, ketoconazole treatment reduced serum calcitriol by half in lactating rats, but this had no effect on the efficiency of intestinal calcium absorption (92), confirming that calcitriol is not necessary for an increased rate of absorption to be achieved during lactation. Intestinal calcium absorption has not been measured in lactating Vdr null and Cyp27b1 null mice.

The increased intestinal calcium absorption contributes to the rebound hypercalcemia after abrupt weaning (396, 718), which also persists in severely vitamin D-deficient rats (915). However, rebound hypercalcemia may have a greater contribution from increased skeletal resorption, which also does not require calcitriol or VDR (see sects. IV and VG).

Estradiol has been proposed to contribute to the stimulation of intestinal calcium absorption during pregnancy because its levels are high, but estradiol is unlikely to be a factor during lactation because its levels are reduced. Moreover, intestinal calcium absorption was unaffected by ovariectomy on the second day after delivery (32).

Intestinal phosphorus and magnesium absorption are both significantly increased during lactation in rats (113, 114). Intestinal phosphorus absorption remains high despite severe vitamin D deficiency (93), confirming that it is independent of calcitriol. During post-weaning recovery, intestinal calcium and phosphorus absorption fall to virgin values in rats (93, 360).

2. Human data

Multiple studies have demonstrated that intestinal calcium absorption is normal (equivalent to nonpregnant values) during lactation, reduced from the doubled rate that occurs during pregnancy (324, 372, 453, 467–469, 764, 873). This fall in the rate coincides with calcitriol also declining to about half the normal value (447, 745). The reduced serum phosphorus in these women may explain the low phosphorus content of milk, without requiring direct role of FGF23 to regulate the phosphorus content of milk.
they are still lactating (453), which supports a role for higher estradiol levels boosting calcium absorption.

Women often increase dietary or supplemental intake of calcium while breastfeeding, and this will increase the total amount of calcium absorbed, even though the efficiency of absorption is not altered (453). However, randomized trials and cohort studies have found that this increases urine calcium excretion without affecting milk calcium or the amount of bone resorbed during lactation (206, 452, 484, 723, 729, 730) (see sects. IV and VI).

The failure of intestinal calcium absorption to increase during lactation does not imply inadequacy of vitamin D intake. Clinical trials of high-dose vitamin D (4,000–6,400 IU daily) resulted in a very high mean 25OHD level of 160 nM (64 ng/ml), but there was no effect on breast milk calcium content (60, 402, 965).

A post-weaning 20% increase in intestinal absorption of calcium has been observed in one study (453), whereas another found a nonsignificant 9% increase (764). These values are well below the doubling that is achieved during pregnancy but may still facilitate restoration of the skeleton after lactation.

3. Summary

Intestinal absorption of calcium and phosphorus was increased during pregnancy, and these high rates continue in rodents throughout lactation. This is likely mediated in part by high levels of calcitriol, but its persistence despite severe vitamin D deficiency indicates that calcitriol-independent mechanisms also stimulate intestinal calcium absorption during lactation.

In contrast, intestinal calcium absorption falls to nonpregnant values during lactation in women, accompanied by a fall in calcitriol. High or low calcium intakes, and high vitamin D intakes, do not seem to alter this rate in lactating women. A modest increase in intestinal calcium absorption may occur during post-weaning recovery.

D. Reduced Renal Excretion of Calcium

1. Animal data

Renal conservation of calcium occurs during lactation in the lactating rat and mouse, with urine calcium concentrations falling to low levels even when a high-calcium diet is consumed (34, 291, 330, 477, 580, 994). Rendering rats severely vitamin D-deficient did not impair the ability of the kidneys to conserve calcium compared with vitamin D-replete rats (93). Similarly, absence of calcitriol, PTH, or calcitonin did not alter urine calcium excretion in the respective mouse models (330, 478, 994).

This relative hypocalciuria is consistently accompanied by phosphaturia in mice (330, 477, 478, 580, 994), which is likely a demonstration of the physiological actions of PTHrP to conserve calcium and excrete phosphorus. A suckling-induced phosphaturia attributable to PTHrP has also been demonstrated in rats, goats, cows, and sheep (56, 226, 907, 1003).

In rats, the persistence of elevated PTH during lactation (see sect. IVA2) likely complements the actions of PTHrP to conserve calcium and excrete phosphorus.

2. Human data

The glomerular filtration rate declines during lactation from the increased rate of pregnancy, the tubular maximum for calcium increases, while fractional excretion of calcium (especially on 24 h collections) falls and may reach hypocalciuric values (18, 168, 207, 221, 304, 439, 454, 467, 468, 470, 632, 757, 764). This is more readily seen in longitudinal studies in which lactating women were compared with themselves at earlier time points, and has also been demonstrated by the urinary response to an oral calcium load in pregnant, lactating, and control women (805). The increase in tubular reabsorption of calcium is attributable to PTHrP. Fractional excretion of calcium did not appear to be reduced in two recent cross-sectional studies from the same authors; however, the results were confounded by the lactating women consuming 60 and 80% more calcium than the unrelated controls (156, 157).

Urine phosphorus excretion may be normal or increased in 24-h urine collections due to the increased filtered load of phosphorus and the phosphaturic actions of PTHrP (206, 207, 439, 454, 468, 470, 757), whereas morning spot urines corrected for creatinine may show decreased values (168). Multiple studies have calculated a significant increase in tubular maximum for phosphate (18, 156, 157, 206, 324, 468, 470, 558), which suggests that the kidneys are actively conserving phosphorus despite the phosphaturic actions of PTHrP and overt phosphaturia. This may imply the presence of an anti-phosphaturic factor during lactation or low levels of FGF23.

The relative hypocalciuria and renal calcium conservation persists during the postweaning interval in some studies (207, 470, 558), whereas the tubular maximum for phosphorus declines to normal (206, 207, 470, 558).

3. Summary

Urine calcium excretion decreases and urine phosphorus excretion may increase during lactation. These actions conserve calcium to provide it to milk while excreting unneeded phosphorus generated from skeletal resorption and intestinal phosphorus absorption. An increase in the renal tubular
maximum for phosphorus suggests the presence of an antiphosphaturic factor (or low FGF23) despite the increased urine phosphorus excretion.

E. Increased Skeletal Resorption and Osteocytic Osteolysis During Lactation

1. Animal data

Multiple lines of evidence have demonstrated that the maternal skeleton is significantly resorbed during lactation to provide calcium and other minerals to milk. Osteoclast-mediated bone resorption is upregulated to resorb mainly trabecular bone and endocortical surfaces, while osteocytic osteolysis is upregulated to resorb mineral from cortical and trabecular bone. Since lactation is a state of rapid bone loss, bone turnover is uncoupled in favor of resorption.

One of the earliest demonstrations that the maternal skeleton provides mineral during lactation involved fixing radioisotopes of calcium and strontium into maternal bone during pregnancy. When the mice later lactated, isotopes appeared in milk and the bodies of cross-fostered pups, with the amount and timing of release of radioactivity suggesting that the last calcium fixed into bone during pregnancy is the first to be removed during lactation (706).

Objective evidence of bone resorption during lactation, and variations in the extent of it among skeletal sites, has come from measurements of bone turnover markers, histomorphometric and electron microscopy studies, ash weight, bone mineral content by DXA, material properties of bone, and bone structure by microCT.

Bone turnover markers are increased during lactation, with bone resorption markers generally showing proportionately greater increases than in bone formation markers (35, 101, 330, 477, 478, 580, 956, 994). These alterations in bone turnover markers can be prevented by treatment with a bisphosphonate, OPG, and high-dose estradiol treatment (37, 956).

Histomorphometric studies have shown that osteoclast-mediated bone resorption is increased primarily in trabecular compartments during lactation in rats (271, 408, 629, 920, 972), beagles (300, 626), and mice (34, 194, 956). Increases in osteoblast number, osteoblast surface, osteoid thickness, osteoclast number, and resorptive surfaces are noted, with the increases being more marked in the resorptive parameters (34, 194, 956). The resorption is particularly marked within the trabecular compartments of the vertebrae and the ends of the long bones, resulting in reduced mineralized tissue volumes, thinning of trabeculae, and decreased trabecular number (34, 194, 626, 956). These histomorphometric changes could be completely prevented by treatment with a bisphosphonate or OPG, and blunted by fivefold physiological levels of estradiol (37, 115, 956). But osteoclast-mediated bone resorption is not the only mechanism by which bone is resorbed to release calcium during lactation. More recent histomorphometric and electron microscopy assessments have shown that osteocytic osteolysis also occurs during lactation, in both trabecular and cortical bone (904, 998). Because cortical bone makes up more than 90% of the skeleton, there is the potential for substantial mineral to be mobilized through osteocytic osteolysis.

Visible enlargement of osteocyte lacunae in certain human pathological states was first described by Rigal and Vignal in 1881 (763). This was reaffirmed by von Recklinghausen’s observations in 1910 (962), who proposed that osteocytes digest their surrounding bone matrix when stressed by such conditions as severe osteomalacia and rickets. Belanger and co-workers (67, 68) rediscovered this concept in the 1960s, named it osteocytic osteolysis, and carried out a detailed series of experiments that confirmed objective loss of mineral and protein content from the enlarged osteocytic lacunae. Furthermore, osteocytic osteolysis was shown to be inhibited by interventions known to suppress bone resorption (calciitonin or a high-calcium diet), and exacerbated by factors known to stimulate bone resorption in rodents (low-calcium diet, parathyroid extract, secondary hyperparathyroidism, pregnancy) and humans (hyperparathyroidism and Paget’s disease) (67, 68). Belanger (67) also recognized that the combined surface area of osteocyte lacunae is ~10 times the trabecular bone surface in humans, which thereby represents a significant storehouse of mineral to be resorbed when needed.

Unfortunately, the concept that osteocytes can function like osteoclasts was disbelieved by many investigators and largely overlooked for decades. More recently, the Bonewald lab has revisited this concept and confirmed with modern techniques that osteocytes express osteoclast-related genes and enzymes, and resorb the mineral and proteinaceous matrix that fills their lacunae (733, 734). Moreover, they showed that this process occurs during lactation in mice (733, 734, 998), and when it was blocked by conditional ablation of the PTH/PTHrP receptor from osteocytes, the amount of mineral lost during lactation was 50% of normal (733). This finding may at first glance be an indication that osteocytic osteolysis contributes ~50% of the mineral that is resorbed from the skeleton during lactation, with the balance achieved by osteoclast-mediated resorption of trabeculae (FIGURE 5). However, loss of PTH signaling in osteocytes also affects nuclear factor kappa-B ligand (RANKL) production and the number and activity of osteoclasts, so osteoclast-mediated bone resorption may have been reduced as well.

The objective loss of bone mass and mineral content from the maternal skeleton has also been demonstrated by bone ash weight measurements, analysis of the calcium content...
of the ash, and radiological assessment of mineral content by DXA. Lactating rats and mice normally resorb 25–35% of bone mineral, primarily from trabecular-rich vertebrae, to a lesser extent from trabecular bone of the femora and tibiae, and much less from purely cortical bone (34, 35, 101, 176, 271, 296, 320, 330, 337, 392, 408, 477, 478, 486, 518, 580, 628, 629, 708, 740, 772, 920, 972, 991, 994). Ewes resorb bone preferentially from the vertebrae and pelvis, resulting in reduced ash weight, while the limbs and digits are relatively spared (69). The African green monkey, which may resemble the human condition more closely, loses 20% of bone mineral density of the lumbar spine during 20 wk of lactation (393). The greater losses of mineral content from trabecular bone demonstrate the ability of osteoclasts to resorb mineral from bone that has the greatest surface area. The proportionately smaller losses of mineral from cortical bone may be due to osteocytic osteolysis.

In rats and mice, the material properties of bone are adversely impacted by the resorption of bone during lactation. The strength, stiffness, and toughness of vertebrae, tibiae, and femora are reduced while ductility is increased (489, 518, 580, 708, 709, 947).

MicroCT measurements of the lumbar vertebrae of lactating mice have revealed 30–50% reductions in trabecular bone volumes, 20% lower trabecular thickness, trabecular perforations, reductions in tissue density, and progression to more rodlike than platelike structures (34, 35, 566). The tibiae and femora show similar changes in trabecular bone, while cortical bone displays reductions in cortical thickness, cross-sectional area, and tissue mineral density, and an increase in cortical porosity and endosteal perimeter (35, 101, 566, 580). However, another microCT study from the same investigators found no significant change in cortical parameters in the femora except for an increase in tissue density (34), while a peripheral QCT (pQCT) study found that lactation increased tibial mid-shaft cortical width, area, periosteal circumference, and cross-sectional moment of inertia in frontal, sagittal, and torsional planes (772). The latter two studies suggest that the weight-bearing limbs have a compensatory improvement in bone volumes and strength during lactation that may offset the potential loss of strength induced by resorption of trabecular microarchitecture.

That resorption of bone during lactation is needed to support milk production has been confirmed by finding that a low-calcium diet initiated at delivery causes greater skeletal losses that can exceed 43% of trabecular bone mineral content in rats and mice (37, 271, 320, 350, 355, 708, 991). Furthermore, nursing larger numbers of pups, which means increased milk production, causes greater resorption of bone in rats (320, 708). Bone strength assessments have demonstrated that a low-calcium diet or nursing more pups each leads to weaker bone at the end of lactation compared with a normal-calcium diet or fewer pups (708). MicroCT studies have confirmed that calcium restriction results in greater reductions in bone volumes, trabecular number and thickness, and tissue density (37). In ewes, a low-calcium diet exacerbates the decline in ash weight (69). Treating lactating rats with a bisphosphonate causes maternal hypocalcemia that is not seen in control rats (115), confirming that skeletal resorption of mineral is required to maintain maternal serum calcium in the setting of milk production. However, this effect was not seen in one study of lactating mice (956).

