Rickets in very-low-birth-weight infants born at Baragwanath Hospital

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Abstract **Disturbed mineral and bone metabolism has been reported to occur frequently in very-low-birth-weight infants fed breast-milk during the first 3 months of life. This study was designed to assess the prevalence of disturbed mineral homeostasis in a breast-milk-fed very-low-birth-weight population at Baragwanath Hospital and to determine whether the addition of a preterm infant formula to the feeds would reduce this prevalence and increase the rate of weight gain. Fifty-three neonates weighing less than 1 200 g at birth were monitored for weight gain, growth and biochemical and radiological evidence of metabolic bone disease at 2-weekly intervals during hospitalisation and for 18 weeks after discharge. The infants were randomised at 2 weeks of age to receive either breast-milk only, or a combination of breast-milk and a premature formula containing 550 mg calcium and 300 mg phosphorus. All infants received 800 IU vitamin D daily from day 14. Weight gain and growth were similar in both groups. Calcium and phosphorus intakes were higher in the mixed feeding group, but did not affect serum mineral levels. Radiological rickets was uncommon in both groups although periosteal reactions and osteopenia occurred frequently and with similar prevalences. Vitamin D deficiency was not found to be a problem.**

In conclusion, overt rickets is not a major problem in very-low-birth-weight infants born at Baragwanath Hospital, although biochemical abnormalities occur frequently. Feeding with breast-milk and a premature infant formula in equal proportions (as opposed to breast-milk only) does not appear to have any effect on weight gain and growth in very-low-birth-weight infants but does partially prevent the pathological rise in alkaline phosphatase levels. It is therefore recommended that breast-feeding of very-low-birth-weight infants be encouraged, provided they are monitored regularly.

S Afr Med J 1994; **84:** 216-220.

Disturbed mineral and bone metabolism is frequently found in very-low-birth-weight infants fed breast-milk during their first 3 months of life. The spectrum of metabolic bone disease (MBD) ranges from mild biochemical abnormalities with no radiological abnormalities, through mild under-mineralisation to severe rickets with fractures.[1,2] In neonates with a birth weight of less than 1 000 g, hypomineralisation has been reported in about 75% of cases at 3 weeks of age and radiological rickets in 57% of these neonates.[3] The pathogenesis has been attributed to a number of factors including deficiencies of calcium, phosphorus and/or vitamin D;[1,4,5] dietary supplementation with calcium and/or phosphate has been shown to decrease the prevalence of MBD.[6] Given the policy of the neonatal service at Baragwanath Hospital, which is to encourage breast-feeding where possible, and the lack of information on the prevalence of the problem in very-low-birth-weight infants born at the hospital, we decided to study the prevalence of MBD in infants under 1 200 g at birth on the basis of biochemical and radiological evidence, as well as assess the effect of a premature infant formula on the prevalence.

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Accepted 15 Apr 1993.

Methods

Patient selection

All neonates weighing less than 1 200 g born at Baragwanath Hospital over a 9-month period were entered into the study if they survived the first 2 weeks post-delivery. Gestational age was assessed according to maternal dates and the Ballard score within 48 hours of delivery. Exclusion criteria were the presence of congenital abnormalities, infections or any diseases which could themselves cause bone disease.

Infant feeding

For the first 2 weeks neonates were fed according to the routine practices of the Baragwanath Neonatal Unit. Parenteral fluids were administered as clinically indicated and enteral feeds consisted of expressed own mother's milk. At 2 weeks of age the neonates were randomised according to their hospital number and entered into the active phase of the study. Patients with even numbers (control group) received own mother's milk only and those with odd numbers (study group) received breast-milk mixed in equal proportions with the premature infant formula Alprem (Nestle SA). The constituents of the feeds are summarised in Table I. Feed volumes were increased according to the tolerance of the infants to a maximum of 200 ml/kg/d. A weight of 1 800 g was used as the end-point of the randomised study and breast-feeding was then encouraged. Vitamin D supplementation of 800 IU/d was introduced from day 14.

