Pregnancy does not alter the metabolic clearance of 1,25-dihydroxyvitamin D in rats

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Pregnancy does not alter the metabolic clearance of 1,25-dihydroxyvitamin D in rats. Am. J. Physiol. 258 (Endocrinol. Metab. 21): E158–E162, 1990.—Increased circulating levels of 1,25-dihydroxyvitamin D [1,25(OH)2D] during pregnancy could be due to an increase in production or decrease in the metabolic clearance rate of 1,25(OH)2D. To answer this question an isotope dilution method was used to determine the clearance rate of 1,25(OH)2D in pregnant and aged-matched nonpregnant female rats. A bolus of 0.146 μCi 1,25(OH)2[3H]D3 was given to 60 pregnant and 60 aged-matched nonpregnant rats and the disappearance of the isotope was followed in these animals over the next 48 h. In 12 pregnant rats vs. 14 nonpregnant controls not injected with tracer, plasma calcium (9.8 ± 0.41 vs. 10.7 ± 0.17 mg/ml) and 25(OH)D (17.1 ± 1.15 vs. 25.4 ± 1.58 ng/ml) levels were significantly lower (P < 0.01 and P < 0.001), whereas plasma 1,25(OH)2D levels (110 ± 16.1 pg/ml vs. 77 ± 6.0 pg/ml) were significantly higher (P < 0.05). Clearance rates of 1,25(OH)2D were elevated in the pregnant rats (2.83 pg/min) compared with the nonpregnant controls (1.55 pg/min). In conclusion, the elevated maternal plasma 1,25(OH)2D level during pregnancy is due to an increase in production and is not due to a decreased clearance.

Vitamin D; production rate; turnover rate; mineral metabolism

1,25-DIHYDOXYVITAMIN D [1,25(OH)2D] is the hormonal form of vitamin D that maintains mineral homeostasis by regulating intestinal calcium and phosphorus transport, and bone resorption. The vitamin D molecule is converted by serial hydroxylations, first in the liver to 25(OH)D, the major circulating metabolite of vitamin D, and subsequently in the proximal tubule of the kidney, resulting in the formation of 1,25(OH)2D. The proximal tubule 25(OH)2D-1-α-hydroxylase (1-hydroxylase), the sole site for 1,25(OH)2D synthesis in the nonpregnant animal, is stimulated by parathyroid hormone and low plasma concentrations of calcium and phosphorus (4). In the pregnant animal the 1-hydroxylase is also present in the fetal kidney (16, 30) and placenta (32).

During pregnancy the mineral requirements for fetal bone growth cause net transfer of calcium from mother to fetus. This large efflux of maternal calcium stores contributes, in part, to the decline in maternal calcium levels (28). The mother adjusts to the drain on her calcium supplies with an increase in fractional intestinal calcium absorption (14). A considerable body of evidence suggests that 1,25(OH)2D is responsible, in part, for this increase in intestinal calcium absorption during pregnancy. Maternal plasma 1,25(OH)2D levels are elevated during pregnancy in both women (17, 31) and laboratory animals (12, 16, 25). Vitamin D deficiency eliminates the majority of the increase in intestinal calcium transport during pregnancy (13). Inadequate supplies of vitamin D during pregnancy can result in maternal osteomalacia and neonatal rickets and hypocalcemia (22).

The mechanism behind the increase in maternal plasma 1,25(OH)2D concentrations during pregnancy is not known but could be a result of an increase in the production or a decrease in the elimination of the hormone. The elevated 1,25(OH)2D levels of pregnancy could also be secondary to the increase in plasma vitamin D binding protein (1, 2). The present report shows that the increase of maternal plasma 1,25(OH)2D levels during pregnancy is due to an increase in the production rate without a significant change in the elimination rate of 1,25(OH)2D.

METHODS

Vitamin D compounds. 1,25-(OH)2[26,27-3H]D3 with a specific activity of 157 Ci/mmol was purchased from Amersham (Arlington Heights, IL). The purity of the [3H]1,25(OH)2D3 was found to be 92% as determined by straight-phase high-performance liquid chromatography (Varian model 5000, Varian Industries, Palo Alto, CA) on a 4.6 mm × 25 cm Zorbax SIL column (Du Pont Industries, Wilmington, DE) using isopropanol-hexane (5:95, vol/vol) at 2 ml/min as the eluting solvent. The 1,25(OH)2D3 was kindly supplied by Dr. Milan Uskokovic (Hoffmann-La Roche, Nutley, NJ).

