RAPID

PUBLICATIONS

Elevated 1,25-Dihydroxyvitamin D Plasma Levels in Normal Human Pregnancy and Lactation

RAJIV KUMAR, WAYNE R. COHEN, PATRICIO SILVA, and FRANKLIN H. EPSTEIN,
Departments of Medicine, Obstetrics, and Gynecology, Thorndike Laboratory of the Harvard Medical School, Beth Israel Hospital, Boston, Massachusetts 02215

ABSTRACT Plasma 1,25-dihydroxyvitamin D levels are elevated in early pregnancy and continue to increase throughout pregnancy. They remain elevated *postpartum* in lactating women. The elevated levels probably represent a physiological response to increased calcium requirements.

INTRODUCTION

Vitamin D_3, through its active hormonal form 1,25-dihydroxyvitamin D_3, increases calcium transport in the intestine (1). Moreover, its synthesis appears to be regulated by the calcium needs of the animal (2). As calcium absorption increases in pregnancy (3) in response to fetal demands, it seems likely to us that 1,25-dihydroxyvitamin D levels would increase. We obtained plasma from normal women during the first, second, and third trimesters of pregnancy as well as from *postpartum* lactating women, to measure levels of 1,25-dihydroxyvitamin D and, in some instances, 25-hydroxyvitamin D.

METHODS

31 normal pregnant women were seen by one of us during the course of antenatal care. Six *postpartum* lactating women were also enrolled in the study. Plasma from 10 age-matched females (mean age 25±4 yr vs. 26±4 yr in experimental subjects, P = NS) was used as a control.

5 ml of plasma were extracted and used in a competitive binding assay for 1,25-dihydroxyvitamin D (4). The only modification was the separation of bound from free hormone. Incubation of cold and radiolabeled 1,25-dihydroxyvitamin D with rachitic chicken duodenum cytosol (1 ml/tube, 1 mg/ml protein) was carried out for 1 h at 25°C. After incubation, 0.2 ml of a 0.5% dextran (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) 5% Norit A (American Norit Co., Jacksonville, Fla.) (wt/vol) suspension, in a 0.05-M phosphate buffer (pH 7.4), was added to the tubes. Exposure time was 15 min. After centrifugation, the supernatant radioactivity was determined. In this assay, plasma samples from anephric humans have undetectable amounts of 1,25-dihydroxyvitamin D. Normal levels in a mixed population are 39.5±24.6 pg/ml (mean±SD). Plasma 25-hydroxyvitamin D levels were determined by a modification of the method of Haddad and Chyu (5). Normal vitamin D replete rat plasma (1:3,000 vol/vol dilution in 0.05 M phosphate buffer) was used instead of rachitic rat kidney cytosol. Plasma from pregnant patients and controls was obtained during the summer months.

Serum calcium was measured with an atomic absorption spectrometer. Serum phosphorus was determined by the method of Chen et al. (6).

Statistical analyses were carried out with Student's t test and linear regression analysis by the method of least squares.

RESULTS

Plasma 1,25-dihydroxyvitamin D levels are elevated early in pregnancy (87.1±52.8 pg/ml (mean±SD) first trimester vs. 52.8±14.8 pg/ml in age-matched controls, $P < 0.05$). Linear regression analysis (Fig. 1) demonstrates that plasma 1,25-dihydroxyvitamin D levels are correlated with the duration of pregnancy ($r = 0.47$,

Dr. Kumar was supported by National Institutes of Health postdoctoral fellowship F32 AM05331. Dr. Silva is an Established Investigator of the American Heart Association. Address all inquiries to Dr. Kumar, Mineral Research Unit, Mayo Clinic, Rochester, Minn. 55901.

Received for publication 30 October 1978 and in revised form 21 November 1978.

