Editorial

Calcium metabolism in pregnancy: Disturbed calcium homeostasis in diabetic pregnancy and preeclampsia

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Summary

1α-dihydroxyvitamin D_3 is a main regulator of calcium metabolism during pregnancy because this metabolite promotes the intestinal calcium absorption to meet the increased calcium demands for fetal mineralization. The mechanisms underlying the increase in 1α-dihydroxyvitamin D_3 may be the stimulation of renal synthesis of this metabolite possibly by human placental lactogen and placental synthesis. Trabecular bone mineral density may be preserved during pregnancy, but it is reduced during lactation due to hypoestrogenic state. Diabetic pregnancy is associated with altered calcium metabolism including the decrease in serum levels of calcium and 1α-dihydroxyvitamin D_3, the impaired duodenal calcium absorption, the increase in serum PTH and hypercalciuria compared with normal pregnant women. The disturbance of transplacental calcium transfer may be a cause of neonatal hypocalcemia. Abnormal intracellular calcium handling in preeclampsia has been discussed in association with the etiology of essential hypertension. The increase in the intracellular calcium concentrations in vascular smooth muscle cells probably caused by parathyroid hormone may contribute to enhanced vascular reactivity.

Key words: Calcium metabolism; Bone metabolism; Pregnancy; Lactation; Diabetes mellitus; Preeclampsia.

Maternal calcium homeostasis is challenged during pregnancy and lactation because calcium demands are increased for the fetal mineralization and milk production. A fetus accumulates as much as 28 g of calcium in the skeleton mainly in late pregnancy, and lactating women lose about 210 mg/day of calcium in breast milk. These increased calcium demands induce the adaptive alterations of maternal calcium and bone metabolism in these periods.

With regard to calcium metabolism in a complicated pregnancy, the infants born to insulin-dependent diabetic mothers have a higher incidence of hypocalcemia, and epidemiologic studies have indicated that altered calcium metabolism plays a primary role in the etiology of preeclampsia. This article summarizes calcium and bone metabolism during normal pregnancy and altered calcium homeostasis in diabetic pregnancy and preeclampsia.

In normal pregnancy, maternal serum total calcium concentrations decrease progressively in parallel with the fall in serum albumin levels during pregnancy [1-4], whereas no significant changes are noted in serum ionized calcium concentrations [1-7]. The urinary excretion of calcium rises during pregnancy [3, 8-11] due to an increased glomerular filtration rate, decreased tubular reabsorption of calcium, and a dissociation between the sodium and calcium handling in Henle's loop [3, 12].

Several studies have reported that serum concentrations of parathyroid hormone (PTH) are increased [13-17], unchanged [2, 4, 8, 10, 18-20] or decreased [5, 21, 22] during pregnancy. However, the descriptions that serum concentrations of intact PTH reflecting a biologically active form of PTH are reduced [5, 21, 22] during pregnancy suggest that PTH may not play a major role in the compensatory mechanisms of calcium loss.

The elevation of serum 1α,25-dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$), a biologically active metabolite of vitamin D_3, has been documented consistently [2, 4, 10, 11, 23-29]. The mechanisms responsible for the

increase in $1\alpha,25(OH)_2D_3$ may be due to the concomitant increase in serum vitamin D binding protein [11, 25, 28]. However, Wilson *et al.* [29] reported that the free $1\alpha,25(OH)_2D_3$ index (the molar ratio of total $1\alpha,25(OH)_2D_3$ and vitamin D binding protein) and free $1\alpha,25(OH)_2D_3$ concentrations were significantly elevated during late pregnancy compared with controls. The cause of the elevation of free $1\alpha,25(OH)_2D_3$ levels may be ascribed to the promoted production of this metabolite in the kidney and the placenta [30-34]. Placental lactogen and prolactin are postulated to stimulate renal 1α-hydroxylase activity during pregnancy, but the factors that promote this enzyme activity in the placenta remain unknown.