Animals. Seventy-two sperm-positive rats at day 6 of gestation and 74 aged-matched female rats were obtained from Holtzman (Madison, WI). Two days after arrival, animals were given deionized distilled water and placed, ad libitum, on a nutritionally complete diet containing 0.6% calcium, 0.6% phosphorus, and 2.2 IU vitamin D3/gm diet (Teklad, Madison, WI). After 11 days, 60 of the pregnant and 60 of the aged-matched control rats were randomly placed in 12 groups of five animals each for...
subsequent clearance experiments. The 12 groups consisted of animals bled at 5, 15, and 30 min and 1, 2, 4, 8, 18, 24, 36, and 48 h after injection of 0.146 μCi 1,25(OH)₂[³H]D₃ in 25 μl of ethanol into the jugular vein. At the designated time periods after tracer injection, the animals were anesthetized with ether and exsanguinated by severing the carotid artery. Serum was obtained from blood by centrifugation and was frozen at -20°C until analysis for [³H]1,25(OH)₂D₃ levels. Twelve pregnant and 14 nonpregnant rats raised concurrently with the rats used above but not injected with tracer were bled by cardiac puncture. Serum from these animals was removed for the determination of calcium, phosphorus, and endogenous 25-hydroxyvitamin D [25(OH)D] and 1,25(OH)₂D levels.

Assay of serum vitamin D metabolites. Serum 25(OH)D and 1,25(OH)₂D concentrations were determined according to the method of Reinhardt et al. (27), as modified by Favus and Langman (8). These assays quantitate both the D₂ and D₃ chemical forms of these metabolites.

Serum containing [³H]1,25(OH)₂D₃ was spiked with 25 μl ethanol containing 100 ng of 1,25(OH)₂D₃ to monitor recoveries. Lipids from serum were sequentially extracted by solid-phase extraction using a C₁₈ Sep-Pak cartridge (Waters Associates, Milford, MA) followed by a silica Sep-Pak cartridge (Waters Associates) (8). This purification technique allows for separation of 1,25(OH)₂D from its more polar metabolite, 1,24,25-trihydroxyvitamin D (T. A. Reinhardt, personal communication). The fractions containing 1,25(OH)₂[³H]D₃ were collected, air dried, and radioactivity determined by scintillation spectrophotometry with a Beckman model L5350 liquid scintillation counter (Palo Alto, CA). Recovery of 1,25(OH)₂[³H]D₃ was determined by quantitating the remainder of the added 100 ng of authentic 1,25(OH)₂D₃ by radioreceptor binding assay utilizing the 100,000 g binding protein from bovine thymus cytosol (27). Because large dilution of sample is necessary to measure the recovered cold 1,25(OH)₂D₃ previously added, there is no interference with the binding assay by the serum concentration of injected tracer 1,25(OH)₂D₃. After correction for recovery, data were expressed as disintegrations per minute per milliliter.

Determination of clearance and turnover rates. The curve that described the concentration of [³H]1,25(OH)₂D₃ vs. time was fitted to a biexponential equation with the assistance of MK Model statistical software (Biosoft, Cambridge, UK). The biexponential equation used to describe the data was

\[ \frac{C}{C_0} = H_1 e^{-kt_1} + H_2 e^{-kt_2} \]

where C is concentration of tracer in the pool, C₀ is concentration of tracer in the pool at time 0 in disintegrations per minute per milliliter, H₁ and H₂ are coefficients that define the starting point of the curve, k₁ and k₂ are rate constants of removal, and t is time. The area under the tracer concentration vs. time curve was determined (29). Maternal metabolic clearance rate of 1,25(OH)₂D is the quotient between the dose of 1,25(OH)₂[³H]D₃ injected and the area under the tracer concentration vs. time curve. Production rate of 1,25(OH)₂D is the product of the metabolic clearance rate and endogenous concentration of the hormone. The V₄ is the quotient of the dose of 1,25(OH)₂[³H]D₃ (dpm) and the sum of values at time 0 for curves representing the α- and β-phases of distribution.

Assay of serum calcium and phosphorus. Calcium was measured in triplicate by fluorometric titration (Calcette, Precision Instruments, Natick, MA). Plasma phosphorus was determined in duplicate according to the method of Fiske and Subbarow (10).

Assay of parathyroid hormone (PTH). Plasma PTH was assayed using a rat mid-molecule PTH radioimmunoassay (Incastar Corporation, Stillwater, MN) as previously described (18).

Statistical analysis. The tracer concentration vs. time curves for the pregnant and control rats were compared using analysis of variance for repeated measures. The differences of the serum biochemistries between the two groups was determined using a one-tailed Student's t test. All analyses were performed by computer-assisted methodologies (MK Model, Biosoft, Cambridge, UK; BMDP, University of California, Berkeley, CA). P values of 0.05 or greater were chosen to denote significance.

