25-Hydroxycholecalciferol serum levels in breast-fed infants

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SUMMARY Serum 25-hydroxycholecalciferol levels were measured longitudinally in a series of breast-feeding mothers and their healthy, term infants for up to 6 months after birth. Although levels both in mothers and infants were lower at 6 weeks' postpartum than at delivery and in cord blood, there was little change thereafter with unsupplemented breast feeding. These findings do not support recommendations for routine supplementation of breast-fed term infants with vitamin D.

Despite increasing enthusiasm for breast feeding, there is still controversy about whether breast-fed infants should receive supplementary vitamin preparation. Although some papers concerning vitamin D levels in the neonate have appeared recently, the effect of breast feeding for longer periods has not been reported. The purpose of this study was to find out if several months of breast feeding, without vitamin supplementation, would maintain adequate plasma levels of vitamin D in healthy, term infants.

Materials and methods

The purpose and methods of this study were explained to a group of women attending the antenatal clinic of the local hospital. Although this is the only area maternity unit, we cannot claim that the volunteers necessarily comprised a representative sample of the local population. Any woman with evidence of pregnancy disease was excluded; all infants were born at term and were healthy at birth. No mother or infant received vitamin D supplements during the study.

Maternal venous and cord blood were sampled during the third stage of delivery. Capillary blood samples were obtained from the infants, if possible, at 3 weeks, 3 months, and 6 months after birth. All infants were solely breast fed during the period of study.

Blood samples were collected without anticoagulant, immediately centrifuged, and the sera were stored at -20°C and analysed as soon as possible. 25-Hydroxycholecalciferol (25-OHCC) was measured by a slight modification of the competitive protein-binding method of Preece *et al.*¹⁻² Repro-

ducibility was examined by replicate analysis of a single sample: 10 replicates gave a mean value of $18.1\text{ }\mu\text{g/l}$ (48.4 nmol/l), with a range of 15.8 to 20.5 (42.2 to 54.8 nmol/l), SD 1.5 and coefficient of variation 8.3 . Serum calcium levels were assayed by atomic absorption spectrophotometry, and inorganic phosphate by a molybdate micromethod of O'Brien *et al.*³

Results

For all samples collected, the mean maternal venous serum 25-OHCC level was $28.5\text{ }\mu\text{g/l}$ (76.2 nmol/l) SD 11.8 ($n=45$), with a range of 9.7 to $56.2\text{ }\mu\text{g/l}$ (25.9 to 150.3 nmol/l). This larger sample showed the expected degree of seasonal variation, mean monthly values ranging from $49.3\text{ }\mu\text{g/l}$ (131.8 nmol/l) in February (late summer) to $17.4\text{ }\mu\text{g/l}$ (46.5 nmol/l) in October (end of winter). The correlation between these levels and total monthly hours of sunshine was $r=+0.59$ ($P<0.001$). However systematic changes in level with stages of late pregnancy or lactation may also produce variation. Cord blood samples in 36 of the infants gave a mean value of $28.4 \pm 10.7\text{ }\mu\text{g/l}$ ($75.9 \pm 28.6\text{ nmol/l}$) range 8.8 to $60.9\text{ }\mu\text{g/l}$ (23.5 to 162.8 nmol/l).

In a smaller number of cases, it was possible to follow mother and infant longitudinally from birth for up to 6 months. The values for serum 25-OHCC, calcium, and inorganic phosphate for these mothers and infants are given in Tables 1 and 2. In some instances inadequate sample size precluded mineral analyses; due to the method of blood collection it was not possible to measure alkaline phosphatase levels.

The correlation between paired maternal and cord 25-OHCC levels was high: $r=+0.91$ ($P<0.001$), as

Table 1 Maternal blood values (mean $\pm$ SD)

Serum	Delivery	Postpartum		
		3 weeks	3 months	6 months
25-OHCC ($\mu\text{g/l}$)	32.5 $\pm$ 13.2 (n=14)	26.1 $\pm$ 13.1 (n=12)	22.8 $\pm$ 6.9 (n=8)	24.3 $\pm$ 2.2 (n=3)
Calcium (mmol/l)	2.08 $\pm$ 0.18 (n=14)	2.39 $\pm$ 0.07 (n=4)	2.02 $\pm$ 0.24 (n=4)	2.13 (n=1)
Phosphate (mmol/l)	1.06 $\pm$ 0.27 (n=13)	1.24 $\pm$ 0.23 (n=10)	1.27 $\pm$ 0.16 (n=5)	1.16 (n=2)

Conversion: SI to traditional units—serum calcium: 1 mmol/l $\approx$ 4 mg/100 ml; phosphate: 1 mmol/l $\approx$ 3.1 mg/100 ml; 25-OHCC: 1 nmol/l $\approx$ 0.374 $\mu\text{g/l}$.