$1\alpha,25(OH)_2D_3$ enhances the active intestinal absorption of calcium by binding with a specific steroid hormone receptor on the intestinal mucosa cells, thereby inducing the transcription and translation of calcium-binding protein 9k ($CaBP_{9k}$, calbindin-D_{9k}) which facilitates calcium diffusion [35].

In human pregnancy, intestinal calcium absorption significantly increases by the second trimester [10], but not during lactation [36, 37] due to the inconsistent elevation of serum $1\alpha,25(OH)_2D_3$ [37]. However, intestinal calcium absorption is increased after weaning or the resumption of menses [38].

In rat pregnancy, active duodenal calcium absorption is increased by midpregnancy until term [39-43], and remains enhanced at lactation [39, 42-44]. The vitamin D-dependent intestinal calcium adenosine triphosphatase (CaATPase) and CaBP mRNA increase 2- to 3-fold at 21 days of gestation and remain elevated at seven days of lactation with a similar pattern of plasma $1\alpha,25(OH)_2D_3$ concentrations [45]. In addition, the intestinal vitamin D receptor content increases 2-fold in late pregnancy and lactation [45]. These data suggest that the active intestinal calcium transport systems are activated during late pregnancy when the fetal skeleton accumulates calcium.

On the other hand, vitamin D-independent factors may be involved in intestinal calcium transport systems in view of the enhanced intestinal calcium absorption observed in vitamin D-deficient lactating rats [44] and the enhanced nonsaturable calcium absorption in the jejunum during lactation [46].

Placental lactogen and prolactin have been assumed to be the vitamin D-independent factors which promote intestinal calcium absorption because serum placental lactogen elevates at term and serum prolactin begins to rise just after parturition in rat pregnancy [47]. Placental lactogen and prolactin significantly increase duodenal calcium absorption in hypophysectomized rats [48]. In addition, human placental lactogen significantly increases serum $1\alpha,25(OH)_2D_3$ in hypophysectomized rats [40], whereas prolactin does not [40, 49]. Thus, placental lactogen may enhance duodenal calcium absorption possibly by stimulating renal synthesis of $1\alpha,25(OH)_2D_3$.

On the other hand, prolactin may enhance intestinal calcium absorption via the vitamin D-independent mechanism because Pahuja and DeLuca [50] reported that intestinal calcium transport increased after an injection of prolactin in vitamin D-deficient rats. Krishnamra *et al.* [51] have reported that prolactin may alter duodenal epithelial handling of calcium by enhancing brush-border uptake of calcium while stimulating $1\alpha,25(OH)_2D_3$. In contrast, Boass *et al.* [43] failed to demonstrate increased intestinal calcium transport after injection of rat prolactin in late pregnancy or reduced intestinal calcium transport by injection of bromocriptine before parturition. Thus, the role of prolactin in intestinal calcium absorption still remains controversial.

Serum concentrations of calcitonin have been reported to increase during human pregnancy [15, 24, 26], but not in other studies [2, 4, 8, 11]. The precise role of calcitonin during pregnancy remains unknown, although the rise in calcitonin may protect the maternal skeleton against the bone-resorptive activities of $1\alpha,25(OH)_2D_3$ [26].

Studies on bone metabolism in human pregnancy are scarce. Previous investigations have demonstrated no substantial loss of cortical bone mineral density during pregnancy, including the metacarpal bone [52], the radius [53], and the lumbar spine [11, 20], excluding a report showing a reduction in trabecular bone mineral density [54]. A recent prospective study has demonstrated no significant femoral bone mineral density loss with pregnancy [55].

In contrast, reduced trabecular bone mineral density accompanies lactating women, including the ultradistal radius [56], the lumbar spine [11, 57-61] and the femoral neck [58, 60]. No significant changes have been noted in the bone mineral content of the distal radius [56], except for one report by Chan *et al.* [62] showing a significant decrease in the bone mineral content of the radius in lactating adolescents with low calcium intake.