Conversely, a high-calcium diet may blunt but not prevent bone loss during lactation, confirming that it is hormonally programmed independent of maternal serum calcium or dietary calcium intake. When lactating rats were studied on diets ranging from 1.2 to 0.3% calcium, there was no change in resorption of skeletal calcium (271, 318),
whereas when lactating mice were studied on a 2 versus 1% calcium diet there was definite blunting of mineral content loss (478). In lactating ewes, a high-calcium diet also blunted the decline in ash weight (69). Bone loss was completely blocked with OPG treatment in mice, but even then milk production remained normal, likely from upregulated intestinal calcium absorption (37). It took the combined effects of OPG treatment and a 0.01% calcium diet to disrupt lactation, resulting in maternal hypocalcemia and deaths in 41% of the mothers by day 12 of lactation (37).

The classic calcitropic hormones do not appear to be required for increased osteoclast-mediated bone resorption and osteocytic osteolysis to occur during lactation. Parathyroidectomy in rats did not blunt the lactational fall in skeletal mineral content (319, 395), while lactating Pth null mice lost the same bone mineral content from whole body, spine, and hindlimb as their WT sisters (478). Vitamin D-deficient and vitamin D-replete rats each resorbed a similar amount of calcium from the femora in two separate studies (362, 598). In one of these cross-sectional studies, the authors concluded that vitamin D-deficient rats lost twice as much mineral compared with unmated rats, but compared with their respective peak values at the end of pregnancy, vitamin D-deficient and vitamin D-replete rats lost a similar amount (598). Similarly, Vdr null mice and their WT sisters lost the same amount of BMC from whole body, lumbar spine, and hindlimb (296, 489). In a preliminary report, the magnitude of bone loss during lactation in Cyp27b1 null females is equivalent to that of their WT sisters (330).

Lactational losses of bone mass were not affected by ovariectomy, adrenalectomy, or immediate pregnancy in rats (32, 118). When ovariectomized, sham-operated, and intact lactating rats were each stressed by a 0.1% calcium diet, there was no difference in the reduction of femoral ash weight among the groups (32). Ovariectomy to create estradiol deficiency leads to modest and slow bone loss in rodents, and not to the rapid loss that occurs during lactation (22, 34, 260). Conversely, treatment with estradiol to achieve fivefold higher levels than normal during lactation blunted bone loss and caused a small decline in milk calcium (956).

Several older studies tested whether calcitonin deficiency, created through thyroidecotomy followed by thyroid hormone replacement, causes greater loss of bone in lactating goats and rats. The results were contradictory, with evidence of increased loss versus no difference between thyroidecotomized animals and sham-operated controls (58, 392, 551, 900). These models were confounded by extrathyroidal production of calcitonin in the mammary glands (131, 938) and pituitary (755), which was not appreciated at the time. A more recent study examined global calcitonin and CGRP-α deficiency in Ctcgrp null mice and found that bone loss increased twofold, accompanied by histomorphometric evidence of increased osteoclast number and activity, and reduced osteoblast parameters (194, 992, 994) (see sect. VH).

Collectively these data show that PTH, calcitriol, VDR, low or high estradiol, and adrenal hormones are not needed for the normal mobilization of skeletal calcium during lactation, but loss of calcitonin can lead to more severe losses of bone mass during lactation. Since ovariectomy in lactating rodents does not increase the amount of bone lost, this implies that estradiol is already low in lactating rodents or that low estradiol is not a relevant stimulus for bone loss during lactation. The comparatively slow bone loss after ovariectomy alone indicates that low estradiol cannot account for the rapidity and extent of bone loss that occurs during lactation. From earlier observations in some of the surgical and vitamin D deficiency models, Brommage and DeLuca (118) correctly proposed in 1985 that the putative hypercalcaemia of malignancy factor (PTHrP) might regulate bone resorption during lactation.

PTHrP and low estradiol have subsequently been shown to play significant roles in increasing skeletal resorption during lactation. Conditional ablation of Pthrp from mammary tissue resulted in reduced bone turnover markers and less bone loss during lactation (953). Conversely, ablation of the calcium receptor from mammary epithelial cells resulted in reduced calcium content of milk, increased mammary gland and circulating PTHrP, markedly increased bone resorption, and greater bone loss compared with normal (591). As noted previously, pharmacological treatment with estradiol reduced bone loss during lactation but did not obliterate it (956). The combined effects of ovariectomy and continuous PTHrP infusions administered through a mini-pump led to more bone loss in the lumbar spine and femora than with either treatment alone in nonlactating mice (34). However, the magnitude of bone loss in this model (ovariectomy + continuous PTHrP infusion) was less than what occurs during lactation (34), which could be an indication that PTHrP and estradiol deficiency do not completely explain lactational bone resorption. However, the combination of ovariectomy with a continuous PTHrP infusion provided no “drain” for calcium to escape the mouse (there was no milk production), so there was no net loss of calcium. Instead, systemic hypercalcaemia resulted, which likely had a dampening effect on further bone loss (34).

Another recent study found that FGF21 circulates at high levels in lactating mice (101). This is a counterregulatory factor that stimulates gluconeogenesis and fatty acid oxidation during substrate deficiency, and a member of a subfamily that includes FGF23 (724). Intriguingly, Fg21 null mice do not resorb bone during lactation, as shown by microCT of the proximal tibiae, histomorphometric analysis of femora, and a lack of increase in bone resorptive markers (101).
The mechanism that leads to the failure to resorb bone is unknown. It may be due to altered energy metabolism or some interaction with PTHrP, which was not measured.

Finally, increased weight bearing has been theorized to reduce skeletal losses during lactation. This was assessed by DXA and pQCT at multiple skeletal sites in rats forced to stand erect to reach their food and water from time of mating through the end of pregnancy, and then switched to cages of normal height, compared with rats housed in cages of normal height (772). Skeletal losses in the lumbar spine, femora, tibiae, and whole body were unaffected by increased weight-bearing, except for a borderline statistically significant and very small relative improvement in mineralization and strength parameters of the tibial diaphysis (772).

2. Human data

The available data from breastfeeding women confirm that hormonally programmed resorption of the maternal skeleton occurs, similar to what has been demonstrated in rodents, sheep, goats, and primates. Careful metabolic studies have shown that women are in a markedly negative calcium balance while breastfeeding, especially during the interval of greatest milk production, and despite any supplemental calcium intake (253, 419).

That the maternal skeleton contributes mineral to breast milk has been inadvertently confirmed by Techa River residents in Russia, who released $^{90}\text{Sr}$ into breast milk years after their exposure from ingestion of contaminated river water (924). Observational data and mathematical models predict that the amounts of $^{90}\text{Sr}$, $^{45}\text{Ca}$, $^{131}\text{I}$, and other isotopes in breast milk will be highest when maternal exposure occurs in late pregnancy or while lactating, confirming that dietary intake remains a significant contributor to breast milk (1, 366, 851, 924).

There are no histomorphometric data from lactating women; instead, serial measurement of bone turnover markers (P1NP, bone-specific alkaline phosphatase, osteocalcin) have been normal in a few studies (207, 769), but generally are also high during lactation, and increased over third trimester values or controls (9, 123, 124, 156, 157, 206, 207, 251, 454, 468, 470, 572, 695, 764, 805, 859, 1002, 1009). Bone formation markers (P1NP, bone-specific alkaline phosphatase, osteocalcin) have been normal in a few studies (207, 769), but generally are also high during lactation, and increased over third trimester values or controls (9, 123, 124, 156, 157, 206, 251, 409, 454, 468, 470, 572, 695, 764, 805, 859, 1002, 1009). Total alkaline phosphatase loses the placental fraction at delivery and falls markedly, but may remain above normal due to the increased bone-specific fraction (769, 1002). These values confirm that bone turnover is significantly increased during lactation in women.

One of the earliest longitudinal studies to assess skeletal changes during lactation used a $^{241}\text{Am}$ source and found that the femoral shaft BMC declined 2.2% over the first 100 days of lactation (40). Since that time, multiple longitudinal studies (observational studies and clinical trials) have been carried using SPA, DPA, or DXA. A consistent decline in BMD or BMC has been reported, with mean values ranging from 3 to 10.0% after 3–6 mo of lactation. The greatest (5–10%) losses occur in the lumbar spine, with more modest (0–5%) losses at less-trabecular-rich sites (hip, femur, and distal radius), and the smallest (0–2%) changes at purely cortical sites such as the whole body (9, 18, 109, 123, 124, 140, 165–168, 180, 193, 206, 251, 256, 371, 403–405, 409, 412, 452, 457, 468, 470, 484, 505, 526, 533–535, 560, 572, 574, 609, 621, 633, 635, 705, 723, 729, 764, 855, 857, 859, 1009). Collectively, these studies suggest that 6 mo of exclusive lactation will result in a median loss of ~6–8% of BMD from the lumbar spine, with half or less those amounts lost from the appendicular skeleton.

It is important to emphasize that these are mean changes from the cohorts of each study; responses of individual women to lactation can vary from a small gain to a 20% decline in the lumbar spine BMC (405, 705). Some women start pregnancy with normal BMD but reach an osteoporotic level (~2.5 SD) at the spine or hip during lactation (705). Some observational studies have included postpartum controls who do not breastfeed; these women do not lose lumbar spine BMD but may show small (0–2%) but statistically significant gains in BMD over the postpartum interval, suggesting a recovery from small losses incurred during pregnancy (9, 140, 371, 412, 451, 505, 572, 633).

Women who breastfeed for more prolonged periods, such as a year or more, may experience even greater bone loss (379, 857, 860). However, this should be mitigated by the infant consuming solid foods by 6 mo of age and a reduction in the amount of milk produced. On the other hand, a professional “wet nurse” can have high volumes of milk output that are sustained for long intervals (419, 583, 838), so ongoing bone loss seems inevitable. However, no bone density data have been reported for such women.

HR-pQCT has been used in very few studies to date, and the only sites that can be assessed by this technology are peripheral appendicular sites such as the radius and distal femur, sites that lose much less mineral content than the lumbar spine. These studies have confirmed 0–2% reductions in trabecular thickness, and cortical thickness and volume, with the losses being greater in those who lactate longer (84, 109). Trabeicular number and volumetric BMD appeared to...
increase, which may be an artifact from trabecularization of cortical bone (84, 109).

Peripheral ultrasound is not a useful technique to assess bone loss during lactation. Although DXA and HR-pQCT have confirmed that bone mass declines significantly during lactation, ultrasound of the os calcis or midtibial shaft showed no changes (533, 917, 1015).

Few studies have looked at lactating adolescents. A 15% decrease in radial BMC over 16 wk of lactation was found in teenaged mothers whose calcium intake was below the recommended amount, whereas lactating adults showed no change in radial BMC; the lumbar spine was not assessed (166, 167). A follow-up study of lactating adolescents found that a calcium intake of 900 mg daily led to a 10% loss of radial BMC over 16 wk compared with no loss when 1,600 mg calcium was consumed; lactating adults who consumed 1,500 mg calcium daily also lost no radial BMD (164). A more recent study of lactating adolescents (mean age 16 yr) found no significant changes in BMD of the lumbar spine, femoral neck, and total hip by 6 mo of lactation with a calcium intake of 1,200 mg daily (590). Another study of lactating adolescents (mean age 17) found that randomization to a supplement containing 500 mg calcium and 200 IU vitamin D resulted in a higher lumbar spine BMD by 20 wk of lactation compared with placebo; however, women receiving the supplement had significantly reduced their breastfeeding frequency and intensity, and twice as many of them had resumed menses, compared with the placebo group (250). These differences mean that the women in the treatment arm would have lost less bone due to reduced breast milk output and recovery from low estradiol, and not necessarily as a direct result of the supplement.

Two small studies by the same investigators in Spain reported contradictory results when lactating adolescents and adults were compared. The first reported that adolescents and adults lost a similar amount of BMD during lactation but had recovered it fully by 9 mo postpartum (159). The second study reported that adolescent lumbar spine BMD increased by 9% at 3 mo and 15% by 12 mo postpartum, compared with adult women who lost only 2% of lumbar spine BMD at 3 mo and achieved a net increase of 4% by 12 mo postpartum (798). The second study implies that adolescent accrual of bone mass is not impaired by simultaneous lactation, but the marked differences between the two studies are unexplained. Lactation in adolescents has been an ongoing concern with regard to poor dietary intake of calcium, and potential interference in reaching peak bone mass (see sect. IVG).