RESULTS

Body weights and serum Ca, P, PTH, 25(OH)D, and 1,25(OH)₂D levels. The body weights and serum concentrations of Ca, P, 25(OH)D, and 1,25(OH)₂D in the 12 pregnant and 14 nonpregnant female rats that were not given an intravenous injection of 1,25(OH)₂[³H]D₃ are shown in Table 1. Pregnancy resulted in an expected increase in body weight. The body weights of the pregnant and aged-matched nonpregnant rats that received radiotracer 1,25(OH)₂[³H]D₃ (Table 2) were not significantly different from the body weights of their counterparts described in Table 1. Pregnant animals had lower serum levels of Ca and 25(OH)D but a significantly higher serum level of 1,25(OH)₂D. Plasma PTH levels were significantly higher in the pregnant rats vs. the nonpregnant controls. Serum P concentration was not altered by pregnancy.

1,25-(OH)₂D clearance and turnover rate. The disappearance of [³H]1,25(OH)₂D₃ from the serum of control female and pregnant rats is portrayed graphically in Fig. 1. The disappearance of 1,25(OH)₂[³H]D₃ from serum in both of these groups was biphasic and thus fit to the biexponential equation described in the METHODS section. The tracer concentration vs. time curves for the pregnant and the nonpregnant aged-matched control animals was found to be the same by analysis of variance for repeated measures (F = 3.92, P = NS). Table 2 presents the 1,25(OH)₂D apparent (V₄), clearance rate, and production rate 1,25(OH)₂D determined from the data in Fig. 1. The clearance rate and V₄ of 1,25(OH)₂D were the same in pregnant and aged-matched control female rats. The production rate of 1,25(OH)₂D was higher in pregnant vs. aged-matched control female rats as indicated by the significantly higher serum 1,25(OH)₂D levels and similar 1,25(OH)₂D clearance rates.
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TABLE 1. Body weights and plasma concentrations of calcium, phosphorus, PTH, 25(OH)D, and 1,25(OH)2D in pregnant and aged-matched control female rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Body wt, g</th>
<th>Calcium, mg/dl</th>
<th>Phosphorus, mg/dl</th>
<th>PTH, pM</th>
<th>25(OH)D, ng/ml</th>
<th>1,25(OH)2D, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>289±6.5 (14)</td>
<td>10.7±0.17</td>
<td>8.1±0.52</td>
<td>202±8.3 (24)*</td>
<td>25.4±1.56 (11)</td>
<td>77±6.0 (11)</td>
</tr>
<tr>
<td>Pregnant</td>
<td>387±6.4† (12)</td>
<td>9.6±0.41‡</td>
<td>8.4±0.33 (11)</td>
<td>258±12.7† (25)*</td>
<td>17.1±1.15† (11)</td>
<td>110±16.1§ (11)</td>
</tr>
</tbody>
</table>

Data are means ± SE; nos. in parentheses represent no. of observations. PTH, parathyroid hormone. * No. of animals tested; PTH levels were measured in 25 rats per group of animals used in clearance study (see Table 2). † Significantly different from controls P < 0.001. ‡ Significantly different from controls P < 0.01. § Significantly different from controls P < 0.05.

TABLE 2. The V_d, clearance rate, and turnover rate of 1,25(OH)2D in pregnant and aged-matched control female rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body wt, g</th>
<th>Plasma levels, pg/ml</th>
<th>1,25(OH)2D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Clearance, ml/min</td>
<td>V_d, ml</td>
</tr>
<tr>
<td>Control</td>
<td>60</td>
<td>296±1.6</td>
<td>77±5.9 (11)</td>
<td>20.2±1.38</td>
</tr>
<tr>
<td>Pregnant</td>
<td>60</td>
<td>388±3.8†</td>
<td>110±16.1† (11)</td>
<td>25.8±1.31</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, no. of observations. V_d, apparent volume of distribution. * No. of animals. † Significantly different from controls P < 0.05.

FIG. 1. The disappearance of 1,25(OH)2[3H]D3 from plasma of pregnant (○) and nonpregnant, aged-matched female (△) rats. Each point represents mean ± SD of 5 data points. SD of data points without bars lie within points.

DISCUSSION

Vitamin D helps maintain calcium homeostasis and bony metabolism of the mother and newborn during pregnancy. Inadequate supplies of vitamin D during pregnancy can result in maternal osteomalacia and neonatal hypocalcemia (22). This is especially prevalent in geographic locations where sunlight is restricted (2, 23) and in cultures where clothing reduces the exposure to the sun (23). In diabetic pregnancy, maternal 1,25(OH)2D levels are decreased (20, 34), and infants from diabetic mothers have a high incidence of hypocalcemia (33) and reduced bone mineral content (19). Because of the importance of 1,25(OH)2D to normal calcium homeostasis in mothers and newborns, we determined the production and clearance rates of 1,25(OH)2D in the pregnant rat.