Kalkwarf *et al.* [59] reported that lactating women lost bone in the total body (-2.8%) and lumbar spine (-3.9%) during the first six months postpartum. However, bone mineral density of the lumbar spine [11, 59] and forearm trabecular bone [56] increased after weaning. Calcium supplementation has no protective effects on bone mineral density of the lumbar spine during lactation [57].

Bone turnover is increased during lactation and lowered postweaning because markers for bone formation (procollagen I carboxypeptides and osteocalcin) and a marker for bone resorption (tartrate resistant alkaline phosphatase) are higher during lactation compared with postweaning [61]. Bone mineral loss associated with lactation may be due to the hypoestrogenic state [57, 59], and the gain postweaning may be attributable to the return of normal serum estrogen, menstruation, and an increase in PTH [61].

These collective data suggest that the transplacental transport of calcium required for fetal bone mineralization appears to be met by the enhancement of active intestinal calcium absorption during pregnancy, resulting in the preservation of maternal bone mineral density. In contrast, intestinal calcium absorption does not increase during lactation, thus lactating women meet calcium demands for milk production by increasing mobilization of calcium from bone. However, the recovery of bone mineral density is presumably ensured postweaning.

The infants of diabetic mothers have an increased incidence of neonatal hypocalcemia, hypomagnesemia, and hypercalcitonemia. The cause of neonatal hypocalcemia may include abnormalities in maternal calcium metabolism, abnormalities in fetal calcitrophic hormones, disturbance of placental calcium transport, and fetal delayed bone maturation.

Calcium metabolism in diabetic pregnant women is characterized by lower concentrations of serum total calcium and $1\alpha,25(OH)_2D_3$, elevated serum PTH levels, and increased urinary excretion of calcium compared with normal pregnant women [63]. Similarly, streptozotocin-induced diabetic pregnant rats are associated with a marked reduction of serum total $1\alpha,25(OH)_2D_3$ concentrations, decreased active duodenal calcium absorption, and hypocalcemia of the fetuses [64, 65].

However, insulin therapy recovers serum levels of ionized calcium [65] and $1\alpha,25(OH)_2D_3$ [65-67] and intestinal calcium absorption [65] in untreated diabetic pregnant rats. In addition, diabetic rats exhibit hypercalciuria [68-72], which is ascribed to osmotic diuresis caused by glycosuria, a streptozotocin-induced renal tubular defect in calcium transport [67], and a reduction in calcium absorption in the loop of Henle [73]. However, insulin therapy can correct increased urinary loss of calcium and phosphorus in diabetic rats [68].

Decreased serum total $1\alpha,25(OH)_2D_3$ concentrations have been reported in non-pregnant and pregnant diabetic rats [65, 66, 68, 69, 71, 74, 75]. Diabetes may impair renal $1\alpha,25(OH)_2D_3$ production due to the deficiency of insulin necessary for the PTH-stimulated cyclic AMP-dependent mechanism [76]. In diabetic pregnant rats, the additional cause of reduced serum total $1\alpha,25(OH)_2D_3$ levels may be the disturbed placental synthesis of $1\alpha,25(OH)_2D_3$ due to the functional immaturity of the placenta, and the reduced serum vitamin D binding protein levels [68, 69, 71] caused by impaired liver synthesis [77]. However, the free $1\alpha,25(OH)_2D_3$ index is not significantly different from that of non-diabetic rats [68, 69, 74].