The studies in adolescents indicate that a higher calcium intake prevents bone resorption in the radius during lactation, a site that normally shows proportionately little resorption during lactation. Several blinded, randomized interventional studies have shown that the lumbar spine and hip are preferentially resorbed during lactation such that taking a 1 g calcium supplement in addition to usual intake will not reduce the amount of bone lost. One study compared 1,800 to 800 mg calcium intake daily and the lumbar spine declined ~4% over 6 mo of lactation with no difference between the groups (452). A second compared with 2,400 to 1,200 mg calcium daily, and the lumbar spine, radius, and ulna declined ~6% in both groups (206). A third study compared 1,400 to <300 mg calcium, and the intervention caused an increase in urinary calcium excretion but neither group lost radial BMD or experienced a change in markers of bone resorption during up to 18 mo of lactation (the spine was not assessed) (729, 730). A fourth also used a 1 g supplement but did not measure dietary calcium intake; the women lost ~4% of lumbar spine BMD with no difference between the groups (723). In two observational cohort studies in which the lumbar spine declined 4% during 3 mo of lactation (533) and 6% during 6 mo of lactation (484), the relative losses were not affected by maternal calcium intake. One of these cohort studies found that the skeletal losses were inversely proportionate to breast milk output and, thereby, maternal calcium losses (533), whereas the other noted a significant correlation to duration of lactation and time to resumption of menses (484). A randomized study using ultrasound of the os calcis compared 2,400 with 1,200 mg calcium and noted a 15% reduction in urinary NTX, but no change in apparent bone loss after 1 mo of lactation (post hoc analyses suggested that higher compliers had a significant increase in SOS of the heel) (274). Another randomized study compared calcium intakes of 1,850 to 850 mg, and although PTH and calcitriol were lower in the calcium-supplemented women, there was no effect of calcium supplementation on bone turnover markers during 6 mo of lactation (454). Overall, the results of these randomized interventions and cohort studies indicate that increased skeletal resorption seems to be programmed during lactation and, unlike rodents, not suppressible by high calcium intakes.

Conversely, low calcium intakes (<300 mg/day) do not appear to increase maternal bone loss or markers of bone resorption during lactation, nor do they alter breast milk calcium content (728–731). These findings are consistent with most of the milk calcium content programmed to come from resorption of the maternal skeleton, such that calcium intakes ranging from low to high do not affect these parameters. However, greater intensity (number of feeds per day) and exclusivity (all of baby’s nutrition comes from milk) of lactation, which are indicators of increased breast milk output, lead to greater loss of maternal bone mass (535).

Intervening to correct calcium intake in women who habitually consume low calcium may be detrimental. A randomized intervention administered 1,500 mg calcium or placebo during pregnancy but not postpartum to Gambian
women with habitual intakes of ~350 mg calcium. There was no effect on bone density at any skeletal site at the end of pregnancy, but during 12 mo of lactation, the women who had received the calcium supplement during pregnancy more than doubled their losses in BMD of the lumbar spine, total hip, and distal radius and had greater increases in bone turnover markers (439). A followup study showed that these effects persisted long term, including through a subsequent pregnancy and lactation cycle (441). These results suggest that Gambian women are beneficially adapted to their low calcium intakes, while use of a calcium supplement during pregnancy disrupts this adaptation to create an adverse response when the supplement is subsequently withheld during lactation.

Weight-bearing exercise has been proposed to reduce the amount of bone resorbed during lactation. A small randomized trial of 20 lactating women found a small benefit on lumbar spine BMD (4.8 vs. 7.0% loss in the usual activity group), but no effect on whole body or total hip BMD (574). However, a subsequent study by the same investigators found no beneficial effect of exercise on any skeletal site (193). A cohort study comparing exercising to sedentary women also showed no impact of exercise on lactational BMD losses (560).

Deficiency of estradiol contributes to bone loss during lactation, but the extent to which it does so is uncertain. Some analyses have suggested that low estradiol, and a longer duration of lactational amenorrhea, predict the speed and magnitude of bone loss during lactation (140, 301, 409, 451, 484, 723, 857, 863, 1025). Earlier resumption of menses or use of an oral contraceptive are associated with reduced skeletal losses, while bone turnover markers normalize after recovery of menses and bone loss at the lumbar spine slows or begins to reverse (140, 451, 484, 723, 764, 863). On the other hand, in another study there was no difference in calcitropic hormones, bone turnover markers, bone density, and intensity of lactation between amenorrheic and eumenorrheic women during lactation, suggesting that resumption of menses does not predict reduced bone loss during lactation (572). Bone density has also continued to decline in women who breastfed for an extended period after their menses resumed (301, 857).

It is difficult to analyze the independent effects of low estradiol on bone turnover during lactation. More intense lactation causes more breast milk output and bone loss, but more intense lactation is also associated with lower estradiol and prolonged amenorrhea. The impact of isolated low estradiol is that it causes 1–2% annual losses of BMD in recently menopausal women (331), but the older ages of these women may not reflect what happens to reproductive age women with low estradiol.

In this context the effects of GnRH analog-induced estradiol deficiency in reproductive-age women are illuminating. This treatment induces marked estradiol deficiency, which can be beneficial to treat endometriosis, uterine leiomyomata, and premenstrual syndrome. However, its effects differ from what is observed during lactation (TABLE 3). Six months of acute estradiol deficiency causes 2–4% reductions in BMD of the spine with no losses at cortical sites (288, 415, 640, 657, 684, 696, 758, 762, 781, 891, 942). This is accompanied by increased serum calcium and phosphorus (265, 288, 640, 657, 781), increased fractional excretion of calcium (265, 288, 640, 657, 781, 891, 942), and low PTH and calcitriol (265, 657, 781). In contrast, 6 mo of lactation typically causes greater loss of BMD from both trabecular and cortical sites, low PTH but normal calcitriol, and reduced fractional excretion of calcium.

In summary, clinical data are consistent with low estradiol playing a permissive role in bone loss during lactation, but with the more excessive bone loss and other changes in calcium metabolism likely contributed to by high circulating levels of PTHrP (FIGURE 6). This would explain the increased bone turnover markers, accelerated bone loss, and reduced urinary calcium excretion, while the normal calcitriol and intestinal calcium absorption may be explained by PTHrP’s apparent reduced efficacy at stimulating these relative to PTH. Consistent with this, higher plasma PTHrP predicted greater loss of BMD at

Table 3. Comparison of the effects of 6 months of lactation versus 6 months of GnRH agonist therapy

<table>
<thead>
<tr>
<th></th>
<th>Lactation</th>
<th>GnRH Agonist</th>
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</thead>
<tbody>
<tr>
<td>Serum calcium</td>
<td>Increased</td>
<td>Increased</td>
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<tr>
<td>Serum phosphorus</td>
<td>Increased</td>
<td>Increased</td>
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<tr>
<td>PTH</td>
<td>Decreased</td>
<td>Decreased</td>
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<tr>
<td>Calcitriol</td>
<td>Normal</td>
<td>Decreased</td>
</tr>
<tr>
<td>24-h urine calcium</td>
<td>Decreased</td>
<td>Increased</td>
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<tr>
<td>Urinary Ca/Cr</td>
<td>Decreased</td>
<td>Increased</td>
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<tr>
<td>Bone resorption markers</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>aBMD changes (DXA)</td>
<td>5–10% at trabecular-rich sites; less at cortical</td>
<td>2–4% at trabecular-rich sites</td>
</tr>
<tr>
<td>Recovery of aBMD at 1 yr</td>
<td>Complete in most</td>
<td>Incomplete in most</td>
</tr>
</tbody>
</table>
the lumbar spine and femoral neck in breastfeeding women, after adjusting for low estradiol, PTH, and intensity of breastfeeding (863). Higher plasma PTHrP in turn was predicted by more intense lactation, higher prolactin, and lower estradiol (863). Some studies of men and nonlactating women have found that hyperprolactinemia due to pituitary adenomas is associated with elevated PTHrP, and that increased PTHrP predicts a higher serum calcium, and lower PTH and lumbar spine BMD (497, 880).

3. Summary

Bone turnover is markedly increased during lactation, but uncoupled to cause net resorption. Mean losses of 25–35% occur in the lumbar spine during 3 wk of lactation in rodents, while 5–10% losses at the lumbar spine are induced by 6 mo of breastfeeding, with smaller losses at other skeletal sites. Bone loss results from a combination of osteoclast-mediated bone resorption and osteocytic osteolysis; how much each contributes to the decline in BMD during lactation is uncertain. The very modest to zero change in BMD at cortical sites in lactating women may mean that osteocytic osteolysis is less important in humans compared with osteoclast-mediated bone resorption. Bone loss during lactation is driven by the combined effects of low estradiol and increased circulating PTHrP. Animal data support that both of these factors are important in regulating skeletal homeostasis during lactation. Conversely in women, the more modest effects of isolated low estradiol, ongoing bone loss despite resumption of menses or use of an oral contraceptive, and the effects of lactational release of PTHrP to normalize hypoparathyroidism (see sect. VD) and induce pseudohypoparathyroidism (see sect. VF) may all point to PTHrP having a greater effect on regulating skeletal metabolism during lactation.

F. Breast-Brain-Bone Circuit Controlling Bone Metabolism During Lactation

The preceding animal and human data are consistent with an important physiological interaction and cross-talk among breast, brain, and bone during lactation. The breast may be the central driver of this interaction. The summary model (FIGURE 7), which also draws on data discussed in sections IV and V, is as follows.

High prolactin and suckling-induced reflex arcs each act on the GnRH pulse center in the hypothalamus to suppress the pituitary gonadotropins [luteinizing hormone (LH) and follicle stimulating hormone (FSH)] and, in turn, ovarian function. This leads to low estradiol levels in early lactation that may persist or normalize despite ongoing lactation. Low estradiol upregulates RANKL and downregulates OPG in osteoblasts; consequently, differentiation, recruitment, and function of osteoclasts are stimulated, thereby resulting in increased bone resorption. Suckling, prolactin, and low estradiol also stimulate mammary epithelial cells to produce PTHrP. While most of the PTHrP is released into milk, some of it is released into the maternal bloodstream, both continuously and with additional surges induced by suckling. Increased PTHrP synergizes with the effects of low estradiol to stimulate osteoclast-mediated bone resorption and osteocytic osteolysis. The calcium receptor may modulate the release of PTHrP from breast tissue somewhat to reduce the likelihood that milk production produces systemic hypocalcemia; however, it is clear from some clinical cases that mammary tissue PTHrP production may be autonomous.

That mammary tissue plays a central role in regulating skeletal physiology during lactation has been made most clear by the finding that mammary-specific ablation of...
PTHrP reduces bone turnover and loss, while mammary-specific ablation of the calcium receptor leads to markedly increased mammary expression of PTHrP, higher systemic PTHrP levels, increased bone turnover, and greater net bone loss (591, 953). Suckling induces production and release of PTHrP from mammary tissue, whereas milk stasis causes the entire process to shut down.

Lactating mammary epithelial cells also produce high concentrations of calcitonin, which may act locally to reduce the calcium content of milk or inhibit PTHrP, as suggested by Ctcgrp null mice (see sect. VH). Calcitonin also has systemic actions to suppress osteoclast-mediated bone resorption and osteocytic osteolysis in bone. Furthermore, calcitonin may inhibit the pituitary lactotrophs and reduce the release of prolactin, which in turn would lessen ovarian suppression and inhibit production of PTHrP. A conditional deletion of calcitonin from mammary tissue is needed to determine its role during lactation.

Additional players in the cross-talk among breast, brain, and bone include oxytocin and calcitonin, and there may be others. In addition to its critical role in stimulating milk ejection, oxytocin may affect the differentiation and function of osteoblasts and osteoclasts. Serotonin may stimulate the production of PTHrP and have other direct and indirect effects on bone metabolism during lactation.
G. Bone Formation and Skeletal Recovery Post-Weaning

1. Animal data

After the pups are weaned, the maternal skeleton undergoes a rapid phase of remineralization. Bone turnover continues to be increased over nonpregnant values, but it is now uncoupled in the opposite direction from lactation, favoring net bone gain.

When weaning is forced by the sudden removal of pups before the natural end of lactation, within 24–48 h there is widespread apoptosis of osteoclasts that prompts a fall in osteoclast number and activity below prepregnant values, a marked decrease in RANKL and RANK expression, and a substantial increase in osteoblast precursors, osteoblast number, osteoid surface, and bone formation rate (35, 107, 194, 627). Transient hypercalcemia can occur over the first several days due to continued increased levels of bone resorption and intestinal calcium absorption despite loss of milk output (35, 396, 718), during which time PTH and calcitriol fall and calcitonin surges (35, 627, 718). Bone resorption markers decline rapidly while bone formation markers increase (35, 477, 478, 580, 956, 992, 994). Osteocytic osteolysis also ceases, but now osteocytes express osteoblast-specific genes, tetracycline-labeled bands become evident in their lacunae, and the matrix is restored to its prior mineral content (FIGURE 5) (733, 734). This anabolic activity of osteoblasts and osteocytes is sustained for several weeks, as a result of which bone strength, mineralization, and microarchitecture improve and may reach prepregnancy values or better.

The extent of skeletal recovery has been documented in several ways.

Serial studies using DXA, and cross-sectional studies using skeletal ash weight, indicate that rodents restore skeletal mineral content within 2–4 wk after weaning (35, 362, 994). Black Swiss and C57BL/6 mice regain their prepregnancy BMC in whole body, lumbar spine, and hindlimb within 14 days of weaning (296, 477, 478, 580, 992, 994), whereas CD1 mice regain their prepregnancy BMD by 28 days after weaning, but remain below the values for age-matched virgin mice (35, 37). The most remarkable change occurs in Ctgcrp null mice, which lose 55% of trabecular (lumbar spine) BMC and 30% of cortical (whole body and hindlimb) BMC during lactation, but restore this within 18 days of weaning, taking just 4 days longer than their WT sisters that experienced half the losses (992, 994).

