Seasonal changes in perinatal vitamin D metabolism: maternal and cord blood biochemistry in normal pregnancies

C M VERITY, D BURMAN, P C BEADLE, J B HOLTON, AND AUDREY MORRIS

Bristol Royal Hospital for Sick Children, British Aerospace, Bristol, Southmead Hospital, Bristol, and Department of Community Medicine, University of Bristol

SUMMARY Two groups of white, primiparous women and their babies were studied: one group in April 1979 and the other in September 1979. They were selected to be as near normal as possible. In each case maternal and cord blood samples were taken at delivery and analysed for serum 25-hydroxycholecalciferol (25-OHD), calcium, magnesium, phosphate, alkaline phosphatase, total protein, and albumin. Follow-up was by questionnaire at 6 weeks. The study showed a highly significant increase in maternal and cord serum 25-OHD levels in September. The few mothers who had taken vitamin D supplements had significantly higher serum 25-OHD values. Some of the unsupplemented women studied in April had low serum 25-OHD levels suggesting that oral vitamin D supplements should be given to pregnant white women in Britain, at least during the winter.

Recent work suggests that the health of a newborn infant is affected by the vitamin D status of his mother during pregnancy. In 1973 Purvis *et al.*¹ in Edinburgh reported an increased incidence of neonatal tetany in babies born after the 3 months of the year with the fewest sunshine hours. They showed an increased incidence of severe enamel hypoplasia of the teeth in such infants, and suggested that the changes might be related to seasonal variations in skin vitamin D formation. In 1974 Hillman and Haddad² showed a highly significant correlation between the concentration of 25-hydroxyvitamin D (25-OHD) in the mother's serum and that in the cord serum of her infant. In 1976 data were published which suggested that ultraviolet exposure was the major determinant of maternal serum 25-OHD levels in St Louis.³ The aim of our study was to determine the normal seasonal variation in serum 25-OHD in pregnant women at delivery, to relate this to cord levels of 25-OHD, and to relevant serum chemistry in mother and infant.

Methods

Patients. Two groups of pregnant women were studied—one during April 1979 and the other during September 1979. Women who were either already in labour or who had been admitted for induction were assessed on arrival at Bristol Maternity Hospital.

Only fit primiparous, white mothers delivering appropriately grown, term infants without significant birth asphyxia were included. Those living further than 20 miles from the centre of Bristol and those who had left the area for longer than 2 weeks during the last trimester of their pregnancies were excluded. Blood (15 ml) was taken from the mother by venepuncture generally without stasis, and from the umbilical vein usually before expulsion of the placenta. Serum was separated and frozen at -20°C within an hour of the sample being taken.

On the day after delivery each mother was interviewed and her baby examined. Social class assessment was on the basis of the husband's occupation, and for an unmarried woman on the basis of her own employment. Results were similar for the April and September mothers but compared with the whole country there was an excess of social classes I and II. Enquiry was made about dietary supplements of vitamin D but detailed dietary histories were not taken. The obstetric assessment was used to estimate gestational age. If there was serious doubt about the gestational age or if it was less than 37 weeks, the patient was excluded. Any infant with an Apgar score of less than 6 at one minute or five minutes was excluded too.

Each mother was asked to complete a short questionnaire which was posted to her 6 weeks after delivery. This questionnaire required her to answer

questions about the baby's feeds and asked if she had consulted a doctor about her baby. She was asked for permission to question the doctor about the baby's health.

Analyses. Chemical estimations were made by the SMAC multichannel autoanalyser in the Department of Clinical Chemistry at Southmead Hospital, Bristol. The following were measured: calcium, phosphate, alkaline phosphatase, magnesium, total protein, and albumin. Samples were analysed in weekly batches. Quality control showed that phosphate was the only measurement to differ between April and September. Phosphate levels were 6.6% higher in September and this figure was used for calculating the significance (Table 2).