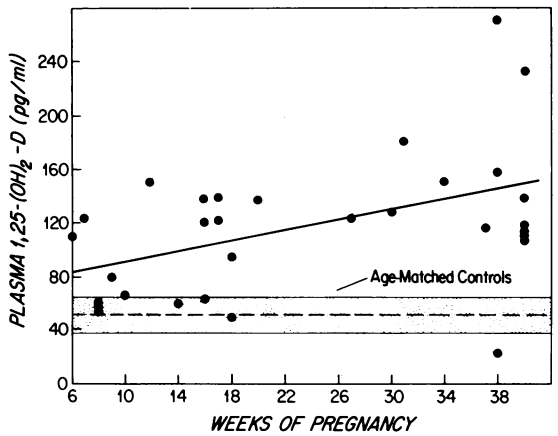

FIGURE 1 Plasma 1,25-dihydroxyvitamin D[1,25-$(OH)_2$-D] levels in normal pregnant women. Shaded area is the mean ±SD in age-matched females.

$P < 0.01$). They remain elevated in the *postpartum* period in lactating women (103.0±56.6 pg/ml *postpartum* vs. 52.8±14.8 pg/ml, $P < 0.025$). The increase in plasma 1,25-dihydroxyvitamin D levels during pregnancy occurs concurrently with the increase in calcium absorption noted in normal pregnant women (3). Four women were studied longitudinally through pregnancy. In all but one, levels of 1,25-dihydroxyvitamin D increased. Plasma 25-hydroxyvitamin D levels were normal (41.7±7.9 ng/ml in 6 pregnant women in the first trimester or early second trimester vs. 38.9±18.7 ng/ml in 15 normal women, P = NS). The serum calcium in women throughout pregnancy was 8.87±0.6 mg/dl (vs. 8.8±0.4 mg/dl in normal nonpregnant women). Vitamin D intakes were approximately 700 IU/d and did not change during pregnancy. Daily calcium intake averaged 1,100 mg/d.

DISCUSSION

1,25-dihydroxyvitamin D increases calcium absorption in the intestine (1). Calcium absorption increases during pregnancy in response to fetal demands, and the observed increases of plasma 1,25-dihydroxyvitamin D are therefore physiologically appropriate. The elevation of plasma 1,25-dihydroxyvitamin D could be a result of either increased synthesis or decreased degradation. Of the two possibilities, the first is more likely. The exact nature of the stimulus to increased 25-hydroxyvitamin D 1α-hydroxylase activity is unclear. The circulating levels of estrogens (7), human placental lactogen (8), and parathyroid hormone (9) increase during pregnancy. An elevation of serum levels of 1α, 25-dihydroxyvitamin D has been reported in lactating rats (10). Estrogens (11), prolactin (12), parathyroid hormone (13), and growth hormone (14) increase 25-hydroxyvitamin D 1α-hydroxylase activity in various animal models, and the observed increases in these hormones could increase 1,25-dihydroxyvitamin D synthesis. The role of other pregnancy-related hormone changes in altering 25-hydroxyvitamin D 1α-hydroxylase activity is unknown. The other possibility, that of decreased metabolism of 1,25-dihydroxyvitamin D by suppression of the rate of side-chain oxidation (15), or 1,25-dihydroxyvitamin D 24-hydroxylation in the intestine (16) was not investigated. 25-hydroxyvitamin D levels in some of the women in early pregnancy showed no change compared with normal. Others (17) have shown that 25-hydroxyvitamin D levels are similar in pregnant white women in the early and late parts of pregnancy when compared with normal controls. It is unlikely that a change in the substrate for the 25-hydroxyvitamin D 1α-hydroxylase enzyme can be invoked to account for the rise in 1,25-dihydroxyvitamin D levels. The levels of 1,25-dihydroxyvitamin D observed in our young female control group are higher than published levels (4) of 29±2 pg/ml. The latter values were obtained in older (mean age of 55 yr) male and female subjects. 1,25-dihydroxyvitamin D levels in adolescents are 37.1±11.9 pg/ml (18). To our knowledge, no values are available in the literature for young, normal women in whom circulating estrogen levels are high. In an older, mixed, male and female population our values for 1,25-dihydroxyvitamin D are 39.5±24.6 pg/ml.

The increase in the level of 1,25-dihydroxyvitamin D observed in plasma probably serves a useful purpose in pregnancy and during lactation. It is likely that the increase in calcium absorption observed during pregnancy occurs as a result of increased production of 1,25-dihydroxyvitamin D.

ACKNOWLEDGMENT

This study was aided by U. S. Public Health Service grant AM 18078.