Nevertheless, the active intestinal calcium transport system is severely impaired in diabetic pregnant rats [65, 71, 77]. Duodenal hyperplasia has been identified in diabetic pregnant rats [64], suggesting enhanced passive calcium absorption to compensate for decreased active intestinal calcium absorption and hypercalciuria. In non-pregnant diabetic rats, intestinal $CaBP_{9k}$ decreases [71, 77]. Intestinal $CaBP_{9k}$ mRNA increases during pregnancy in rats, whereas diabetic pregnant rats have lower duodenal $CaBP_{9k}$ than controls [78]. These data suggest the possibility of disturbed intestinal calcium transfer in diabetic pregnant rats. Thus, impairment of the duodenal calcium absorption places diabetic pregnant rats in a state of negative calcium balance, leading to insufficient transplacental calcium transfer to the fetuses.

Fetal wet weight from untreated diabetic pregnant rats is significantly lower than that from control and insulin-treated groups [65]. In addition, fetal serum ionized calcium levels have a significantly negative correlation with serum glucose levels in diabetic pregnant rats when maternal glucose levels are higher than 220 to 240 mg/dl [79].

Transplacental calcium transport at term is by an active mechanism because of the higher serum ionized calcium levels of the fetuses compared with those of maternal levels [65].

Rat placenta contains a CaBP identical to intestinal $CaBP_{9k}$ [80]. Placental CaBP is localized in the

columnar epithelial cells of the intraplacental yolk sac [81]. Mathieu *et al.* [81] described that placental CaBP plays a role in maternal-fetal calcium transport because the induction of CaBP in rat placentas coincided with the time of exponential fetal bone growth and maximal fetal accumulation of calcium. The presence of $1\alpha,25(OH)_2D_3$ receptors in the placenta [82, 83] suggests that $1\alpha,25(OH)_2D_3$ may be a main regulator in the synthesis of the placental CaBP. The rat placental CaBP mRNA that increases between 15 and 22 days of gestation appears to facilitate the diffusion of calcium through the cytosol of calcium transporting epithelium [84]. However, decreased placental CaBP has been reported in diabetic rats [75, 78, 85, 86]. Husain *et al.* [85] reported that placental $CaBP_{9k}$ mRNA was 11- to 12-fold lower in untreated diabetic rats compared with control and insulin-treated diabetic rats. They also demonstrated that fetal calcium content was significantly lower in the untreated diabetic group compared with control and insulin-treated diabetic rats, suggesting that the decreased calcium content of the diabetic rat fetus could be a result of either a decreased unidirectional maternofetal flux or an increased unidirectional fetomaternal flux. Thus, decreased placental CaBP concentrations, impaired transplacental calcium flux, and reduced uteroplacental blood flow [87] may be involved in fetal hypocalcemia, hypotrophy, and fetal bone demineralization.

In summary, insulin therapy may stimulate the production of placental CaBP by recovering the synthesis of $1\alpha,25(OH)_2D_3$ in diabetic pregnant rats, the effects of which could increase fetal wet weight and calcium content through the promotion of the transplacental calcium transfer.

Epidemiologic studies have suggested an inverse relationship between dietary calcium intake and the incidence of preeclampsia [88]. Low calcium diet is also associated with high blood pressure in pregnant rats [89] and ewes [90]. Pathological alterations in calcium metabolism have been reported in essential hypertension [91, 92].

We observed that pregnant women with severe pregnancy-induced hypertension are characterized by low concentrations of serum ionized calcium and total $1\alpha,25(OH)_2D_3$, elevated serum PTH levels and hypocalciuria compared with normotensive pregnant women [4, 93, 94]. Furthermore, we found a higher incidence of osteopenia in severe pregnancy-induced hypertension [95, 96]. These findings on calcium metabolism in severe pregnancy-induced hypertension are in accord with those reported by several authors [97-100]. Furthermore, Lalau *et al.* [99] reported that patients with pregnancy-induced hypertension had a lower free $1\alpha,25(OH)_2D_3$ index despite higher serum PTH levels and comparable vitamin D-binding protein levels compared with normotensive pregnant women.