Serum 25-OHD was assayed in batches after

completion of the sampling period in April and September. The serum was extracted with chloroform, methanol, and water, then it was purified by silicic acid chromatography, and the dried extract made up in absolute ethanol before assay. A competitive protein-binding assay was used, in which normal human serum provided the binding protein.⁴ In order to check the variation between the April and September groups, samples from a common serum pool were analysed. When the first group was assayed the serum pool gave the value 28.7 ± 1.4 ng/ml ($n = 7$) and for the second group the value was 31.2 ± 2.9 ng/ml ($n = 4$); this represents a variation of 8.3% of the mean value. Allowance was made for interassay variation when comparing values from different assays. In measuring the September group the correction was applied to each separate assay.

Table 1 *Biochemical results obtained in April and September 1979*

	Total no of cases	Mean	Standard deviation	Range
<i>April and September groups combined</i>				
Maternal				
Calcium (mmol/l) (uncorrected)	103	2.30	0.13	1.82 — 2.64
Alkaline phosphatase (IU)	100	207	56.6	92 — 415
Total protein (g/l)	104	62.6	4.6	43 — 76
Albumin (g/l)	104	34.7	2.3	27 — 41
Magnesium (mmol/l)	103	0.77	0.14	0.50 — 1.72
Cord				
Calcium (mmol/l) (uncorrected)	102	2.75	0.17	2.34 — 3.20
Calcium (mmol/l) (corrected)	102	2.83	0.18	2.49 — 3.69
Alkaline phosphatase (IU)	99	157	42.0	83 — 329
Total protein (g/l)	102	59.6	4.9	48 — 77
Magnesium (mmol/l)	102	0.75	0.12	0.50 — 1.23
<i>April group</i>				
Maternal				
25-OHD (ng/ml)				
With supplements	11	22.4	4.1	14.8 — 28.6
Without supplements	41	16.7	4.7	7.5 — 24.9
Calcium (corrected) all cases	52	2.45	0.09	2.13 — 2.62
Phosphate (mmol/l)				
With supplements	11	1.09	0.12	0.92 — 1.25
Without supplements	41	0.98	0.15	0.56 — 1.35
Cord				
25-OHD (ng/ml) all cases	52	10.6	3.70	4.7 — 21.5
Phosphate (mmol/l) all cases	52	1.48	0.20	0.96 — 1.86
Albumin (g/l) all cases	52	37.1	3.3	23 — 43
<i>September group</i>				
Maternal				
25-OHD (ng/ml)				
With supplements	9	33.0	10.6	16.2 — 48.6
Without supplements	45	25.1	7.0	14.2 — 42.9
Calcium (corrected) all cases	51	2.4	0.11	2.11 — 2.58
Phosphate (mmol/l) all cases	52	1.14	0.22	0.81 — 1.65
Cord				
25-OHD (ng/ml)				
With supplements	9	22.9	7.1	14.4 — 38.9
Without supplements	45	16.7	5.28	6.2 — 37.3
Phosphate (mmol/l) all cases	49	1.72	0.22	1.29 — 2.45
Albumin (g/l) all cases	50	38.5	3.5	33 — 47

If there was no significant difference for season or for supplements, the results are combined as shown at the top. If significant differences existed for the season the results are given in the lower part of the table. These significant differences are further subdivided according to whether supplements were taken. Numbers vary because not all estimations were available.

Values are given for both corrected and uncorrected calcium. Corrected maternal values are given separately for April and September because of a seasonal difference not present in uncorrected values.

Conversion: traditional to SI units—25-OHD: 1 ng/ml $\approx$ 2.5 nmol/l.

Results

Calcium values were adjusted for albumin concentrations using the method described by Payne *et al.*⁵ Because such a correction has not been universally applied in previous studies, both corrected and uncorrected calcium results are given here. After correcting calcium values there was a significant difference in maternal samples with season and a less significant positive correlation between maternal and cord calcium levels than with the uncorrected results.

Biochemical values are shown in Table 1 and significant differences in Table 2.

In cases where vitamin supplements had not been taken maternal and cord blood 25-OHD levels were significantly higher in September than in April (Figure). Phosphate levels were significantly higher in September in both maternal and cord blood.

Women who had taken vitamin D supplements had significantly higher 25-OHD levels in both April and September. Cord blood 25-OHD was also higher in supplemented cases in September but not in April. Maternal phosphate was higher in the supplemented group in April.