Histomorphometry has revealed increased osteoid seams covering 50–70% of bone surfaces, while dynamic histomorphometry has shown widely spaced tetracycline labels (107, 194, 628, 947). The combination of increased osteoid surface and widened seams corresponds to a markedly increased bone formation rate. Bone volumes, trabecular thickness, and mineral content are recovered within 2–4 wk of weaning in the mouse and 4–8 wk of weaning in the rat (107, 194, 628, 947). Detailed histomorphometric assessment of vertebrae and cortical bone of femora at 8 wk after weaning in rats showed that bone formation rates and mineralizing surfaces on trabecular, periosteal, and endosteal surfaces had declined to age-matched nulliparous values, while bone mass, cortical thickness, and cortical area had increased to virgin values (947).

Microradiographs, scanning electron microscopy, and microCT have been used to assess recovery of microarchitecture, which have revealed normalization at some sites, and permanent alterations at others. The vertebrae fully recover their microarchitecture, including bone volumes, trabecular number and thickness, mineralization, stiffness, and reversion from a rodlike back to a platelike structure (35, 566). The femora and tibiae significantly improve trabecular number and thickness, bone volumes, mineralization, cortical thickness, porosity, and stiffness (35, 106, 107, 355, 566, 628, 947, 994). In some studies, the values in tibiae and femora become equivalent to age-matched virgins (35, 628), whereas in other studies there were permanent reductions in these parameters compared with virgin mice or prepregnancy values (566, 628). Recovery is full or better when the end-of-pregnancy value is the baseline measurement (107). There are also region-specific differences in the degree of recovery, since in rats the proximal and distal femora regained the mineral content of nulliparous controls while the femoral midshaft did not completely recover (355), and there was a permanent reduction in the trabecular content of the femora (355, 628).

Intriguingly, in some studies the cross-sectional diameters, cortical perimeters, and volumes of the tibiae or femora were significantly greater at post-weaning compared with prepregnancy, or post-weaning compared with age-matched controls (106, 947, 994). Such changes in the macro-architecture of bone, especially the cortical diameter, will increase the cross-sectional and polar moments of inertia, and, thereby, the breaking strength in bending and torsion (FIGURE 8). This may contribute to maintenance of bone strengths of the tibiae and femora despite any failure to fully recover the trabecular microarchitecture (477).

Other biomechanical testing has shown vertebral and femoral strength and stiffness parameters to improve significantly in rats by 8 wk after weaning, and to equal the values of nulliparous controls (947).

The rapidity and relative completeness of this post-weaning phase of bone recovery has prompted numerous approaches to try to identify the factors that stimulate bone formation and post-weaning recovery. Severely vitamin D-deficient rats improve bone mass and architecture after lac-
tation, appearing to regain the amount that was lost during lactation (628); however, an earlier study from the same investigators suggested that there was no recovery of ash weight or calcium content (362). Vdr null mice and Cyp27b1 null mice display osteomalacia and reduced bone mass before pregnancy, but after lactation they upregulate bone formation markers and increase BMC substantially to values 40–50% higher than prepregnancy (296, 330). Pth null mice have low bone turnover prior to pregnancy, but upregulate bone formation markers normally during post-weaning, and increase BMC after lactation to values 20% higher than prepregnancy (478). Ctcggrp null mice lose 55% of skeletal mineral content during lactation but fully regain it post-weaning (194, 992, 994). Osteoblast-specific ablation of Pthrp results in an osteoporotic phenotype in adult mice, but these obPthrp null mice lactate normally and have full recovery of bone mass afterward (477). Collectively these studies indicate that PTH, osteoblast-derived PTHrP, vitamin D, VDR, calcitriol, and calcitonin are not required full remineralization of the maternal skeleton to be achieved after lactation (194, 296, 477, 478, 992, 994).

Adequate calcium is required, since a low-calcium diet prevented skeletal recovery in rats, whereas normalizing the diet 3 wk later allowed normal recovery to occur (355). An increased rate of mineral absorption is evidently not required since both intestinal calcium and phosphorus absorption are normal during post-weaning recovery in rats (93, 360). The marked increase in bone mass of Vdr nulls and Cyp27b1 nulls (296, 330) may imply that intestinal calcium absorption is increased, since these mice have a reduced, rachitic bone structure on the same diet when non-pregnant. However, measurements of intestinal calcium absorption have not been done in mice during this interval.

Theoretically, increased weight bearing during this anabolic phase might lead to a greater increment in bone mass. In a preliminary report, rats forced to stand upright to reach their food during lactation and 6 wk of post-weaning showed significantly higher femoral BMD and a trend toward increased tibial bone volumes, compared with rats housed in normal cages (829).

In rats that are not permitted to lactate, normalization of BMC and skeletal microarchitecture (as assessed by histomorphometry) has been observed within a week of parturition (408). This is evidence of skeletal recovery from losses incurred during pregnancy. Conversely, no post-weaning improvement resulted after lactational bone loss was completely blocked by treatment with OPG (37). This may simply mean that if no bone is lost during lactation, then there is nothing to restore, and no signal is sent to upregulate bone formation.

2. Human data

Women also undergo a substantial increase in bone mass and mineralization after weaning, evidently reversing the losses that transiently occur during lactation. There are no histomorphometric studies or direct tests of bone strength in women, as there are in the rodent models. Instead, skeletal recovery after lactation has largely been determined by longitudinal assessment of bone mass and mineralization during lactation and post-weaning by DXA at the total body, lumbar spine, and hip. More recently, changes in microarchitecture by HR-pQCT have been assessed at peripheral sites. Many dozens of studies have examined the effect that lactation has on predicting future risk of low bone density, osteoporosis, or fracture.

Analysis of women who inadvertently ingested isotopes while living near the Techa River in Russia revealed that women who were pregnant and lactated during the time of exposure had a greater content of $^{90}$Sr in the maternal skeleton years after the exposure, compared with women living in the region who were pregnant prior to the exposure or had never been pregnant (923). This result is compatible with increased uptake of isotope into the maternal skeleton during post-weaning recovery.

The available DXA data suggest that lactational loss of bone density is completely reversed by 12 mo after weaning in most women (140, 168, 206, 379, 403–405, 412, 452, 457, 470, 484, 505, 532, 572, 635, 705, 723, 729, 764, 856, 857, 860, 1009), whereas recovery has been incomplete at some skeletal sites when assessed at 6 mo or less after weaning the baby (9, 123, 124, 168, 403–405, 457, 484, 532, 633, 635, 1009). A small longitudinal study of 22 women found a marked loss of 8% in the distal radius that
was still reduced 5% by 12 mo after weaning (609), which suggests that the radius is slower to recover BMD. Note that these studies report the mean responses of a cohort; the response of individual women will vary, such that some women may not return to baseline after lactation, while others may end up with a BMD even higher than prior to pregnancy. Recovery is fast enough that women who breastfeed for 6 mo or more and have a second pregnancy within 18 mo do not have reduced BMD of the spine or hip at the end of the second pregnancy (534, 860).

A study from China took a different approach of doing a baseline BMD reading in 40 women with habitually low calcium intakes, who had either stopped lactating or were still lactating but had recovered their menses. They were randomized to receive 600 mg of calcium versus placebo, and subgrouped by genotyping for a VDR polymorphism (FokI) (1012). A second BMD was obtained a year later and revealed a net increase in bone mass with additional benefits attributable to calcium supplementation and the FF genotype. BMD of the lumbar spine increased by as little as 7.9% in the placebo group with ff genotype to as great as 17.4% in the FF genotype receiving calcium, while the femoral neck BMD increased 4.4% in the ff genotype on placebo versus 14.7% in FF genotype on calcium. This study provides confirmation that a substantial increase can occur in BMD post-weaning. The suggested additional benefits of calcium supplementation and the VDR polymorphism require independent confirmation.

HR-pQCT of the radius and ultradistal femur reveal recovery of trabecular microarchitecture and cortical parameters in women who lactate for shorter intervals, but incomplete recovery in women who lactate for longer (84, 109). However, follow-up in these studies was for less than 6 mo after lactation ended for women who lactated for a longer duration, which is not sufficient time for recovery or to determine if permanent changes in structure resulted. Similar to studies in animals, some clinical studies have found that the cross-sectional diameter of the femur increased after lactation or post-weaning recovery, and that cortical bone area is restored or increased (869, 986). An increase in bone volumes will improve bone strength and may compensate for any permanent loss of trabecular microarchitecture (FIGURE 8).

Hip structural analysis of data from DXA scans provides direct measurements of femoral geometry and derived measures of femoral strength. A longitudinal study obtained these measurements at 2 wk, 6 mo, and 12 mo postpartum in lactating women, while similar timing of measurements were carried out in nonpregnant, nonlactating women (537). In lactating women there was an ~3% decrease in cross-sectional area, a 1.7% decline in cortical thickness, a 2.1% decrease in section modulus (bending strength), and a 2.3% increase in buckling ratio (instability) by 6 mo of lactation. These changes reversed by ~6 mo after lactation ceased, during which no changes were seen in the controls (537).

If a permanent reduction in BMD or skeletal strength were to occur from lactation, then lactation should be a strong risk factor for low BMD or fracture in women of all ages. However, over five dozen epidemiologic studies of pre-, peri-, and postmenopausal women have found a neutral effect (16, 38, 63, 158, 203, 214, 215, 290, 292, 299, 341, 356, 368, 379, 383, 397, 444, 445, 448, 463, 481, 506, 509, 541, 547, 611, 619, 625, 636, 648, 683, 700, 761, 828, 845, 846, 861, 864, 865, 869, 879, 934, 935, 944, 951, 969, 1020) or a protective effect (25, 27, 71, 83, 144, 169, 212, 244, 279, 327, 365, 416, 421, 482, 507, 700, 804, 833, 879, 986, 1018) of lactation on peak bone mass, BMD, and fracture risk. This includes a study in which extended duration of breastfeeding per child conferred a progressively greater protection against hip fractures (421). A study of 1,852 twins and their females relatives, including 83 twins who were discordant for pregnancy and lactation, found no effect of breastfeeding history on BMD or BMC between twins, but that parous women who had breastfed had higher BMC and BMD than parous women who had never breastfed (700). A study of 30 women who had had at least six pregnancies, and breastfed each child for at least 6 mo, found no effect of lactation on BMD (379).

A previously cited NHANES study of 819 women ages 20–25 yr found that women who had breastfed as adolescents had higher bone mass than those who had not breastfed, indicating a protective effect (169). This study is particularly informative because of the shorter interval of time elapsed between lactation and measurement of BMD, compared with most of the dozens of studies that examined women decades later. It also suggests that adolescent pregnancy does not adversely impact the achievement of peak bone mass, as previously feared.

There are, of course, a few contrary studies that suggest lactation predicts lower BMD (261, 336, 352, 472, 559, 686, 770, 946, 971, 973) or increases the risk of fracture (97, 525, 559, 699). Smoking may cause breastfeeding to have a negative effect on BMD that is not seen in nonsmokers who breastfed (448). Fractures do occur rarely during lactation while bone mass and strength are temporarily reduced, so these lactational losses of bone structure are not always benign in the short term (see sect. VA). Overall, it appears that any harm of lactational bone loss is transient in most women, such that lactation generally confers a long-term neutral or protective effect on future risk of fracture. The fewer studies suggesting a long-term adverse effect may point to ethnic or other differences among the cohorts that remain to be explained;
measurements of bone strength after weaning have been sectional diameters of these bones. Although no formal effects of which may be offset by an increase in the cross-trabecular microarchitecture of the femora and tibiae, the human studies, there may be incomplete recovery of the this closer to 1 yr after weaning. In both the animal and rodents appear to fully regain mineralization and strength within 2–8 wk, whereas women likely achieve substantial improvements in bone mass, mineralization, favor bone formation. The maternal skeleton undergoes turnover is also increased during the post-weaning phase, whereas another study found a modest increase in intestinal calcium absorption post-weaning skeletal recovery. As noted earlier, one study found no effect of resumption of menses on bone loss during lactation or recovery afterward (572).

It is evident from the many longitudinal DXA studies that women who lactate for shorter intervals recover sooner, and this is also likely because they lost less bone.

A small but statistically significant benefit on bone mass gained during post-weaning was conferred by randomization to 1 g calcium supplementation versus placebo, despite no beneficial effect of the supplement during lactation (452). No study has prospectively examined the potential benefit of weight-bearing exercise to increase BMD gains after weaning. However, in retrospective studies of premenopausal women in which breastfeeding conferred a higher lumbar spine BMD, increased weight-bearing also conferred a significant, independent benefit (327, 1018). No other studies have examined factors that may stimulate post-weaning skeletal recovery. As noted earlier, one study found a modest increase in intestinal calcium absorption during post-weaning (453), whereas another study found no significant increase (764).

3. Summary

Extensive animal data and human data indicate that bone turnover is also increased during the post-weaning phase, but uncoupled in the reverse direction from lactation to favor bone formation. The maternal skeleton undergoes substantial improvements in bone mass, mineralization, and strength. Rodents appear to fully regain mineralization and strength within 2–8 wk, whereas women likely achieve this closer to 1 yr after weaning. In both the animal and human studies, there may be incomplete recovery of the trabecular microarchitecture of the femora and tibiae, the effects of which may be offset by an increase in the cross-sectional diameters of these bones. Although no formal measurements of bone strength after weaning have been done in women, the fact that more than five dozen epidemiological studies found that lactation confers a neutral or protective effect against low BMD and fragility suggests that recovery is effectively complete, without long-term adverse effects on bone strength. Some data are compatible with the possibility that weight-bearing exercise and increased calcium intake during post-weaning may contribute to an even greater BMD being achieved.