Table 2 Blood values (mean $\pm$ SD) in infants

Serum	Cord	3 weeks	3 months	6 months
25-OHCC ($\mu\text{g/l}$)	27.8 $\pm$ 11.1 (n=14)	19.8 $\pm$ 7.3 (n=12)	18.5 $\pm$ 3.7 (n=8)	17.2 $\pm$ 5.2 (n=3)
Calcium (mmol/l)	2.38 $\pm$ 0.30 (n=14)	2.55 $\pm$ 0.10 (n=4)	2.22 $\pm$ 0.17 (n=4)	2.28 (n=1)
Phosphate (mmol/l)	1.55 $\pm$ 0.37 (n=13)	2.06 $\pm$ 0.11 (n=10)	1.82 $\pm$ 0.16 (n=5)	1.69 (n=2)

Figure Relationship between maternal and cord 25-OHCC levels.

shown in the Figure. In virtually every instance maternal levels were slightly higher than cord values. Correlations between maternal and infant levels from 3 weeks' postpartum onwards were not significant, although the number of measurements was small.

Although maternal levels of 25-OHCC did fall in these fully breast-feeding women between delivery and 3 weeks' postpartum, there was no change thereafter. Infants showed a similar trend. In no case was the infant value considered to represent a hazardous level.

Few infants in New Zealand remain solely breast fed at age 6 months. In view of the small number of babies no attempt was made to relate the longitudinal

changes to season. All values for serum calcium and phosphate both in mothers and infants were within recognised limits for age.

Discussion

Harrison⁴ stated: 'The rare cases of rickets seen now in the United States are usually in breast-fed infants whose mothers have failed to realise that human milk is as deficient in vitamin D as is cows' milk.'... 'Babies fed human milk therefore need vitamin D supplements'. Although these forthright statements are based on erroneous premises, it should have been apparent that the implication, that the great majority of the world's infants were vitamin-D deficient, must be absurd.

The fallacy that human milk contained little of the vitamin began when Macy and Kelly⁵ reported a value of 4.2 USPU*/l, although, as with most of their data, this was quoted from a much earlier source⁶⁻⁷ which reported bioassay values for human milk *fat*. It was assumed that a fat-soluble vitamin would be present only in the lipid phase. Lakdawala and Widdowson⁸ have pointed out that this value, equivalent to about 0.1 $\mu\text{g/l}$, was substantially below other contemporary bioassay values for whole milk of up to 1.5 $\mu\text{g/l}$ (for example Drummond *et al.*⁹). This discrepancy was overlooked although Macy and Kelly⁵ themselves commented that 'even without additional vitamin D, rickets is rare in breast-fed full-term infants except in the Negro'.

The demonstration of aqueous-phase cholecalciferol sulphate in human milk¹⁰⁻¹¹ in substantial amounts,^{8,12} coupled with evidence that this derivative is biologically active,¹⁰ has resolved the apparent paradox. Vitamin D sulphate is indeed also present in the aqueous phase of bovine milk.¹³ Furthermore the quantitative findings of Widdowson¹² suggest that a breast-fed infant of about age 3 months could receive about 7.5 μg vitamin D a day, well above the daily level of 2.5–5 μg which was felt to be adequate for most healthy, term infants¹⁴ and from which the very cautious 10 μg recommended daily intake was derived.

In recent years there have been several reports of rickets seen in unsupplemented breast-fed infants, and it has been implied or stated that all breast-fed infants require routine vitamin D supplementation. Several of these reports relate to markedly preterm infants (for example, Lewin *et al.*,¹⁵ Tulloch,¹⁶ Glasgow and Thomas,¹⁷ Davies *et al.*¹⁸). Some of these infants were receiving supplementary vitamin D, others were shown to have adequate blood levels of 25-OHCC. O'Connor¹⁹ reported two cases in term infants: a 4½-month-old black infant who initially

* United States pharmacopoeia units.