Comparing cord with maternal levels, albumin and calcium (corrected and uncorrected) were higher in cord samples; alkaline phosphatase and total protein were higher in maternal samples. There was no significant difference between maternal and cord magnesium levels.

Where there was no significant difference in measurements between April and September and supplementation had no effect, correlations of

Table 2 Significant differences

	t	P
September compared with April group		
Unsupplemented cases		
Maternal 25-OHD	6.43	< 0.001
Maternal phosphate	2.28	< 0.05
Cord 25-OHD	6.18	< 0.001
Cord phosphate	2.87	< 0.01
All cases—maternal calcium (corrected)	2.09	< 0.05
Supplements compared with no supplements		
April		
Maternal 25-OHD	3.65	< 0.01
Maternal phosphate	2.35	< 0.05
September		
Maternal 25-OHD	2.80	< 0.01
Cord	3.03	< 0.01
Cord compared with maternal levels		
All cases		
Calcium (uncorrected)	26.0	< 0.001
Alkaline phosphatase	7.82	< 0.001
Total protein	5.67	< 0.001
April		
Calcium (corrected)	17.47	< 0.001
Albumin	6.51	< 0.001
September		
Calcium (corrected)	14.46	< 0.001
Albumin	5.87	< 0.001

Figure 25-Hydroxycholecalciferol levels in maternal serum and cord serum in April and September.

maternal with cord values were studied for all 106 cases. There was a highly significant positive correlation for total protein ($r = 0.381$; $P < 0.001$) and for uncorrected calcium ($r = 0.377$; $P < 0.001$). There was a significant correlation for magnesium ($r = 0.308$; $P < 0.01$). After correcting calcium values for albumin there was no significant correlation between maternal and cord levels in April, but there was still a significant correlation in September ($r = 0.387$; $P < 0.01$).

In the unsupplemented cases there was a positive correlation between maternal and cord 25-OHD. This correlation was highly significant in September ($r = 0.648$; $P < 0.001$) and just significant in April ($r = 0.340$; $P < 0.05$).

In both April and September there was a highly significant correlation ($r = 0.668$ and 0.669 respectively; $P < 0.001$) between the maternal 25-OHD level and the difference between maternal and cord levels.

There was no correlation between calcium and vitamin D levels in maternal or cord samples for corrected or uncorrected calcium results.

Follow-up questionnaire. Replies were received from 51 mothers in the April group and from 48 in the

September one. Although some babies had been taken to a doctor in the 6 weeks after delivery, none had developed symptoms suspicious of neonatal hypocalcaemia.

Discussion

Because of the complexity of perinatal vitamin D metabolism we tried to find as normal a group of pregnancies as possible. Primigravidae were chosen because of the possible depletion of maternal vitamin D stores in multigravidae.⁶ Any woman with diabetes or with suspected placental insufficiency was excluded because of the greater likelihood of hypocalcaemia in her infant.⁷ For the same reason, any pregnancy that resulted in a preterm, small-for-dates, or asphyxiated infant was excluded.^{8,9} Any woman with hypertension, pre-eclamptic toxemia, or renal disease was excluded because of possible disturbances in the renal regulation of vitamin D metabolism.¹⁰

Unlike some previous reports^{3,10} most of the women were not taking vitamin D supplements.

Because of the known seasonal variation in vitamin D levels^{3,10-14} our two groups of samples were collected over fairly short periods: the months of April and September. As we were interested in the effect of season on the last trimester of pregnancy our collection periods followed the two periods of the year previously shown to have maximum variation in 25-OHD levels.

Maternal samples were all taken at term in the early stages of labour as there is evidence that 25-OHD, calcium, magnesium, and phosphate levels change during pregnancy.^{7,14-16}

To ensure that all the women had been exposed to the same seasonal influences we chose women who had spent the last trimester in the Bristol area. The Meteorological Office at Filton in Bristol confirmed that there had been more bright sunshine hours in the 3 months preceding September 1979 than in the 3 months preceding April. The year was typical for the area.