The question arises as to whether or not the skeletal losses invoked by lactation and the recovery post-weaning are the same in a first compared with a second or third reproductive cycle, or in younger versus older mothers. In areas where the trabecular microarchitecture is not completely restored, such as the tibia or femur, there may be less resorption of bone in subsequent lactation cycles. Conversely, the trabecular microarchitecture is completely restored in the spine, so subsequent lactation cycles can be expected to result in a similar magnitude of resorption and recovery. Available data from human studies are consistent with a similar degree of skeletal resorption occurring in all lactation episodes, and a similar degree of recovery occurring afterwards regardless of maternal age. This includes observational studies that included women of various reproductive ages and parity, and the dozens of epidemiological studies that have not found an association among parity, lifetime extent of lactation, and risk of osteoporosis or fractures. However, there have been no systematic comparisons in animal or human studies of younger versus older mothers, or primiparas versus multiparas, so differences in the skeletal responses to lactation and recovery invoked by age or parity cannot be ruled out.

V. DISORDERS OF BONE AND MINERAL METABOLISM DURING LACTATION

A. Osteoporosis of Lactation

1. Animal data

Despite significant skeletal resorption during lactation that is most marked in the lumbar spine, it is unusual for fractures to be found. This is likely because the rodent ambulates on four limbs, thereby not loading the spine in the same orientation or to the same degree as in a bipedal human.

2. Human data

Multiple case reports and series have confirmed that, although rare, osteoporotic fractures can occur in breastfeeding women (501). Consistent with most bone loss occurring in the axial spine, vertebral compression fractures have been reported, sometimes with as many as 6–10 such fractures evident at presentation in one individual (98, 501,
677, 687). These fractures can be quite debilitating. The literature is confusing because many cases are described as “pregnancy-associated osteoporosis” despite the fractures occurring several months into lactation.

In some breastfeeding women, the physiological bone resorption induced by PTHrP and low estradiol may be a sufficient explanation for reduced skeletal strength that predisposes to fragility fractures. As noted earlier, in some individuals the lumbar spine BMD has been shown to drop from normal to osteoporotic values during otherwise normal lactation. In some instances release of PTHrP by the breasts may be more exuberant than normal, leading to more marked resorption of bone. Three such breastfeeding women presented with hypercalcemia, increased PTHrP, and vertebral compression fractures (29, 751). The hypercalcemia and increased PTHrP resolved in all three women after weaning (29, 751), but in one woman the plasma PTHrP level remained elevated for at least 4 mo after lactation ceased (751). Three other cases of compression fractures during lactation also had hypercalcemia that resolved after weaning, which is suspicious for elevated PTHrP as the cause (928). PTHrP was undetectable in one case and appropriate for lactation in two others, but common problems with sample collection and processing (detailed in sect. IIA3b) may explain why high levels of PTHrP were not found. It is also conceivable that relative sensitivity to the skeletal-resorptive effects of PTHrP may differ among women, thereby contributing to differences in the amount of bone lost during lactation.

On the other hand, review of the individual cases indicates that in many women there are additional factors that contribute to skeletal fragility, including causes of low bone mass or fragility that precede pregnancy, and bone loss that likely occurs during pregnancy (such as with very low calcium intake). This leads to the consideration that if a woman is known to have skeletal fragility or very low bone mass prior to pregnancy or at delivery, it may be reasonable to advise against breastfeeding in case the programmed skeletal resorption proves to be too much for that woman’s skeleton (501).

When fractures do occur, it may also be reasonable to advise that breastfeeding cease to stop further bone loss and initiate the expected post-weaning skeletal recovery. Individual cases have reported spontaneous improvements in bone density by 20–70% (29, 433, 713, 811, 928), so pharmacological therapy may be reasonably withheld for 12–18 mo to determine the magnitude of spontaneous recovery (501). An anti-remodeling agent such as a bisphosphonate or denosumab might blunt the spontaneous bone formation that is expected during the post-weaning interval.

As noted earlier, individual case reports have described use of nasal calcitonin (263, 687, 885), bisphosphonates (43, 143, 178, 263, 438, 475, 671, 677, 685, 799, 885, 897), strontium ranelate (897, 1016), and teriparatide (98, 178, 377, 527, 544, 885) to treat osteoporosis diagnosed during pregnancy or lactation. In each of these uncontrolled cases, the achieved BMD increase was assumed to be due to pharmacological therapy, but remained within the expected range of increase that can occur spontaneously during post-weaning recovery.

3. Summary

Fragility fractures of the spine and other sites rarely occur during lactation and may result from the normal physiological resorption of the skeleton during lactation, combined with effects of low bone mass or skeletal fragility that preceded pregnancy, and skeletal resorption that may have occurred during pregnancy (501). A spontaneous and substantial improvement in bone mass occurs after weaning even in women who have fractured, so pharmacological therapy should probably be withheld for at least a year to determine if it is really needed.

B. Primary Hyperparathyroidism

1. Animal data

Animal models of primary hyperparathyroidism have not been studied during lactation, although studies in Casr\(^{+/−}\) mice are relevant (see sect. VC). As described earlier, rebound hypercalcemia occurs after delivery and again at weaning in normal rodents; the increase in serum calcium may be even greater in rodents that are hypercalcemic from primary hyperparathyroidism.

2. Human data

Severe hypercalcemia, also termed a parathyroid crisis, has occurred during the postpartum interval in women with primary hyperparathyroidism who were not operated on during pregnancy, and in some women who go on to breastfeed (54, 153, 186, 612, 662, 795). The sudden rise in serum calcium likely results from several factors, including abrupt loss of the placenta (a significant route of calcium loss), the initiation of low estradiol and PTHrP-induced skeletal resorption to support milk production, the effect of low estradiol to increase skeletal sensitivity to excess PTH, and physiological resorption induced by physical inactivity and bedrest during the puerperium. Consequently, significant worsening of hypercalcemia has occurred after delivery and in women who go on to breastfeed. However, there is variability in everything, and in at least one case the serum calcium improved during lactation, likely because milk production drains calcium from the maternal circulation (511).

3. Summary

Primary hyperparathyroidism can worsen significantly postpartum, with variable effects in women who breast-
feed. The clinician should be alert to this and consider whether parathyroidectomy or medical therapy might be needed during the postpartum interval.

C. Familial Hypocalciuric Hypercalcemia

1. Animal data

Castr mice have life-long hypercalcemia and hypocalciuria and mimic the human condition of FHH (394). They lactate normally, and their pups grow at the expected rate. However, mammary tissue shows twofold increased PTHrP expression while milk has twice the normal content of PTHrP but modestly reduced calcium content (36). These results imply that skeletal resorption increases to compensate for reduced entry of the calcium into milk. A mammary-specific deletion of Casr confirmed this by finding increased PTHrP in mammary tissue, plasma, and milk; suppressed PTH; reduced milk calcium content; reduced bone volumes and BMD; and increased urine calcium excretion, compared with lactating controls (591). These findings point to a central role of the CaSR in mammary tissue to control skeletal resorption during lactation, and the milk content of PTHrP and calcium.

Treatment of mammary epithelial cells with a calcimimetic drug that activates the calcium receptor results in increased milk calcium content and reduced PTHrP content (36, 952). Therefore, activating mutations of the calcium receptor, which cause systemic hypocalcemia, may be expected to result in milk with increased calcium but reduced PTHrP content.

2. Human data

The available mouse data predict that women with FHH who breastfeed will have increased PTHrP, more bone loss, increased renal calcium excretion, but a lower calcium content of milk. However, there are no published case reports describing changes in serum minerals, bone density, or milk composition in these women.

3. Summary

FHH likely causes increased PTHrP-mediated bone loss and reduced milk calcium content, but confirmatory human data are lacking.

D. Hypoparathyroidism

1. Animal data

Lactation appears to improve mineral and skeletal homeostasis in rodents that lack PTH. This has been most clearly shown in Pth null mice, which lactate normally on a standard 1% calcium diet, normalize serum calcium and phosphorus, and experience a normal loss of BMC at whole body, lumbar spine, and hindlimb (478). After weaning, they fully regain the mineral content lost during lactation, reach a BMC that is 10–15% higher than the prepregnancy baseline value, and redevelop hypocalcemia and hyperphosphatemia. They are somewhat more prone to anesthetic-related deaths associated with serial DXA readings (133). A 2% calcium diet blunted the lactational decline in BMC but did not reduce the post-lactation gain in BMC (478). Overall, the studies in Pth null mice indicate that loss of PTH has no significant effect on lactational or post-weaning mineral homeostasis. Calcitriol, PTHrP, and intestinal calcium absorption have not been measured in lactating Pth null mice.

Parathyroidectomy has been carried out during established lactation in rats, resulting in sudden PTH deficiency that was not present prior to pregnancy or lactation. As discussed in the following paragraphs, parathyroidectomy results in improved values compared with nonlactating, parathyroidectomized rats, but worsening of some parameters compared with intact or sham-operated rats. The calcium content of the diet appears to be an important confounding variable.

In a series of experiments carried out by one team of investigators, lactating, parathyroidectomized rats consuming a 2% calcium diet had normal serum calcium and phosphorus that was also different from values in lactating, intact rats on the same diet (395). On this diet the femoral ash weight declined ~40% during lactation in both intact and parathyroidectomized rats, confirming that resorption of bone during lactation does not require PTH and is not made worse by its absence (395). Substitution of a 0.02% calcium diet caused the serum calcium to fall to half normal in lactating, parathyroidectomized rats, and 60% of them died suddenly from presumed tetany or arrhythmias. In contrast, none of the nonlactating parathyroidectomized rats died, nor did any intact rats (395).

In other studies, parathyroidectomized rats in lactation caused a 50% fall in serum or ionized calcium and an increase in serum phosphorus when 0.4 and 1% calcium diets were consumed (295, 569, 718). The magnitude of hyperphosphatemia in parathyroidectomized rats lessened as lactation progressed (295). Calcitriol increased in lactating, parathyroidectomized rats to at least double the value of nonlactating, parathyroidectomized rats and nonlactating, sham-operated rats, but remained below the concentration of lactating sham-operated rats (569, 718). Intestinal calcium absorption increased normally during lactation despite simultaneous parathyroidectomy and vitamin D deficiency (93). Milk calcium content was significantly higher at day 6 of lactation and normal during the remainder of lactation in parathyroidectomized rats, despite the serum
calcium being 50% of normal (295). This confirms that achieving a normal calcium content in milk does not require PTH. Based on studies in mice, systemic hypocalcemia may cause increased milk PTHrP content (36, 952).

Weight gain of the pups was normal when lactating, parathyroidectomized rats consumed a 2% calcium diet (395), whereas weight gain was modestly reduced as early as 24 h after parathyroidectomy when the mothers consumed 1 or 0.4% calcium diets (295, 569). This reduced weight gain was not seen in pups nursed by Pth null mice compared with WT mice; the ash weight and calcium content were no different at the end of 3 wk of lactation (133). Since the calcium content of milk is normal in parathyroidectomized rats, other factor(s) must be contributing to reduced weight gain in their pups. Since the parathyroidectomized mothers are hypocalcemic on the diets associated with reduced pup weight gain, maternal tetany may be preventing prolonged suckling, or causing reduced milk volume. Another possibility is that the expected increased milk PTHrP content (36, 952) is causing reduced weight gain, because milk content of PTHrP has been shown to be inversely related to the rate of skeletal mineral accrual in nurses pups, and independent of milk calcium content (591). The physiological role of PTHrP in milk may be to reduce or modulate skeletal mineral accrual in the neonate (492).

2. Human data

Since the 1970s it has been appreciated that mineral and skeletal metabolism substantially improves in hypoparathyroid women who are breastfeeding (30, 148, 161, 607, 790, 796, 811, 837, 890, 1008), and these observations contributed to the hypothesis that the lactating breast expresses a PTH-like hormone. Occasionally women have been diagnosed to have hypoparathyroidism due to abrupt onset of hypocalcemia when lactation ceases (72), which is also compatible with loss of a calciotropic hormone produced by the breasts.

That hypothesis proved to be correct when PTHrP was identified and confirmed to be expressed at high levels in lactating mammary tissue, the maternal circulation, and milk. Furthermore, the animal data previously discussed have confirmed the physiological role of PTHrP during lactation. One clinical case documented that hypoparathyroid women can experience transient hypocalcemia in the first day or two postpartum, presumably from the abrupt loss of placental PTHrP (890). This hypocalcemia resolves as lactation, and expression of PTHrP in the breast, becomes more fully established. However, most published cases of hypoparathyroidism have not reported such a worsening during the puerperium, but instead have noted that the requirement for supplemental calcium and calcitriol drops substantially as milk production is upregulated. Significant iatrogenic hypercalcemia and vertebral compression fractures have occurred when attention has not been paid to monitoring the serum calcium and the anticipated requirement for the doses of calcium and calcitriol to be decreased (148, 161, 790, 796, 811, 837, 1008). In some women calcium and calcitriol both need to be stopped, whereas in other women reduced doses may be all that is required.

The reason for the improvement in mineral and skeletal homeostasis is that PTHrP, released from lactating breast tissue, stimulates bone turnover, renal tubular calcium conservation, and production of calcitriol by the kidneys (148, 607, 837). Calcitriol does not increase above normal, and this may be because in some studies PTHrP appears less potent than PTH in activating the PTH/PTHrP receptor and stimulating Cyp27b1 (308, 413, 414, 494, 988). Another potential reason is that the hormonal milieu of lactation, including high prolactin and low estradiol, conceivably alters how Cyp27b1 responds to PTHrP during lactation.

