Vitamin D Deficiency in Guinea Pigs: Exacerbation of Bone Phenotype During Pregnancy and Disturbed Fetal Mineralization, with Recovery by 1,25(OH)_2D_3 Infusion or Dietary Calcium-Phosphate Supplementation

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Abstract. Vitamin D (D) deficiency during human pregnancy appears to disturb fetal growth and mineralization, but fetal development is normal in D-deficient rats and vitamin D receptor gene-ablated mice. We used the guinea pig model to investigate maternal and fetal effects of D deficiency. Pregnant (Pr) and nonpregnant (NP) animals were fed a D-replete (+D) or D-deficient diet (−D) for 8 weeks. We further studied whether the effects of a −D diet are reversed by continuous 1,25(OH)_2D_3 infusion (−D + 1.25) and/or by a lactose-, Ca- and P-enriched D-deficient diet (−D + Ca/P). Bone analyses included histomorphometry of the proximal tibiae, dual-energy X-ray absorptiometry (DXA), and quantitative computed tomography (QCT) of the femora. Depletion of 25(OH)D_3 and 1,25(OH)_2D_3 levels and the D-deficiency syndrome were more severe in pregnant animals. Indeed, Pr/−D but not NP/−D guinea pigs were hypophosphatemic, and showed robust increases in growth plate width and osteoid surface and thickness; in addition, bone mineral density on DXA was lower in Pr/−D animals only, which was exclusively in cortical bone on QCT. Bone phenotype was partly normalized in Pr/−D + 1.25 and Pr/−D + Ca/P animals. Compared with + D fetuses, −D fetuses had very low or undetectable 25(OH)D_3 and 1,25(OH)_2D_3, were hypercalcemic and hypophosphatemic, and had lower osteocalcin levels. In addition, body weight and total body bone mineral content were 10–15% lower; histomorphometry showed hypertrophic chondrocyte zone expansion and hyperosteoidosis. 1,25(OH)_2D_3 levels were restored in −D + 1.25 fetuses, and the phenotype was partially corrected. Similarly, the fetal + D phenotype was rescued in large part in −D + Ca/P fetuses, despite undetectable circulating 25(OH)D_3 and 1,25(OH)_2D_3. We conclude that pregnancy markedly exacerbates D deficiency, and that augmenting Ca and P intake overcomes the deleterious effects of D deficiency on fetal development.

Keywords: Vitamin D deficiency — Guinea pig — Pregnancy — Fetal mineralization — Histomorphometry.

D deficiency is common in women during the reproductive period and pregnancy. A report of a high prevalence of D deficiency in pregnant Asian women living in Britain [1] has been followed by the recognition that D deficiency is also common in women of reproductive age in more southern regions with abundant sunlight [2–5]. Avoidance of ultraviolet (UV) light exposure — by wearing traditional dress and staying indoors — appears to be the primary risk factor [4, 5], with lower dietary D intake as a secondary factor [5, 6].

In humans, there is fragmentary evidence that D deficiency disturbs fetal development. Cases of ‘fetal rickets’ have been reported in severely D-deficient pregnant women [7]. Babies of D-deficient mothers were found to be more frequently small-for-gestational age [1], and their birth weight was correlated with maternal ionized calcium (Ca^{2+}) levels [8]. Vitamin D supplements during the third trimester of pregnancy increased birth weight in Asian women [9]. Also, cord plasma alkaline phosphatase levels were raised in D-deficient pregnancies, the newborns had a larger anterior fontanelle, and symptomatic early neonatal hypocalcemia was more common [1]. Furthermore, total-body bone mineral content (BMC) was 6% lower in winter-born than in summer-born newborns in South Korea, and was correlated with cord serum 25-hydroxyvitamin D_3 [25(OH)D_3] levels [10].

Studies in small rodents failed to show an effect of D deficiency on fetal growth and mineralization. In D-deficient rats, fetal body weight and Ca content/
weight [11], trabecular bone volume on tibial histomorphometry [12] and plasma Ca and phosphate (P) levels [13] were found to be normal, although there was a significant increase in osteoid area [12]. In fact, when corrected for lower milk intake, D deficiency did not affect growth and mineralization until day 20 of postnatal life [14]. Similarly, growth, bone histology, and plasma Ca and parathyroid hormone (PTH) levels were normal before postnatal day 21 in mice with a vitamin D receptor (VDR) gene knockout; however, an expansion in the hypertrophic chondrocyte width was observed by day 15 [15, 16].

Fetuses of small rodents are born chemically and physiologically immature, and growth and bone formation increase rapidly during the suckling and early postweaning stages [17]. In contrast, guinea pig fetuses (gestation length 63–68 days) are mineralized comparably to human fetuses by the end of gestation, and show active bone formation [18]. Herein we examined the effects of a D-deficient diet in guinea pigs on maternal and fetal bone and mineral metabolism. The diet was started on the first day of pregnancy to avoid infertility [19]; we also studied nonpregnant guinea pigs fed the diet during a similar period of 8 weeks. Also, we studied the effect of maternal treatment with 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], the only vitamin D metabolite required to prevent rickets in postnatal rats fed a D-deficient diet [20]. We used a dose (5 ng/100 g/day, s.c.) that was shown to maximally prevent rickets in D-deficient rats; indeed, at higher doses, 1,25(OH)₂D₃ may induce, rather than reverse, osteomalacia [21]. 1,25(OH)₂D₃ crosses the placental barrier in humans [22], and we have reported a highly significant correlation between maternal and fetal 1,25(OH)₂D₃ levels in humans [23], guinea pigs [24], and rats [25]. 1,25(OH)₂D₃ treatment was given during the last 4 weeks of pregnancy only, since ossification centers are first detected from fetal day 26 in guinea pigs [26]. Finally, we studied the effect of a high lactose, Ca- and P-enriched D-deficient diet. A comparable diet reduced or even normalized the secondary hyperparathyroidism and the skeletal abnormalities (rickets and osteomalacia) in weaned nutritionally D-deficient rats [27, 28] as well as in weaned mice with an ablated VDR gene [29, 30].