Plasma calcium values were fairly constant despite pronounced seasonal variations in 25-OHD. This has previously been shown in healthy non-pregnant subjects¹² and in pregnant women.³ We found no correlation between 25-OHD and calcium values in maternal or cord blood samples and this agrees with previous observations,^{17,18} but a weak positive correlation between 25-OHD and calcium in cord blood has been shown.^{19,20} Rosen *et al.*⁹ found low 25-OHD values too in a group of hypocalcaemic preterm infants and their mothers. The mean corrected calcium level of 2.45 mmol/l (9.8 mg/100 ml) in our April group was lower than

the mean level of 2.51 mmol/l (10.04 mg/100 ml) found in a group of unsupplemented pregnant Asian women with evidence of vitamin D deficiency.²⁰

It may be important to measure ionised calcium in perinatal studies^{7,16,21} although this was not done in our study nor was it done in those quoted in the previous paragraph.

We found no significant difference between maternal and cord values for magnesium, nor was there any variation with season or supplement. There are reports that cord magnesium levels are higher than maternal levels.^{16,22} Hillman and Haddad³ found magnesium levels were lower in summer in white, pregnant women.

The higher levels of phosphate found in September and in supplemented women in April may be related to vitamin D status as Watney *et al.*²³ found significantly higher values of serum phosphorus in Asian mothers taking vitamin supplements compared with those who were not.

Both Watney *et al.*²³ and Brooke *et al.*²⁰ found higher alkaline phosphatase levels in pregnant Asian women who were not taking vitamin D supplements. We found no significant changes in alkaline phosphatase with season or with supplement. Presumably abnormal alkaline phosphatase levels reflect subclinical bone disease secondary to fairly prolonged and severe vitamin D deficiency which we would not expect to find in our patients.

We found a significant increase in maternal and cord levels of 25-OHD in September compared with April. Previous workers did not measure these in carefully controlled groups of normal pregnancies nor did they relate them to other biochemical values in spring and autumn. The most important time to measure serum 25-OHD is at the end of winter. The mean maternal level in our unsupplemented April group was 16.7 ng/ml and the corresponding mean cord level was 10.6 ng/ml. Studies performed in winter or spring are available for comparison. Hillman and Haddad³ quoted a mean 25-OHD level of 16.2 ng/ml in a group of third trimester white women in St Louis in February; an unspecified number was taking vitamin D supplements. Hillman *et al.*¹⁹ found a mean cord value of 14.2 ng/ml for 10 term infants born in February after a varying vitamin intake during pregnancy. Weisman *et al.*¹⁰ noted a maternal mean of 20.0 ng/ml in a group of white women in Florida who had taken 400 IU vitamin D during pregnancy and who delivered in January or February. Paunier *et al.*¹⁷ discovered much lower 25-OHD levels in Switzerland, where samples taken from healthy unsupplemented mothers at delivery had a mean of 9.1 ng/ml.

Fourteen of the 41 unsupplemented women in our April group had levels in the 'low' maternal range

defined by Hillman and Haddad² as 2–15 ng/ml and the April mean was only just above that. Five of these women were in the 'osteomalacic range' quoted by Heckmatt *et al.*¹⁸ as less than 10 ng/ml. However so were a proportion of the healthy, white British subjects reported by Stamp and Round.¹¹ None of our mothers had levels as low as the mean maternal level of 16.2 nmol/l (about 6.5 ng/ml) quoted by Brooke *et al.* for unsupplemented Asians at term.²⁰

Like other workers^{2 10 17 18 20 24} we found that cord 25-OHD was correlated with maternal levels. We also found a highly significant positive correlation between maternal serum 25-OHD and the difference between maternal and cord 25-OHD. This supports the hypothesis that there is an active process regulating fetal 25-OHD levels as has been observed and discussed previously.^{2 17}