Since the identification of PTHrP, many clinical reports have deduced the presence of increased circulating PTHrP but have not measured it (30, 890, 1008). However, a rise and decline in plasma PTHrP has been shown to correspond to the rise and decline in serum calcium, calcitriol, and bone turnover markers (607, 811, 837). In all published cases, the improvement in mineral homeostasis and decline afterwards correlates respectively with the increasing and lessening intensity of lactation. The less exclusively or intensively a hypoparathyroid woman breastfeeding, the more likely it is that supplemental calcium and calcitriol are still needed.

The ameliorating effect of breast-derived PTHrP wanes at varying rates as lactation lessens and the baby is weaned. In some women the calcium and calcitriol has to be reinstated while breastfeeding was still ongoing, whereas in others it may not be required until days, weeks, or months after lactation has ceased. In most cases the need to reinstitute or increase the doses of calcium and calcitriol occurs within days prior to or after weaning, but in one unpublished case that the author was involved in, serum calcium remained normal off all supplements for more than 1 yr post-weaning, before hypocalcemia abruptly recurred.

One clinical report documented a spontaneous 40% increase in lumbar spine BMD and 7.5% increase in femoral neck BMD in a surgically hypoparathyroid woman after lactation, confirming that substantial skeletal recovery is possible after weaning without PTH (811).

3. Summary

Animal and human data are consistent with mammary-derived PTHrP stimulating bone turnover, renal calcium conservation, and synthesis of calcitriol during lactation.

Rodents require both increased intestinal calcium absorption and skeletal resorption to meet the calcium require-
ments of lactation. A standard 1% calcium diet is sufficient for lactating mice to normalize mineral homeostasis without PTH, whereas lactating rats appear to need a 2% calcium diet for normalization to occur without PTH. There may be species differences that contribute to why mice and rats do not have identical responses to absence of PTH.

Women rely mainly on skeletal resorption to meet the calcium requirements of lactation. The available case reports make very clear that lactation usually results in normalization or near-normalization of mineral and skeletal homeostasis in hypoparathyroid women. It is hazardous to blindly maintain prepregnancy doses of calcitriol and calcium in hypoparathyroid women who plan to breastfeed; the albumin-adjusted calcium or ionized calcium must be followed so that appropriate reductions in the doses of these supplements can be made. As lactation lessens, and especially after it ceases, the requirement for calcium and calcitriol can be expected to revert to prepregnancy levels.

E. Pseudohypoparathyroidism

1. Animal data

A mouse model of pseudohypoparathyroidism has not been studied during lactation (322).

2. Human data

There are no animal or human data regarding mineral homeostasis and bone metabolism in animal models or women with pseudohypoparathyroidism. It is expected that lactation should lead to an overall improvement due to the release of PTHrP from mammary tissue. Since pseudohypoparathyroidism leads largely to renal but not skeletal resistance to PTH, it is possible that lactation may lead to increased skeletal resorption compared with normal when the effects of PTH and PTHrP are combined.

3. Summary

Pseudohypoparathyroidism can be anticipated to improve during lactation, mimicking the clinical experience with hypoparathyroidism, but this has not been documented.

F. Pseudohyperparathyroidism

1. Animal data

Preceding sections have confirmed that lactating mammary tissue produces PTHrP, and that suckling leads to increased release of PTHrP and systemic effects on mineral homeostasis. However, there are no data on pseudohyperparathyroidism from lactating animals.

2. Human data

The physiological release of PTHrP from lactating breasts alters mineral and skeletal homeostasis, thereby contributing to a small increase in serum calcium and phosphorus, suppression of PTH, increased bone resorption, and reduced renal calcium excretion. But most women and their clinicians are unaware of the presence of PTHrP or its effects, because these physiological changes are silent and without consequences. As noted in section VD, the presence of PTHrP becomes apparent in hypoparathyroid women who normalize mineral and skeletal homeostasis, and will become hypercalcemic if the doses of calcium and calcitriol are not decreased or stopped.

Occasionally the effects of breast-derived PTHrP become symptomatically obvious in otherwise normal breastfeeding women. PTHrP-mediated hypercalcemia is called pseudohyperparathyroidism because it mimics the effects of primary hyperparathyroidism but is not due to excess PTH. It has developed during normal lactation (179, 549, 751, 847), in a woman who recently delivered a baby but was unable to breastfeed because of a critical illness in the baby (800), and even in nonlactating women who simply have large breasts (435, 474, 603, 950). The mechanism is that high levels of PTHrP cause excess skeletal resorption of calcium and renal reabsorption of calcium; intestinal calcium absorption may also be increased if the achieved levels of PTHrP are sufficient to cause high levels of calcitriol.

One woman developed hypercalcemia during each of two pregnancies; it worsened and persisted while she breastfed and resolved with weaning. During the second lactation interval, high circulating levels of PTHrP were confirmed, which normalized as the hypercalcemia resolved (549). A second woman had lactation-induced hypercalcemia and vertebral compression fractures, high PTHrP of 10.9 pg/ml, low calcitriol, and undetectable PTH. The hypercalcemia improved with weaning while the PTHrP level stayed elevated for several months before becoming undetectable (751). In a third case, severe postpartum hypercalcemia (4.85 mM) worsened over the first 3 days postpartum, accompanied by a very high plasma PTHrP (28.4 pM) with undetectable PTH. The hypercalcemia persisted for 10 days despite treatment with intravenous pamidronate, before subsiding to normal, accompanied by a fall in PTHrP to undetectable, and a rise in PTH to normal. The authors of that report incorrectly attributed placental PTHrP as the explanation for her hypercalcemia (800). However, PTHrP has a half-life of minutes in the circulation, and its placental source was abruptly lost with the afterbirth; therefore, placental PTHrP cannot explain maternal hypercalcemia that worsened over the first 3 days after delivery and persisted for 10 days. The time course is consistent with release of PTHrP from the breasts, which upregulates in response to regulatory cues during late gestation and parturition, and subsides when breastfeeding does not occur. A fourth case
involved a woman who developed hypercalcemia with suppressed PTH during two successive lactation episodes (179). The hypercalcemia resolved and PTH rose to normal with weaning each time; no measurements of PTHrP were done. A fifth and much older case from 1981 (prior to the discovery of PTHrP) also involved lactational hypercalcemia with low PTH; a bone biopsy revealed active skeletal resorption suggestive of hyperparathyroidism (847). The hypercalcemia resolved after weaning, and the authors correctly deduced that the breasts must be producing a PTH-like hormone.

These cases emphasize the extreme of women developing symptomatic hypercalcemia during lactation. This appears to be uncommon; however, some cases may go undiagnosed because hypercalcemia causes nonspecific constitutional symptoms that will not be obviously different from normal symptoms in a woman who is feeding a baby on demand around the clock. Recall that the serum calcium and ionized calcium have both been shown to rise during lactation in longitudinal studies, so the prevalence of high serum calcium during lactation may be more common than what these isolated cases would otherwise suggest.

Weaning the baby, combined with judicious use of breast binders or dopaminergic medications that suppress prolactin (cabergoline, bromocriptine), should reverse the excess production of PTHrP and consequent hypercalcemia. A bisphosphonate or denosumab may be needed to acutely shut down the increased bone turnover. However, excess production of PTHrP can last for months or longer, which explains why a few cases have even required bilateral mastectomies to correct the problem (435, 474, 603, 950).

3. Summary

Release of PTHrP from lactating breast tissue causes the serum and ionized calcium to rise slightly in most women; uncommonly, this has caused symptomatic hypercalcemia. The prevalence of PTHrP-mediated hypercalcemia during lactation may be underestimated since serum calcium is not normally measured, and symptoms of hypercalcemia may be misattributed to other aspects of life with a new baby. When hypercalcemia does occur, it should resolve with weaning, but occasionally surgical reduction in the amount of breast tissue may be necessary.

G. Vitamin D Deficiency and Genetic Vitamin D Resistance Syndromes

1. Animal data

The effect of extreme disturbances in maternal vitamin D physiology during lactation and post-weaning recovery have been investigated in Vdr null and Cyp27b1 null mice as well as vitamin D-deficient mice and rats.

In longitudinal studies wherein Vdr null mice were raised on a standard 1% calcium diet to allow them to develop rickets, but switched to a 2% calcium diet prior mating to maximize fertility, Vdr null mice increased BMC by 55% during pregnancy, and lactated normally, losing a normal amount of BMC compared with their WT sisters on the same diet (296). Serum calcium and phosphorus remained normal throughout lactation and post-weaning. Within 14 days after weaning, the Vdr null mice restored all that had been lost to achieve a BMC that was 50% higher than their prepregnant baseline but equal to the WT value. Histomorphometry during late pregnancy compared with prepregnancy showed that the pregnancy-related increase in BMC was largely attributable to mineralization of osteoid (296). The subsequent increase in BMC after weaning implies that new bone formation has occurred; however, histomorphometric studies have not yet been carried out in this time frame. Preliminary and ongoing studies in the author’s lab have shown that the post-weaning increase in BMC is no different between WT and Vdr null mice when both are switched to a 1% calcium diet after delivery, confirming that the high calcium content of the diet is not required for skeletal recovery to occur after weaning.

The same Vdr null mice have also been studied after being raised on a 2% calcium diet from 3 wk of age (489), so they did not have rickets prior to pregnancy. Micro-CT studies, biomechanical tests, and femoral ash weight were carried out during pregnancy and lactation. WT mice gained trabecular bone volume and thickness during pregnancy while the Vdr nulls did not, and this difference persisted to day 5 of lactation but was gone by day 10 (489). By the end of lactation, WT and Vdr nulls resorbed a similar amount of bone as determined by microCT and ash weight studies, lost a similar amount of femoral bone strength, and showed no significant differences between the genotypes (489).

Milk calcium content was normal in Vdr null mice compared with WT (1026). But after weaning, Vdr nulls show delayed involution and apoptosis of mammary epithelial cells (1026). This suggests that calcitriol plays a role in regulating involution after lactation.

In a preliminary report, Cyp27b1 null mice raised on a 2% calcium diet from the time of weaning had reduced BMC and microCT evidence of rickets prior to mating, gained 40% BMC during pregnancy, and lost a normal amount of BMC during lactation (330). After weaning, they regained what was lost during lactation and reached a higher BMC value than prior to pregnancy (330). Serum calcium normalized during pregnancy but fell sharply during lactation before recovering slowly to normal during post-weaning. In contrast, phosphorus was low during lactation but normal throughout post-weaning recovery.
Vitamin D-deficient mice from the CD1 background, kept on a 1.2% calcium diet, had normal serum calcium and ionized calcium but lower serum phosphorus during lactation (19). Their pups grew at the same rate as pups from vitamin D-replete mice (19). Milk from these mice showed normal nutritional and calcium content, but there was a lower (0.47%) calcium and phosphorus absorption to lactation; however, the lower (0.47%) calcium absorption during lactation or post-weaning on the 1.2–1.7% calcium diet (93). Serum calcium and phosphorus were normal, and intestinal calcium absorption was no different between vitamin D-deficient and replete rats either during lactation or post-weaning on the 1.2–1.7% calcium diet (93, 360, 362, 628). Lactation proceeds normally, with vitamin D-deficient and vitamin D-replete rats each resorbing a similar amount of calcium from the femora compared with the peak value of pregnancy (628). Histomorphometry revealed that lactating vitamin D-deficient rats have significantly widened osteoid seams as well as increased osteoblast surface, osteoclast number, and resorptive surface (146). After weaning, severely vitamin D-deficient Holtzman rats on a 0.47% calcium diet recovered lactational losses in mineralized bone volumes and cortical width, thereby achieving values that equaled or exceeded those prior to pregnancy (628). But in an earlier study from the same investigators, there was no recovery of ash weight or mineral content at 3 wk (362). The discrepancy in post-weaning skeletal recovery between these two studies is unexplained.

Intestinal absorption of calcium doubled during lactation and declined to virgin values during the recovery phase in rats consuming 0.47 to 1.7% calcium diets (93, 360). Intestinal phosphorus absorption also doubled on the 1.2–1.7% calcium diet (93). Serum calcium and phosphorus were normal, and intestinal calcium absorption was no different between vitamin D-deficient and replete rats either during lactation or post-weaning on the 1.2–1.7% calcium diet (93). On the 0.47% calcium diet, vitamin D-deficient rats had lower serum calcium and phosphorus, and achieved a doubling of intestinal calcium absorption during lactation; they were studied separately from vitamin D-replete rats and may have had a slightly lower peak efficiency of intestinal calcium absorption (360). These results indicate that calcitriol is not required for a doubling of efficiency of intestinal calcium and phosphorus absorption to occur during lactation; however, the lower (0.47%) calcium diet likely reduces passive absorption of calcium (360) compared with the 1.2–1.7% calcium diet (360). Both studies indicate that the normal rate of intestinal mineral absorption in virgin rats is sufficient to facilitate skeletal recovery during post-weaning (93, 360).