had pronounced hypocalcaemia (with convulsive tetany) but without hypophosphataemia; the other infant was not diagnosed until aged 16 months and the relationship to breast feeding was less clear: in this case hypophosphataemia was pronounced but hypocalcaemia was negligible. Castile *et al.*²⁰ reported two cases of 'nutritional' rickets, one of whom was breast fed until 8 months, had vitamin D supplementation until 9 months, and was diagnosed as rachitic at 12 months. Arnaud *et al.*²¹ described 9 cases of infantile rickets seen in Canada. Six of these babies were breast fed. One, aged 2 months, a 'mild' case, had been breast fed for only 1 month, and had diarrhoeal disease. This child was markedly hypocalcaemic but normophosphataemic with only questionable skull demineralisation on x-rays. It was suggested that mild (stage 1) rickets was associated with serum 25-OHCC levels of 18 ± 3 ng/ml (48 ± 8 nmol/l) (quoting their normal range as 36 ± 12 ng/ml (96 ± 32 nmol/l), moderate (stage 2) with 12 ± 2 ng/ml (32 ± 5 nmol/l), and severe (stage 3) about 8 ng/ml (21 nmol/l)). These values seem rather high to be associated with rickets and suggest differences in the way the measurements were taken. Arnaud *et al.*²¹ also described a 6-month-old infant, breast fed and unsupplemented, with early long bone changes, raised alkaline phosphatase, normal parathyroid hormone level, and relatively normal calcium and phosphate levels. Very few authors have reported urinary amino-acid levels, but Glasgow and Thomas¹⁷ noted considerable amino-aciduria in their 4 preterm babies, which certainly did not disappear quickly with adequate treatment. No family metabolic studies were reported.

There is increasing evidence that vitamin D metabolism and action in fetal life are different from those in postnatal life; it is possible that the hormonally-active metabolite may not be 1α , 25-dihydroxycholecalciferol, but for example 24, 25-DHCC.²² In fetal life the principal tasks of such hormonal mechanisms would presumably be to enhance calcium transfer across the placenta, known to be an active process,²³ and to promote bone mineralisation. 24, 25-DHCC, which is bound by transcalfiferin about as strongly as 25-DHCC,⁴¹⁻⁴² although relatively inactive in promoting bone resorption, does strongly promote intestinal calcium absorption²⁴ and by inhibiting parathyroid hormone secretion may enhance bone mineralisation.²⁵⁻²⁶ Thus 24, 25-DHCC might better qualify for fetal use than 1α , 25-DHCC: further work is clearly necessary. However this does mean that vitamin D metabolism in the preterm infant, at least initially, may be fundamentally different from that of term infants. For this reason the present study, and the conclusions therefrom, are confined to term infants.

There is some uncertainty about the influence of late pregnancy on maternal 25-OHCC levels. Turton *et al.*²⁷ reported that nonpregnant white women had a mean 25-OHCC level of 9 ng/ml; 24 nmol/l ($7-11$ ng/ml ($18.7-29.4$ nmol/l) ± 1 SEM after log transformation), and while at 20-30 weeks of pregnancy the mean value was 7 ng/ml; 18.7 nmol/l ($6-9$ ng/ml ($16-24$ nmol/l)), by 30-40 weeks it had returned to 9 ng/ml ($6-12$). All values were performed in February, so seasonal effects were excluded. However of course these are not longitudinal values in individual women.

Weisman *et al.*²⁸ reported mean maternal serum 25-OHCC at term of 20.0 ± 7.0 ng/ml (53.5 ± 18.7 nmol/l) ($\pm$ SD) in white women compared with 31.8 ± 6.7 ng/ml (85.0 ± 17.9 nmol/l) in non-pregnant controls, and 13.8 ± 1.5 ng/ml (36.9 ± 4.0 nmol/l) and 19.4 ± 6.0 ng/ml (51.9 ± 16.0 nmol/l) respectively in black term pregnant and nonpregnant women.

Fairney *et al.*,²⁹ while noting a wide variation in serum 25-OHCC in recently-delivered women, found that such women had a somewhat higher value than men or nonpregnant women in the same season. Lactating women had higher values both then and 4-6 weeks later than similar women who were not lactating. Lactating women just after delivery had a mean value of 30 ng/ml; 80 nmol/l (range 15-44 ng/ml ($40-118$ nmol/l)) and nonlactating women 23 ng/ml; 62 nmol/l (range 18-29 ng/ml ($48-77.5$ nmol/l)). These values were unchanged after 4-6 weeks. The method used did not separate 25-OHCC from 24, 25-DHCC. Serum calcium level (2.3 ± 0.12 mmol/l; 9.2 ± 0.48 mg/100 ml ($\pm$ SD)) and phosphate (1.26 ± 0.27 mmol/l; 3.9 ± 0.8 mg/100 ml) also did not change during the 4-6 week period in lactating women. Calcium absorption from the intestine however is known to be enhanced during pregnancy,³⁰ and prolactin enhances renal 1α -hydroxylation of 25-OHCC.³¹ Plasma transcalfiferin is also raised,³² so it appears that the dynamics of vitamin D metabolism and action are probably much changed in pregnancy and perhaps in lactation too.