Although we found fairly low levels of 25-OHD in our April group, cord calcium levels were not abnormally low, and we found no clinical evidence of hypocalcaemia. Neonatal calcium levels decline in the first 48 hours of life and this decline has been shown to be greater in preterm infants.¹⁹ We did not collect data after cord blood and therefore cannot relate variations in vitamin D status to subsequent changes in the neonate. Low 25-OHD levels have been reported in preterm infants with neonatal hypocalcaemia^{9 19} but there is little evidence directly linking maternal 25-OHD deficiency with early neonatal hypocalcaemia. Watney *et al.*²³ suggested that maternal vitamin D deficiency might influence fetal calcium levels measured on the sixth day of life. In the long term, vitamin D deficiency may contribute to enamel hypoplasia of the teeth¹ and to the development of infantile rickets in Asians in Britain.^{14 20}

Finding normal cord calcium levels does not in itself disprove the need for vitamin D supplementation. The question is whether supplements have any appreciable effect. In 1974 Hillman and Haddad² found little correlation between maternal vitamin D intake and serum 25-OHD but later they showed that this correlation existed in white subjects in winter.³ Other workers have shown that oral vitamin supplementation improves serum 25-OHD levels to a lesser extent than exposure to sunlight.^{11 13} We have shown a significant increase in serum 25-OHD levels in mothers taking supplements although numbers were few and there were wide variations in the supplements taken. Cord blood 25-OHD levels were higher in supplemented cases in September.

Paunier *et al.*¹⁷ found that mothers with high vitamin intakes had higher serum 25-OHD levels and so did their fetuses but the differences were not statistically significant. They also found that mothers with fairly low vitamin D intakes had infants with

lower total plasma calcium levels on the fourth day of life than those born to mothers supplemented with vitamin D. In Asian women in Britain, Brooke *et al.*²⁰ found no effect of vitamin D supplementation on cord blood calcium, but infant calcium levels at 3 and 6 days were significantly higher and the incidence of clinical hypocalcaemia was lower.

It is not possible to predict which women will deliver prematurely and preterm infants may be particularly susceptible to the effects of low maternal 25-OHD levels.^{9 19} We found that levels were low in normal pregnancies in the spring. It is wise to prescribe oral vitamin D supplements to pregnant white women in Britain, at least during the winter. In normal white pregnancies 400–500 IU/day seems adequate and safe.^{1 6 17 25} There are stronger indications for supplementing Asians in Britain²⁶ and 1000 IU of calciferol has been given to them with good effect and no reported toxicity.^{18 20}

We thank the nursing staff in the delivery suite of the Bristol Maternity Hospital for help with the collection of specimens, and the consultant obstetricians for allowing us to study their patients.

References

- 1 Purvis R J, Mackay G S, Cockburn F, *et al.* Enamel hypoplasia of the teeth associated with neonatal tetany: a manifestation of maternal vitamin-D deficiency. *Lancet* 1973; ii: 811–4.
- 2 Hillman L S, Haddad J G. Human perinatal vitamin D metabolism. I. 25-hydroxyvitamin D in maternal and cord blood. *J Pediatr* 1974; 84: 742–9.
- 3 Hillman L S, Haddad J G. Perinatal vitamin D metabolism. III. Factors influencing late gestational human serum 25-hydroxyvitamin D. *Am J Obstet Gynecol* 1976; 125: 196–200.
- 4 Beadle P C, Burton J L, Leach J F. Correlation of seasonal variation of 25-hydroxycalciferol with UV radiation dose. *Br J Dermatol* 1980; 102: 289–93.
- 5 Payne R B, Little A J, Williams R B, Milner J R. Interpretation of serum calcium in patients with abnormal serum proteins. *Br Med J* 1973; iv: 643–6.
- 6 Dent C E, Gupta M M. Plasma 25-hydroxyvitamin D levels during pregnancy in Caucasians, and in vegetarian and non-vegetarian Asians. *Lancet* 1975; ii: 1057–60.
- 7 Pitkin R M. Calcium metabolism in pregnancy: a review. *Am J Obstet Gynecol* 1975; 121: 724–37.
- 8 Tsang R. Early neonatal hypocalcaemia. In: Klaus M H, Fanaroff A A, eds. *Care of the high risk neonate*, second edition. Philadelphia: Saunders, 1979: 233–4.
- 9 Rosen J F, Roginsky M, Nathenson G, Finberg L. 25-dihydroxyvitamin D. Plasma levels in mothers and their premature infants with neonatal hypocalcaemia. *Am J Dis Child* 1974; 127: 220–3.
- 10 Weisman Y, Occhipinti M, Knox G, Reiter E, Root A. Concentrations of 24, 25-dihydroxyvitamin D and 25-hydroxyvitamin D in paired maternal-cord sera. *Am J Obstet Gynecol* 1978; 130: 704–7.
- 11 Stamp T C B, Round J M. Seasonal changes in human plasma levels of 25-hydroxyvitamin D. *Nature* 1974; 247: 563–5.