In one study, vitamin D-deficient rats produced only ~20% of the volume of milk of vitamin D-replete rats, as determined by injecting the rats with oxytocin and then manually expressing the milk. The nutritional content of the milk was normal to enhanced, since it contained more protein, calcium, phosphorus, but somewhat less carbohydrate than normal milk (119). Pups of vitamin D-deficient rats had modestly reduced weight gain, but this was corrected by giving the mothers fewer pups to nurse, confirming that the mother may be producing milk of insufficient quantity to meet the demands of larger litters (119). However, the markedly reduced volume of expressed milk was out of keeping with the modest reduction in pup weight gain, so the experimental technique may have overestimated any difference in milk volumes produced. Hypocalcemic vitamin D-deficient rats may, for example, differ from vitamin D-replete rats in their response to exogenous oxytocin, but not in their response to suckling.

Rat milk contains a very low concentration of vitamin D and 25OHD, which together total ~3–12 IU/liter (190). This is similar to the low vitamin D content of human milk.

Overall, the rodent studies indicate that disrupted vitamin D physiology is more likely to cause hypocalcemia in rats than in mice, regardless of dietary calcium content. Lactation otherwise proceeds normally despite maternal loss of calcitriol, VDR, or vitamin D, with the amount of bone resorbed being comparable to that of their respective WT or vitamin D-sufficient controls. Post-weaning, skeletal recovery occurs at a normal rate in Vdr null mice and (in a preliminary report) Cyp27b1 mice, whereas vitamin D-deficient mice have been found to have discrepant results of both normal skeletal recovery and failure to recover. In vitamin D-deficient mice and rats, and in Vdr null mice, milk content of calcium is normal, but the volume of milk produced was reduced in one study of vitamin D-deficient rats. A reduction in milk volume could explain a reduction in pup growth rate that was seen in some but not all studies.

2. Human data

Observational cohort studies (145, 731, 862, 893) and randomized interventional trials (13, 14, 60, 402, 453, 672, 780, 788, 789, 965) have not shown any effect of higher 25OHD concentrations or vitamin D supplementation to alter maternal mineral or skeletal homeostasis in otherwise healthy, lactating women across a broad range of vitamin D intakes and 25OHD levels. Vitamin D supplementation raises maternal 25OHD levels with a similar dose-response effect as in nonpregnant or nonlactating women. Many of
the randomized trials measured only the achieved 25OHD level as the outcome of interest (13, 60, 402, 780, 788, 965).

Milk normally contains little vitamin D or 25OHD, ~30–40 IU/liter in total, while calcitriol is usually very low to undetectable (400, 410, 515, 545, 587, 748, 872, 939). Consequently, maternal 25OHD should not decline significantly as a consequence of milk production, and this has been confirmed by multiple studies (156, 157, 165–167, 470, 505, 558, 764, 805, 862, 871). Moreover, this means that breast milk is normally not a good source of vitamin D for the neonate or infant. These facts are often disbelieved because marketed milk and other dairy products in the United States and Canada contain ~10 times the concentration of vitamin D, i.e., 400 IU/liter or 100 IU/standard serving (250 ml or cup). However, that is a synthetic vitamin D supplement, which is added to milk and dairy products after pasteurization. It is not put there by the cow or goat.

Although milk vitamin D and 25OHD content are low, there is a correlation between milk 25OHD and neonatal 25OHD after the first month of lactation, indicating that despite its low content of vitamin D, breast milk is an important determinant of neonatal vitamin D stores (145, 540, 780). The estimated half-life of 25OHD in infants is 2–3 wk (717), similar to values in adults (59, 62, 189, 344, 449), which means that a higher cord blood 25OHD will only influence neonatal levels over about the first 6 wk. Randomized intervention studies indicate that typical maternal vitamin D doses of 400–1,000 IU/day do not consistently increase breast milk content of vitamin D or 25OHD, whereas with maternal vitamin D doses of 2,000–4,000 IU/day, an increase in milk content of vitamin D and 25OHD and neonatal 25OHD can be demonstrated (13, 14, 402, 780). Additional randomized trials have shown that even higher maternal vitamin D intake increases the amount of vitamin D and 25OHD in milk (also called “anti-rachitic activity” of milk) (60, 672, 788, 965). A dose of 6,400 IU/day raises the milk vitamin D content sufficiently so that the babies achieved 25OHD values of 115 nM (46 ng/ml), the same level reached by babies who received 300 IU of vitamin D directly in the form of oral drops (965). A daily maternal dose of 5,000 IU/day raised maternal 25OHD to 400 nM (160 ng/ml) and neonatal 25OHD to 98 nM (39 ng/ml), while a single maternal dose of 150,000 IU had a similar effect (672).

These high levels of maternal and neonatal 25OHD have been targeted without evidence that they confer a specific benefit. It is relevant to recall that multiple stable isotope-based studies in children, adolescents, and adults have shown that on an adequate calcium diet, intestinal calcium absorption is already maximal with a 25OHD level above ~20 nM (7, 24, 26, 306, 650, 774). Moreover, intestinal calcium absorption in the breastfed neonate and infant is boosted by the lactose content of milk, which increases passive, paracellular absorption of calcium (479, 480). Therefore, a 25OHD level of 100 nM is far above the value at which intestinal calcium absorption maximizes, and is unlikely to confer any additional benefit on intestinal calcium absorption or bone metabolism in the neonate or infant.

Moreover, in areas where severe vitamin D deficiency rickets is endemic, it has been effectively treated with breast milk as the only form of nutrition in mothers who received 150,000 IU vitamin D every 3 mo, or ~1,800 IU/day (902, 903). These babies and their mothers were protected from sun exposure and had negligible vitamin D in the diet, so a maternal supplement was the main source. Starting 25OHD values were 6 nM (2 ng/ml) or lower in mothers and babies, consistent with extreme vitamin D deficiency. Achieved 25OHD values were ~50 nM (20 ng/ml) in the mothers and 40 nM (16 ng/ml) in their babies, both of which are above the 20 nM threshold that appears to maximize intestinal calcium absorption. The far higher breast milk vitamin D content and infant 25OHD levels achieved by giving a 5,000 or 6,400 IU supplement daily to the mother greatly exceed what has been shown to treat rickets, the severe manifestation of vitamin D deficiency. Therefore, such high doses may be superfluous if no additional benefit is provided to mineral or skeletal metabolism. The question remains as to whether such high vitamin D intakes or achieved 25OHD levels in breastfed babies confer any non-skeletal benefits (773).

Breast milk calcium content is unaffected by maternal 25OHD over a wide range from 25 nM (10 ng/ml) to 160 nM (64 ng/ml) (60, 731). This includes cohort studies that have examined low maternal vitamin D intakes and 25OHD concentrations (731), and randomized interventions which found that maternal intake of 2,000–6,400 IU vitamin D/day failed to change the calcium content of milk compared with an intake of 400 IU/day (60, 402, 965), even though the 6,400 IU dose raised maternal 25OHD to 160 nM (64 ng/ml) (965). On the other hand, several individual cases from India revealed that milk calcium content was lower in mothers presenting with overt vitamin D deficiency with mean 25OHD values of 6 nM (2.5 ng/ml), hypocalcemia, hypophosphatemia, and markedly elevated PTH (903). These results confirm that calcitriol likely does not play a direct role in causing calcium to enter milk, but extremes of vitamin D deficiency (25OHD closer to 6 nM) will impair milk production. As noted earlier, calcium intake does not alter breast milk calcium content (206, 452, 484, 723). Instead, the calcium content of milk is largely regulated by the calcium receptor and PTHR, with the supply being the maternal skeleton and a lesser contribution from diet. In extreme vitamin D deficiency, there is skeletal resistance to the resorptive actions of PTH (550, 577, 624,
Vitamin D deficiency and insufficiency also have not been explicitly examined in any of the cohort studies or large epidemiological studies that looked at the effect of prior lactation history on BMD or fracture risk. However, since those studies largely found a neutral or even a protective effect of lactation on long-term BMD and fracture risk, and the majority of women in those studies are considered to be vitamin D insufficient or deficient by some modern experts, it seems probable that skeletal recovery after weaning is not impaired by vitamin D insufficiency either. Direct studies of the effect of vitamin D supplementation during post-weaning recovery are needed. In the absence of such data, it is prudent to recommend the same intake of vitamin D as in nonpregnant women.

Hereditary absence of Cyp24a1 reduces calcitriol catabolism and has been shown to lead to marked maternal hypercalcemia during pregnancy accompanied by high calcitriol. While breastfeeding, hypercalcemia was milder and serum calcitriol was normal (820). This is consistent with Cyp27b1 stimulation being reduced to nonpregnant levels during lactation.

3. Summary

The animal data indicate that resorption of bone and milk calcium content may be unaffected by extremes of vitamin D physiology, including absence of VDR, calcitriol, and vitamin D. Post-weaning recovery of bone mass also occurs despite absence of VDR, calcitriol, and vitamin D, although data from vitamin D-deficient rats are inconsistent.

Clinical data are largely similar, suggesting that maternal vitamin D stores are not adversely impacted by lactation, that milk calcium content is unaffected by extremes of severe vitamin D deficiency and excess, and that lactational bone loss is likely also unaffected. Given the prevalence of insufficient to low levels of 25OHD in the normal population, the epidemiological studies also suggest that post-weaning recovery of bone mass is also likely not impaired. Overall, there is no clear evidence that the requirement for vitamin D increases during lactation to meet maternal needs. Since milk normally contains little vitamin D or 25OHD, a theoretical benefit of high-dose vitamin D supplementation is that all of a baby’s nutrition could then come from breast milk, rather than requiring that breast-fed babies alone be stigmatized to receive vitamin D supplements. Further study is needed of the effectiveness, safety, and adherence to this route of delivery compared with giving vitamin D supplements directly to the baby. The high doses used in some of these studies are not needed if the neonatal goal is a 25OHD level of only 50 nM, as the Institute of Medicine and pediatric societies have suggested (430).

H. Calcitonin Deficiency

1. Animal data

As noted earlier (sect. IIIH), the theory that calcitonin protects against excessive resorption of the skeleton during pregnancy was originally tested by removing the thyroid glands of goats and rats, autotransplanting the parathyroids, and providing thyroid hormone replacement. The same approach was used for lactation.

In two studies such thyroidectomized rats had ~10% lower femoral ash weight, calcium, and phosphorus content than sham-operated rats after 3 wk of lactation (551, 900). Although there was no difference in basal serum calcium between groups during lactation (900), there was a greater surge in serum calcium after gavage in thyroidectomized compared with sham-operated rats (927), suggesting that calcitonin modulates the postprandial calcemic response during lactation. Intestinal calcium absorption was also reduced 20% in thyroidectomized rats (551). These were small studies and described only at abstract length, with few methodological details and some relevant data not disclosed (551, 900). Whether the surgically altered animals were euparathyroid and euthyroid was not determined, and that may have confounded the results.

These results were refuted by a later study that used a larger sample size of rats, and confirmed that serum thyroxin and calcium were no different between thyroidectomized and sham-operated rats during lactation. Both groups resorbed bone, but there was no difference between them in femoral ash weight or calcium content at the end of lactation (392).

Similar experiments were carried out in lactating, thyroidectomized, thyroxin-replaced goats but without confirmation of thyroid or parathyroid status. The thyroidectomized goats lost 9% more ash weight and calcium from metatarsals and metacarpals compared with sham-operated animals (55). The sample size was quite small, consisting of only five thyroidectomized and four intact goats.

None of these studies in rats or goats examined the trabecular bone of the spine, which is the site that loses the most
mineral during lactation. Moreover, extrathyroidal sources of calcitonin synthesis were not appreciated when these models were created; lactating mammary tissue is a potent source of calcitonin, and there is additional expression in the pituitary.

The postulated physiological role of calcitonin to protect the maternal skeleton during lactation was reexamined in Ctcgrp null mice, which lack calcitonin and calcitonin gene-related peptide-α, but retain calcitonin gene-related peptide-B (394). This global calcitonin deficiency resulted in lactating Ctcgrp null mice losing double the BMC as their WT sisters from whole body, lumbar spine, and hindlimb, with the losses reaching 55% of lumbar spine BMC (994). Treatment with calcitonin through 3 wk of lactation prevented the excess bone loss, whereas CGRP-α had no effect, confirming that loss of calcitonin led to the skeletal phenotype. Increased expression of PTHrP in mammary tissue and increased circulating PTH likely combined to cause increased bone resorption and exaggerated net bone loss in Ctcgrp null mice (992, 994). Histomorphometric studies showed that Ctcgrp nulls had a doubling of osteoclast number and resorptive surfaces, and a halving of osteoblast number and osteoid surface (194). Intestinal calcium absorption was no different between lactating Ctcgrp nulls and WT (994). A doubling of milk calcium content was found that was nonsignificant in early lactation (994) but statistically significant at mid-lactation (992). It remains undetermined whether the primary response to loss of calcitonin is increased bone resorption that leads to increased flux of calcium into mammary tissue and milk, or loss of calcitonin in mammary tissue that increases calcium transport into milk and, thereby, a compensatory increase in PTHrP and PTH. It is also possible that both pathways are involved, and that loss of calcitonin signaling in the pituitary contributes to increased prolactin, which in turn stimulates PTHrP and inhibits ovarian function. Lactating Ctcgrp null mice did not have higher prolactin levels compared with WT (994), while serum estradiol was at the detection limit of the assay in both genotypes at all time points (992). The postulated role of calcitonin to inhibit prolactin is not supported by Ctcgrp null mice.

Remarkably, the 55% loss of BMC during lactation was completely recovered within 18 days of weaning (994). This was accompanied by histomorphometric evidence of a marked reduction in osteoclasts, and a surge in osteoblast numbers and surface to the same values of their WT sisters during the post-weaning recovery phase (194). Marked downregulation of Sost and Dkk1 expression occurred within bone but was reduced even further in Ctcgrp null mice, which likely contributed to the upregulation in osteoblast number and function (194).