REFERENCES

1. DeLuca, H. F., and H. K. Schnoes. 1976. Metabolism and mechanism of action of vitamin D. *Annu. Rev. Biochem.* **45**: 631–666.
2. Hughes, M. R., P. F. Brumbaugh, M. R. Haussler, J. C. Wegerdal, and D. J. Baylink. 1975. Regulation of serum 1α,25-dihydroxyvitamin D_3 by calcium and phosphate in the rat. *Science (Wash. D. C.).* **190**: 578–580.
3. Heany, R. D., and T. G. Skillman. 1971. Calcium metabolism in normal human pregnancy. *J. Clin. Endocrinol. Metab.* **33**: 661–670.
4. Eisman, J. A., A. J. Hamstra, B. E. Kream, and H. F. DeLuca. 1976. A sensitive, precise and convenient assay for 1,25-dihydroxyvitamin D in human plasma. *Arch. Biochem. Biophys.* **176**: 235–243.
5. Haddad, J. G., and K. J. Chyu. 1971. Competitive protein binding radioassay for 25-hydroxycholecalciferol. *J. Clin. Endocrinol. Metab.* **33**: 992–995.

6. Chen, P. S., T. Y. Toribara, and H. Warner. 1956. Microdetermination of phosphorus. *Anal. Chem.* **28:** 1756–1758.
7. Roy, E. J., and R. MacKay. 1962. The concentration of estrogens in blood during pregnancy. *J. Obstet. Gynaecol. Br. Commonw.* **69:** 13–17.
8. Josinovich, J. B. 1969. Human placental lactogen. *In* Fetal Homeostasis. R. M. Wynn, editor. Academic Press, Inc., New York. **4:** 109.
9. Cushard, W. G., M. A. Creditor, J. M. Canterbury, and E. Reiss. 1972. Physiologic hyperparathyroidism in pregnancy. *J. Clin. Endocrinol. Metab.* **34:** 767–771.
10. Boass, A., S. A. Toverud, T. A. McCain, J. W. Pike, and M. R. Haussler. 1977. Elevated serum levels of 1α,25-dihydroxycholecalciferol in lactating rats. *Nature (Lond.).* **267:** 630–632.
11. Kenney, A. D. 1976. Vitamin D metabolism: Physiological regulation in the egg-laying Japanese quail. *Am. J. Physiol.* **230:** 1609–1615.
12. Spanos, E., K. W. Colston, A. Evans, L. S. Galante, S. J. MacAuley, and I. MacIntyre. 1976. Effect of prolactin on vitamin D metabolism. *Nature (Lond.).* **5:** 163–167.
13. Garabedian, M., M. F. Hollick, H. F. DeLuca, and I. T. Boyle. 1972. Control of 25-hydroxycholecalciferol metabolism by parathyroid glands. *Proc. Natl. Acad. Sci. U. S. A.* **69:** 1673–1676.
14. Spanos, E., D. Barrett, I. MacIntyre, J. W. Pike, E. F. Safilian, and M. R. Haussler. 1978. Effect of growth hormone on vitamin D metabolism. *Nature (Lond.).* **273:** 2420–2433.
15. Kumar, R., D. H. Harnden, and H. F. DeLuca. 1976. Metabolism of 1,25-dihydroxyvitamin D_3: Evidence for side chain oxidation. *Biochemistry.* **15:** 2420–2433.
16. Kumar, R., H. K. Schnoes, and H. F. DeLuca. 1978. Rat intestinal 25-hydroxyvitamin D_3 and 1α, 25-hydroxyvitamin D_3-24-hydroxylase. *J. Biol. Chem.* **253:** 3804–3809.
17. Turton, C. W. G., P. Stanley, T. C. B. Stamp, and J. D. Maxwell. 1977. Altered vitamin-D metabolism in pregnancy. *Lancet.* **I:** 222–224.
18. Scriver, C. R., T. M. Reade, H. F. DeLuca, and A. J. Hamstra. 1978. Serum 1,25-dihydroxyvitamin D levels in normal subjects and in patients with hereditary rickets or bone disease. *N. Engl. J. Med.* **299:** 976–979.