- ¹² McLaughlin M, Fairney A, Lester E, Raggatt P R, Brown D J, Wills M R. Seasonal variations in serum 25-hydroxycholecalciferol in healthy people. *Lancet* 1974; i: 536-8.
- ¹³ Poskitt E M E, Cole T J, Lawson D E M. Diet, sunlight, and 25-hydroxyvitamin D in healthy children and adults. *Br Med J* 1979; i: 221-3.
- ¹⁴ MacLennan W J, Hamilton J C, Darmady J M. The effects of season and stage of pregnancy on plasma 25-hydroxy-vitamin D concentrations in pregnant women. *Postgrad Med J* 1980; 56: 75-9.
- ¹⁵ Turton C W G, Stamp T C B, Stanley P, Maxwell J D. Altered vitamin D metabolism in pregnancy. *Lancet* 1977; i: 222-5.
- ¹⁶ Reitz R E, Daane T A, Woods J R, Weinstein R L. Calcium, magnesium, phosphorus, and parathyroid hormone interrelationships in pregnancy and newborn infants. *Obstet Gynecol* 1977; 50: 701-5.
- ¹⁷ Paunier L, Lacourt G, Pilloud P, Schlaeppi P, Sizonenko P C. 25-hydroxyvitamin D and calcium levels in maternal, cord, and infant serum in relation to maternal vitamin D intake. *Helv Paediatr Acta* 1978; 33: 95-103.
- ¹⁸ Heckmatt J Z, Davies A E J, Peacock M, McMurray J, Isherwood D M. Plasma 25-hydroxyvitamin D in pregnant Asian women and their babies. *Lancet* 1979; ii: 546-9.
- ¹⁹ Hillman L S, Rojanasathit S, Slatopolsky E, Haddad J G. Serial measurements of serum calcium, magnesium, parathyroid hormone, calcitonin, and 25-hydroxy-vitamin D in premature and term infants in the first week of life. *Pediatr Res* 1977; 11: 739-44.
- ²⁰ Brooke O G, Brown I R F, Bone C D M, et al. Vitamin D supplements in pregnant Asian women: effects on calcium status and fetal growth. *Br Med J* 1980; 280: 751-4.
- ²¹ Levitt M J. Calcium, ionized. Commentary. In: Meites S, ed. *Pediatric clinical chemistry*. Washington DC: American Association for Clinical Chemistry, 1977: 60-1.
- ²² Levitt M J. Magnesium. Commentary. In: Meites S, ed. *Pediatric clinical chemistry*. Washington DC: American Association for Clinical Chemistry, 1977: 163-5.
- ²³ Watney P J M, Chance G W, Scott P, Thompson J M. Maternal factors in neonatal hypocalcaemia: a study in three ethnic groups. *Br Med J* 1971; ii: 432-6.
- ²⁴ Birkbeck J A, Scott H F. 25-hydroxycholecalciferol serum levels in breast-fed infants. *Arch Dis Child* 1980; 55: 691-5.
- ²⁵ American Food and Nutrition Board. Hazards of overuse of vitamin D. *Am J Clin Nutr* 1975; 28: 512-3.
- ²⁶ Anonymous. Rickets in Asian immigrants. *Br Med J* 1979; i: 1744.

Correspondence to Dr C M Verity, Bristol Royal Hospital for Sick Children, St Michael's Hill, Bristol BS2 8BJ.

Received 21 November 1980

British Paediatric Association

Annual meetings

1982	20-24 April	Aviemore Centre, Scotland
1983	12-16 April	York University
1984	10-14 April	York University
1985	16-20 April	York University
1986	15-19 April	York University