The Ctcgrp null model confirms that physiological levels of calcitonin protect the rodent skeleton from excessive resorption during lactation, but it remains to be determined how much of calcitonin’s actions are at the level of osteoclasts, mammary epithelial cells, and pituitary lactotrophs (994). Moreover, the model is confounded by loss of CGRP-α, which may contribute to the phenotype of altered mineral and skeletal homeostasis. A mouse model in which CGRP-α only was deleted, apparently leaving calcitonin expression intact, resulted in osteopenia and reduced bone formation (801). These mice have not been studied during lactation. A mouse model with selective deletion of calcitonin has not been created. It would be especially informative to selectively delete calcitonin from mammary epithelial cells at the onset of lactation.

Calcitonin acts on the calcitonin receptor, which is expressed by osteoclasts (660), lactating mammary epithelial cells (432, 938), and pituitary (831), as well as numerous other tissues during embryonic and fetal development (437). Pharmacological doses of CGRP and amylin can activate the CTR, but at physiological doses they act on different receptors (787). It is not possible to study the effect of global loss of the calcitonin receptor during lactation because Ctr null mice usually die at the embryonic stage (220, 529), a lethality that is not seen in Ctcgrp null mice. WT and Ctr+/− mice were no different in their responses to lactation (580).

More recently a conditional knockout using a CMV promoter has enabled deletion of Ctr at a later stage in development (223). This results in >90% but not 100% loss of CTR expression in kidneys and osteoclasts; expression in other tissues relevant to lactation, especially mammary tissue and pituitary, were not assessed. During lactation these conditionally deleted Ctr null mice show a greater increase in osteocyte lacunar size compared with WT, suggesting that loss of calcitonin signaling leads to increased osteocytic osteolysis (187). However, there were no significant differences in bone structure by microCT or histomorphometry between Ctr nulls and WT (187). Moreover, prolactin was significantly reduced, which is the opposite of what was expected given that targeted overexpression of calcitonin in the pituitary, and pharmacological treatment with calcitonin, both reduce prolactin. A lower serum prolactin also indicates relative inhibition of lactation in these conditionally deleted Ctr nulls and, therefore, the potential for reduced bone loss as a consequence, but milk calcium content was normal and no significant disturbance in pup growth was noted.

Overall, the conditional knockout of Ctr incompletely resembles the lactational phenotype of Ctcgrp null mice. It remains to be determined if these differences are due to insufficient deletion of CTRs from relevant tissues during lactation; loss of actions of other ligands that may be mediated by CTR; physiological effects of calcitonin that are mediated by other receptors; or that loss of two ligands in
Ctcgrp nulls leads to effects that loss of CTR cannot reproduce. Since the breast appears to be a key controller of the skeletal response to lactation, as explained in section IVF, if CTR is not ablated in mammary tissue or if calcitonin’s actions within mammary tissue are not mediated by CTR, then that could explain the more modest phenotype of the conditional Ctr knockout during lactation.

There is some consistency to the evidence that calcitonin inhibits bone resorption during lactation. Global loss of calcitonin and CGRP-α leads to more marked bone loss than the surgical models, which may be explainable by retention of extrathyroidal (especially mammary) sources of calcitonin in the surgical models. Conditional but incomplete deletion of CTR leads to increased osteocytic osteolysis but does not fully reproduce the phenotype of the Ctcgrp null.

2. Human data

No clinical studies have specifically tested whether calcitonin deficiency leads to increased bone resorption during lactation. No individuals with inactivating mutations of calcitonin or its receptor have been identified. However, it is intriguing to speculate that such women may be among the extreme cases of more marked bone loss or multiple vertebral compression fractures that have been seen during lactation (501). In one report a thyroidectomized woman nursing twins experienced multiple vertebral compression fractures and had marked bone loss confirmed by DXA; the authors speculated that calcitonin deficiency was to blame (811). But thyroidectomized women are not expected to be calcitonin deficient while breastfeeding because calcitonin produced by breast tissue leads to normal circulating calcitonin levels (52, 131, 755).

As noted earlier, long-term followup of thyroidectomized men and women has yielded conflicting results as to whether or not there is increased risk of bone loss and fractures (326, 338, 422, 630, 659). More recent studies are confounded by the use of high doses of thyroid hormone to suppress TSH and reduce the risk of recurrence of thyroid cancer.

3. Summary

Surgical models in rats and goats have provided evidence that loss of thyroidal calcitonin leads to increased bone loss during lactation compared with sham-operated animals; however, these effects were not confirmed by a larger study. More recent use of gene ablation techniques has shown that global loss of calcitonin and CGRP-α leads to a doubling of bone loss during lactation but full recovery afterward. This confirms that calcitonin is needed in the short term to prevent excessive skeletal resorption, but not in the long term since the skeleton recovers fully from these extreme losses of bone. Conditional deletion of CTR did not fully reproduce the effects of loss of calcitonin and CGRP-α. No human studies have tested whether calcitonin deficiency causes excess bone loss during lactation, but it may contribute to some of the extreme cases of lactational bone loss and fractures that have been reported in the literature.

I. Low or High Calcium Intake

1. Animal data

The preceding sections have made clear that lactating rodents require abundant calcium from multiple sources, mediated by increases in intestinal calcium absorption, skeletal resorption, and renal tubal calcium reabsorption. But within some limits, the rodent is capable of extracting more from the skeleton when the diet is deficient, or more from the diet when the skeleton cannot be resorbed. Consequently, a calcium-restricted diet increases skeletal losses and also can lead to hypocalcemia and sudden death from tetany; conversely, a high-calcium diet reduces skeletal losses. Similarly in ewes, a calcium-restricted diet causes a lower serum calcium, increased resorption, and reduced skeletal ash weight within the vertebrae and pelvis by the end of lactation, with relative sparing of the long bones (69). A high-calcium diet reduces the amount of skeletal resorption. In these studies there was no impact on the offspring unless the mother died of hypocalcemia, because the milk calcium content was maintained. Similarly, blocking bone resorption by treating with OPG did not affect milk calcium content unless the animal was also given a low calcium diet, thereby blocking the delivery of needed calcium to mammary tissue (37). These results confirm that rodents and sheep rely on both compartments (skeleton and intestines) to get needed calcium, but can get by with one of these compartments alone. However, if mobilization of calcium from both compartments is blocked, then milk cannot be produced adequately and severe maternal hypocalcemia is inevitable.

Low calcium intake in the post-weaning phase has been shown to impair skeletal recovery in rats, but full recovery was achieved when a normal calcium diet was administered later (355).

2. Human data

The calcium content of milk appears to be largely derived from skeletal resorption. Low calcium intake does not reduce breast milk calcium, nor does it lead to increased resorption of the maternal skeleton during lactation (440, 535, 728–731). Conversely, high calcium intake similarly fails to increase breast milk calcium or reduce skeletal resorption during lactation (206, 275, 440, 452, 484, 535, 723, 729, 730); its only effect may be to increase urinary
calcium excretion. These data from randomized clinical trials and cohort studies indicate that it does not matter how much calcium is consumed during lactation because maternal skeletal resorption is hormonally programmed to supply needed calcium. There is no evidence that women require a higher intake of calcium during lactation compared with nonpregnant women.

No studies have examined calcium requirements during post-weaning, which may be the more critical time for adequate calcium intake. However, a study of adolescents recovering from lactation found that there was a substantial increment in bone mass despite a habitual intake of <500 mg calcium/day (78). Randomization to a 1 g calcium supplement conferred a small benefit in bone mass gain during 6 mo of recovery (452), which confirms that calcium intake can have a positive impact on bone mass accrual during this interval.

3. Summary

Whereas rodents will increase or decrease skeletal resorption during lactation in inverse proportion to calcium intake, in breastfeeding women the magnitude of skeletal resorption appears to be independent of the extremes of low and high calcium intake. Consequently, it is quite evident that women do not require increased intakes of calcium during lactation because there is no benefit on the skeletal response or on the calcium content of milk. Maintaining adequate calcium intake is probably more crucial during the post-weaning phase when the skeleton is reversing the uncoupled state of bone turnover from net resorption to net formation.

J. FGF23-Related Disorders

1. Animal data

Phex+/− females remain hypophosphatemic and normocalcemic during lactation, nurse the same number of pups as normal controls, and their pups gain weight normally (240). Analysis of expressed milk from Phex+/− females has shown normal phosphorus, calcium, and protein content (240).

Hyperphosphatemic disorders due to absence of FGF23 have not been studied during lactation because of the inability to obtain pregnancies in Fgf23 null or Klotho null mice. The effects of experimental renal failure causing maternal hyperphosphatemia have not been studied during lactation.

2. Human data

Serum phosphorus was low during pregnancy and immediately postpartum in a woman with XLH, but normalized once lactation was fully established (447). This supports the idea that skeletal resorption is responsible for the increase in serum phosphorus that normally occurs during lactation. However, despite normalization of serum phosphorus, the phosphorus content in expressed milk was 50% of normal in two cases, whereas the calcium content was modestly reduced in one but normal in the other (447, 745). Oral phosphorus supplementation normalized the milk composition (447). In both cases, the babies inherited XLH and subsequently became hypophosphatemic with skeletal evidence of rickets, which was likely due to the combined effects of the mutation and the low phosphorus content of milk (447, 745). This contrasts with apparently normal milk phosphorus content in the mouse model.

There are no data from lactating women with hyperphosphatemic disorders, but it is conceivable that phosphorus content of milk will be elevated.

3. Summary

Although hypophosphatemic mice produce milk with normal mineral composition, XLH in women reduces the phosphorus content of milk by 50%, and inconsistently reduces the calcium content. These changes will contribute to the development of neonatal rickets. However, the phosphorus content of milk can be normalized with oral phosphorus supplementation.

VI. CONCLUSIONS

Pregnancy and lactation invoke novel regulatory systems in women to meet the challenges for increased delivery of minerals (FIGURE 9).

A doubling of intestinal calcium and phosphorus absorption during pregnancy meets the fetal mineral demand. This adaptation may be only partially explained by a severalfold increase in calcitriol and may persist despite vitamin D deficiency and inherited disorders of vitamin D physiology. Minimal skeletal calcium is normally resorbed during pregnancy, but this will increase significantly and lead to skeletal fragility if maternal calcium intake is very low, because the placenta acts to maintain calcium delivery to the fetus even if the mother is hypocalcemic. The maternal kidneys, not the placenta, supply most of the increased calcitriol output during pregnancy, but do not seem to aid calcium conservation because absorptive hypercalciuria is commonly found. Spot and fasting urines will not detect this hypercalciuria.

A marked uncoupling of bone turnover to favor skeletal resorption is the main mechanism by which calcium is supplied to the breast milk, aided by renal calcium conservation and (in women) independent of dietary calcium intake. The lactational response is driven by breast-produced PTHrP in the setting of low estradiol, while PTH, vitamin D, and
calcitriol are not required. Osteoclast-mediated bone resorption and (at least in rodents) osteocytic osteolysis contribute to the loss of skeletal mineral content. A similar magnitude of bone loss occurs during lactation in women with very low or high calcium intakes. In some women the normal skeletal resorption during lactation may be sufficient to cause fragility fractures, whereas for the majority of women it is a silent process that they are never aware of. There is normally very little vitamin D or 25OHD in milk, which explains why breast-fed babies are at higher risk for rickets than formula-fed babies. Very high intakes of vitamin D will increase the breast milk content of vitamin D, but will not alter breast milk calcium content.

After weaning the baby, an uncoupling of bone turnover in the reverse direction from lactation is initiated, thereby favoring net bone formation. Osteoblasts upregulate number and function while (at least in rodents) osteocytes function like osteoblasts to remineralize their lacunae. The maternal skeleton rapidly restores itself such that by 6–12 mo after lactation, the bone density has usually returned to baseline or better, even in women who suffered fragility fractures. This is an otherwise unprecedented interval of recovery since most causes of bone loss in adults, once the insult is removed, result in slow and partial recovery of bone mass and structure. It appears that weaning triggers a programmed, sustained interval of bone formation, but the mechanisms that regulate this recovery remain to be elucidated.

In the long term, most studies show that parity and lactation pose no adverse risk of low bone mass or fractures, and in fact a number of studies have suggested that parity and lactation confer a protective effect. These results may imply that the cyclical resorption and rebuilding of the maternal skeleton leads to beneficial changes in bone structure and strength that are not seen in women who never bear a child or breastfeed.

There are important differences among the various animal models and in how they compare to and inform about the human condition (TABLES 1 and 2). These issues must be considered whenever animal studies are used to model the response of women to pregnancy or lactation.

Lastly, these adaptations during pregnancy and lactation have important effects on preexisting disorders of bone and mineral metabolism. The presenting symptoms, signs, diagnostic indices, and treatment thresholds may be altered. This is best exemplified by the normalization in mineral homeostasis that occurs in hypoparathyroid women during lactation, but which has led to iatrogenic and life-threatening hypercalcemia when unappreciated and left unmonitored.

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FIGURE 9. Comparison of the adaptive processes of calcium and bone homeostasis during human pregnancy and lactation, compared with normal (nonpregnant, nonlactating). The thickness of arrows indicates a relative increase or decrease with respect to normal. [Adapted from Kovacs and Kronenberg (499). Copyright 1997 The Endocrine Society. Permission conveyed through Copyright Clearance Center, Inc.]
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