Materials and Methods

Animals

All experiments were approved by the ethical committee for animal research at the K.U. Leuven. Two- to three-month-old (young adult) female guinea pigs, bred at the K.U. Leuven Proefdierencentrum, were housed in a room with a constant temperature of 22°C and a 12 hour light/12 hour dark cycle. All animals were fed a standard guinea-pig chow ad libitum, containing 0.96% Ca, 0.49% P, 2400 IU vitamin D₃/kg, and 1600 mg vitamin C/kg (Hope Farms, Woerden, the Netherlands). The animals received deionized water with added vitamin C (400 mg/L), as guinea pigs are susceptible to development of vitamin C deficiency. Part of the animals were mated during the day; the presence of a copulation plug was checked twice daily on those days. Once a vaginal plug was detected, the animal was kept in a separate cage and labeled; this day was defined as day 1 of pregnancy. The pregnant animals were divided into four experimental groups: (1) a D-replete group (+D group), which continued on the standard guinea pig diet; (2) a D-deficient group (+D group), fed an experimental diet containing 0.97% Ca, 0.38% P, no vitamin D₃, and 2300 mg vitamin C/kg (Harlan/Teklad, Zeist, the Netherlands); (3) a D-deficient group, which were infused s.c. with 1.25(OH)₂D₃ (0.12 nmol/day) via an Alzet miniosmotic pump (model 2ML4, Charles River, Palo Alto, CA) placed in the back region under anesthesia (ketamine hydrochloride, 48 mg/kg, and xylazine hydrochloride, 6 mg/kg, both i.m.) from day 30 of pregnancy onward (−D+1.25 group); (4) a D-deficient group fed an experimental diet containing 20% lactose as carbohydrate, 1.9% Ca, 1.0% P, and 2300 mg vitamin C/kg (Harlan/Teklad) (−D + Ca/P group). All animals in groups 2–4 were housed in a UV light-free room. The experimental diets in groups 2–4 were introduced over a 7-day period, in a 50% standard-50% experimental diet mixture, to avoid food deprivation; the experimental diet was then continued for 7 weeks. Nonpregnant animals received the experimental diets for the same duration. All animals received fluorochrome labeling with calcine (Sigma, St. Louis, MO), 16 mg/kg i.m., 8 and 1 and day (24 hours prior to the end of the study).

Eight weeks after the start of the experiment (day 57 for pregnant animals), the animals were transported to the UV light-deprived surgical room, and were anesthetized as described. The anesthetized animals were weighed, and xylcaine 2% was injected s.c. in the midline abdominal area, prior to laparotomy. In pregnant animals, the uterus was exposed, and amniotic fluid was collected from the gestational sacs and pooled. The fetuses were exposed through a small uterine incision from left to right, and a midline laparotomy was performed in each fetus to obtain a blood sample from the abdominal aorta, with the umbilical cord still attached to the placenta. All fetuses and placentas were weighed. The sample from the last exposed fetus was kept for measurement of Ca²⁺, total Ca, and P; the previous samples were kept for measurement of 25(OH)D₃, 1,25(OH)₂D₃, and osteocalcin. The last two fetuses (or last fetus in case there were only two fetuses per mother) were eviscerated, weighed again, and stored at −20°C for measurement of whole-body BMC. The tibiae were removed from the other fetuses for histomorphometry. A blood sample was taken from the abdominal aorta of the mother, and the animal was euthanized by cervical dislocation. Both tibiae were removed for histomorphometry and both femora were removed for measurement of weight and dual-energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) analysis. The pQCT procedure did not exceed 45 min; the Ca²⁺ measurements were done within 10 min of the maternal sample collection.

Assays

All assays, except Ca²⁺ and 1,25(OH)₂D₃, were done in a single run. Ca²⁺ was measured in whole blood by the ion selective electrode method on the Ciba Corning 288 Blood Gas System (Bayer Diagnostics, Brussels, Belgium); plasma P was measured by colorimetry [31]. Plasma 25(OH)D₃ was measured by direct competitive protein binding assay [32]; the detection limit of this assay is about 2.5 µg/L (6.25 nmol/L). Plasma 1,25(OH)₂D₃ was determined by radioimmunoassay (RIA) after HPLC purification, as described [23], with later modifications [31]; the detection limit is around 18 pg for 1 mL plasma (43 pmol/L). Vitamin D-binding protein (DBP) was measured by a single radial immunodiffusion assay specific for guinea pig DBP [18]; the free 1,25(OH)₂D₃ index was calculated as the 1,25(OH)₂D₃/DBP molar index (×10⁻²) [23]. Plasma osteocalcin was determined by RIA, with osteocalcin from the rabbit as the standard and a rabbit polyclonal antisera