It is well known that the maternal plasma 1,25(OH)2D levels increase during pregnancy in both women (17, 31) and laboratory animals (12, 16, 25). Similar results were obtained in the present study. The mechanism(s) responsible for this increase is (are) not known. Elevated maternal plasma 1,25(OH)2D levels during pregnancy could be a result of one, or a combination of, the following possibilities: 1) an increase in maternal kidney 1-hydroxylase activity above nonpregnancy levels, 2) the presence of placental and/or fetal kidney 1-hydroxylase activity, 3) a secondary effect due to the increase in the plasma concentration of the vitamin D binding protein (DBP), or 4) a decrease in the plasma elimination rate of 1,25(OH)2D. The results of the present study indicate that the elevated 1,25(OH)2D levels characteristic of pregnancy are due to an increased production of 1,25(OH)2D and not secondary to a decrease in the metabolic clearance rate of the hormone. Similar findings have been reported in the rabbit (6).

The source of the elevated 1-hydroxylase activity during pregnancy is not known but could be from the 1-hydroxylase present in the maternal kidney, fetal kidney, or placenta (23). The kidney is generally recognized as the unique source of 1-hydroxylase activity in the nonpregnant animal (26). However, in the pregnant animal, in vitro production of 1,25(OH)2D occurs also in the fetal kidney and placenta. Fetal 1-hydroxylase activity has been found in rabbit (16), guinea pig (9), pig (30), and rat kidney (32). Placental 1-hydroxylase activity has been observed in a number of species, including vitamin D-depleted rats (32), guinea pig (9), and humans (6, 36) but not in rabbits (16) or yolk sac of vitamin D-replete rats (3). The presence of functional fetal kidney and/or placental 1-hydroxylase in vivo has been demonstrated by several groups of investigators (11, 35, 37). Increased
maternal kidney 1-hydroxylase activity may also be the source of the elevated maternal 1,25(OH)2D levels in the pregnant animal. Low plasma calcium levels, characteristic of pregnancy, acting directly or through PTH, may increase maternal kidney 1-hydroxylase activity. In support of this latter hypothesis, we found PTH levels to be elevated in the pregnant rats.

It is possible that the high levels of 1,25(OH)2D during pregnancy are due entirely to an elevation in plasma DBP concentration, and increased 1-hydroxylase activity is not required to maintain the elevated plasma hormone concentration. Increased binding of 1,25(OH)2D to its specific carrier protein may protect the hormone from catabolism and result in a prolonged clearance rate and elevated plasma levels. Even though plasma levels of DBP are increased during pregnancy (1, 2) and with estrogen therapy (2), the present results show that pregnancy does not prolong the clearance rate of 1,25(OH)2D. Therefore, it seems unlikely that the elevation of DBP alone can explain the increased 1,25(OH)2D levels that we saw during pregnancy.

In the present study we used an isotope dilution method to determine maternal 1,25(OH)2D turnover rate. It should be recognized that the 1,25(OH)2D turnover rate in the pregnant animal, as determined by the present methodology, represents not only maternal utilization but also hormone transfer to the fetal-placental unit. Because we find the clearance rate of 1,25(OH)2D to be the same in pregnant as in nonpregnant age-matched animals, hormone transfer to the fetal-placental unit must represent an insignificantly small percentage of maternal 1,25(OH)2D turnover. Therefore, maternal 1,25(OH)2D turnover rate as determined in this study is synonymous with maternal 1,25(OH)2D utilization.

The present study also provides valuable data concerning the pharmacokinetics of 1,25(OH)2D during pregnancy. We find the apparent Vd of 1,25(OH)2D in the pregnant animal to be similar to that of nonpregnant controls. This indicates that only a very small percentage of maternal 1,25(OH)2D turnover rate as determined in this study is thought to be responsible for the decreased 25(OH)D levels of pregnancy, consistent with our demonstration of increased 1,25(OH)2D turnover in pregnancy.

In summary, we find the elevated serum 1,25(OH)2D levels observed in the pregnant mother to be a result of an increase in production rate and not due to a decrease in the clearance rate of the hormone. Maternal 1,25(OH)2D flux to the fetal-placental unit represents a small percentage of maternal 1,25(OH)2D turnover rate.

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REFERENCES


17. KUMAR, R., W. R. COHEN, R. SILVA, AND F. H. EPSTEIN. Elevated 1,25-dihydroxyvitamin D plasma levels in normal human preg-
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