The cause of reduced serum $1\alpha,25(OH)_2D_3$ in severe pregnancy-induced hypertension may be due to diminished renal and/or placental synthesis of this metabolite. A possible hypothesis in the pathogenesis of severe pregnancy-induced hypertension [4, 93, 94] is that the suboptimal intestinal calcium absorption due to reduced $1\alpha,25(OH)_2D_3$ may cause maternal hypocalcemia, the subsequent elevation of serum PTH and hypocalciuria. However, this hypothesis is currently being challenged because conflicting data on calcium metabolism in preeclampsia have been reported [8, 101-104].

Nonetheless, hypocalciuria in preeclampsia has been observed [8, 12, 94, 98, 100, 102, 105-112], excluding one study [3]. A decreased percent of tubular reabsorption of phosphate has also been noted in preeclampsia [109]. Hypocalciuria may be caused by increased distal tubular reabsorption of calcium [105], a decreased glomerular filtration rate [8], and decreased intestinal calcium absorption. However, a recent study has reported that measuring urinary calcium may not be useful in predicting preeclampsia in normal pregnant women and gestational hypertensives [111].

The divergent results concerning calcium metabolism in preeclampsia make it difficult to unify the mechanisms responsible for the development of preeclampsia. However, recent studies of the intracellular calcium handling in preeclampsia have pointed out characteristic findings, including increased intracellular calcium concentrations in erythrocytes [112, 113], platelets [114, 115] and lymphocytes [116]; increased sensitivity of platelet intracellular calcium to arginine vasopressin [117] and angiotensin II [114, 118] in pregnant women who subsequently become hypertensive; increased erythrocyte membrane calcium content [119, 120]; and decreased CaATPase activity of erythrocytes [112, 121, 122].

Intracellular ionized calcium is a determinant of vascular tone, and an increase in intracellular ionized calcium is related to the enhanced vascular reactivity observed in essential hypertension and preeclampsia. Nardulli *et al.* [121] have suggested that the diminished calcium pump of erythrocytes by the decreased activity of CaATPase leads to increased cytoplasmic free calcium concentrations.

Three authors [113, 116, 121] have suggested a potential role for PTH in the increased cellular uptake of calcium in vascular smooth muscle because PTH is a well-defined calcium ionophore [123]. Kawashima [124] demonstrated that PTH caused a transient rise in intracellular calcium in vascular smooth muscle cells through its receptor via opening calcium channels. Elevation of serum PTH has been reported in preeclampsia [4, 93, 94, 97-100], except for one study [102]. Increased PTH may contribute to an increase in intracellular calcium concentrations, thereby enhancing vascular reactivity.

Kilby *et al.* [115] speculated that transmembrane calcium flux was altered in hypertensive pregnancy by a specific mechanism, probably of placental origin. Recently, Page *et al.* [125] have detected potent vasoactive neurokinin B in the placenta. They suggest the involvement of neurokinin B in the clinical manifestation of preeclampsia because plasma concentrations of neurokinin B are elevated in pregnancy-induced hypertension and preeclampsia [125].

Beliźan *et al.* [126] reported that calcium supplementation could reduce intracellular calcium concentrations in vascular smooth muscle cells by lowing serum PTH. Many clinical trials and meta-analyses have suggested that calcium supplementation during pregnancy may reduce the risk of hypertension and preeclampsia [126-137]. However, differences in study design and a low dietary calcium intake in the populations studied limit acceptance of the data [138]. A recent large randomized controlled trial in the USA of 4,589 healthy nulliparous women provides conclusive evidence that calcium supplementation during pregnancy does not prevent preeclampsia and pregnancy-associated hypertension [138], although it is unknown as to whether calcium supplementation can prevent preeclampsia in women at increased risk, such as those with prior preeclampsia or chronic hypertension [139].

Further considerations are to clarify the effects of vasoconstrictive substances related to preeclampsia on intracellular calcium handling, and to establish a treatment to suppress possible causal substances that increase intracellular calcium concentrations in vascular muscle cells.

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