There are numerous reports of the levels and relationship of maternal and cord blood 25-OHCC. Rosen *et al.*³³ reported immediate postnatal 25-OHCC levels of 28 ± 2 ng/ml; 75 ± 5 nmol/l (SE) in mothers ($n=11$) and 23 ± 2 ng/ml in their term infants (not cord samples, but collected before any milk feed); the correlation between the two was $r = \pm 0.97$. Hillman and Haddad³⁴ reported in 7 term singletons a mean cord level of about 19 ng/ml; 51 nmol/l (range 6-33 ng/ml ($16-88$ nmol/l)) in cord blood. Levels changed little in the first 2 postnatal weeks. Hillman *et al.*³⁵ reported cord blood 25-OHCC level in 10 term infants of 14.2 ng/ml;

38 nmol/l (SE 2.5) with a range of 4–26 ng/ml (11–70 nmol/l), and at 7 days a mean of 11.4 ± 0.8 ng/ml (30.5 ± 2.1 nmol/l) with a range of 9–16 ng/ml (24–43 nmol/l) in 9 infants. Weisman *et al.*²⁸ reported a mean maternal 25-OHCC at term of 20.0 ± 7.0 ng/ml; 53.5 ± 18.7 nmol/l ($\pm$ SD) and 13.8 ± 1.5 ng/ml (36.9 ± 4.0 nmol/l) in the cord blood of their white infants. Values in blacks were distinctly lower. They found that 24,25-DHCC levels were about 10% of the 25-OHCC levels (2.3 ± 1.1 and 20.0 ± 7.0 ng/ml (5.9 ± 2.8 and 53.5 ± 18.7 nmol/l) respectively). The correlation between maternal and cord values was $r = +0.67$. These were contrasted with values in nonpregnant whites of 31.8 ± 6.7 ng/ml (85.0 ± 17.9 nmol/l) in white and 19.4 ± 6.0 ng/ml (51.9 ± 16.0 nmol/l) in black women. The levels of 24,25-DHCC also tended to be higher in nonpregnant women. The seasonal variation in cord blood 25-OHCC level was stressed by Frdrich *et al.*³⁶ who found a correlation of $r = +0.92$ with hours of sunshine.

At present there are no data available on the quantitative relationship between vitamin D levels in maternal blood and in her milk, although in view of the wide range of 25-OHCC levels seen in healthy adults one might assume that some relationship was likely. While dietary evaluation was not undertaken in these mothers, studies on young adult New Zealand women suggest³⁷ that a median dietary intake of only 0.8 μ g/day would be expected. Clearly skin synthesis is the major source, and maternal 25-OHCC levels suggest that this was fully adequate during pregnancy at least.

An insufficient oral intake of vitamin D is not the sole factor in rickets. Inadequate intake or inappropriate proportions of calcium or phosphate may be important.^{38–43} It is not clear to what extent solar irradiation of the infant itself may provide some vitamin synthesis: there is certainly a tradition favouring this in New Zealand. Finally it is still uncertain to what extent genetic factors determine individual susceptibility to rickets. There is some evidence^{39–40} that the renal tubular aminoaciduria seen in most rachitic infants may in part have a genetic basis, since usually some close relatives of the affected infant manifest excessive urinary amino-acid and sometimes also phosphate excretion; also adequate treatment of the rickets frequently reduces, but does not abolish, the aminoaciduria in the affected infant. Perhaps the genetic anomaly results in susceptibility to rachitic abnormalities at levels of circulating vitamin D little below the normal range.

It is unfortunate that cases of 'nutritional' rickets are still reported without measurement either of serum 25-OHCC levels or of urinary amino-acid excretion. If we assume that the serum 25-OHCC

level accurately reflects adequacy of intake or production of vitamin D as well as of the hepatic hydroxylation step, the present study suggests that in optimum circumstances human milk alone can provide sufficient dietary vitamin D for the needs of the term infant for up to the first 6 months of life. There is thus no justification for the routine administration of vitamin D supplements to such infants, but rather these should be reserved for instances where environmental factors may place the infant in jeopardy.

It is probable that 'dietary vitamin D deficiency' represents an oversimplification of the problem of rickets, and that more detailed investigation into the interrelationship of the vitamin, calcium, and phosphate intake and into hormonal and renal tubular mechanisms is needed.

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