TABLE I.
Calcium, phosphorus and energy content of own mother's milk (OMM), Alprem and mixed feeds (MF)

Constituents	OMM	Alprem	MF
Calcium (mg/l)	255	550	400
Phosphorus (mg/l)	170	300	240
Protein (g/l)	12	24	18
Energy (kcal/l)	650	700	680
Fat (g/l)	33	33	33

Monitoring and management

Management of neonates was determined by the clinician in charge. Postnatal problems were recorded. Parenteral and enteral feeds were recorded daily and

daily calcium, phosphorus and energy intakes were calculated. Two weekly assessments were performed until the infants weighed 1 800 g; thereafter assessments were performed at 6-weekly intervals on three occasions following discharge. Anthropometric measurements (height, weight and skull circumference), serum calcium (total and ionised), phosphorus, magnesium, alkaline phosphatase (AP) and albumin levels were determined at the above intervals. Serum 25-hydroxyvitamin D (25-OHD) and 1,25-dihydroxyvitamin D ($1,25\text{-}(OH)_2D$) were measured at 2 weeks of age, on hospital discharge (at 1 800 g) and at the final follow-up visit. Radiographs of the wrist and distal femur were obtained on hospital discharge and at the final visit, or when the AP level was greater than 750 IU/l at any stage. Radiographs were reviewed blind and the presence of periosteal reactions, osteopenia and/or rickets recorded. Data were analysed by means of analysis of variance and χ^2 procedures.

Results

Fifty-six neonates weighing less than 1 200 g at birth were entered into the study over a 9-month period. Twenty-seven received own mother's milk (controls) and 29 received the mixed feed (study group). Three patients in the control group were subsequently excluded because of incorrect feeding. Comparison of clinical features showed the 2 groups to be similar (Table II). Most of the infants (45/53; 84%) were small for gestational age.

Anthropometric measurements showed similar increases in the 2 groups throughout the study (Table III). Weight gain in both groups followed the expected pattern when plotted on the Dancis Premature Birth Weight Chart.[7] Increase in length was not statistically different between the control and study groups during hospitalisation, although those in the study group were statistically longer at the second and third follow-up visits.

TABLE II.
Comparison of the clinical characteristics of the neonates in the control group and study group

	OMM group	MF group
No. in randomised study	27	29
No. completing hospital study	24	29
No. completing follow-up	16	14
Mean gestational age (wks)	32,8 ± 1,3	32,6 ± 1,3
Mean birth weight (g)	1 095 ± 99	1 091 ± 80
AGA	4	4
SGA	25	20
Multiple pregnancies	4	2
Ventilated	3	2
Septicaemia	6	3
Exchange transfusion	3	2
Enterocolitis	1	1
Hospital stay (days)	52 ± 11	55 ± 14

OMM = own mother's milk; MF = mixed feeds; AGA = appropriate for gestational age; SGA = small for gestational age.

TABLE III.
Changes in weight, length and biochemical data in both groups during the study (mean ± SD)

	Time post-delivery							
Group	2 wks	4 wks	6 wks	8 wks	10 wks	6 wks FU	12 wks FU	8 wks FU
Weight (g)								
Control	1 080 ± 108 (24)	1 275 ± 188 (24)	1 556 ± 245 (23)	1 750 ± 192 (15)	1 933 ± 73 (8)	3 028 ± 465 (18)	4 133 ± 515 (17)	5 060 ± 672 (16)
Study	1 047 ± 108 (29)	1 252 ± 182 (29)	1 558 ± 238 (29)	1 703 ± 205 (18)	1 814 ± 193 (9)	3 156 ± 447 (22)	4 410 ± 638 (16)	5 304 ± 579 (14)
Length (cm)								
Control	38,2 ± 1,3	39,7 ± 1,7	41,6 ± 1,8	42,4 ± 1,5	43,4 ± 1,3	47,8 ± 2,2	52,5 ± 1,9	56,7 ± 2,9
Study	38,1 ± 1,3	39,7 ± 1,4	41,3 ± 1,6	42,3 ± 1,7	42,8 ± 1,8	48,9 ± 1,8	54,1 ± 1,6‡	59 ± 2,8‡
Albumin (g/l)								
Control	31,5 ± 4,4	31,9 ± 4,1	32 ± 3,1	29,6 ± 2,1	28,6 ± 3,5	36,5 ± 5,7	41,2 ± 3,2	45,1 ± 2,4
Study	31,3 ± 4	32,7 ± 3,9	33,6 ± 3	33,6 ± 3,6*	33,7 ± 3,7†	39,1 ± 3,9	42,7 ± 3,3	43,3 ± 3,6
Ionised calcium (mEq/l)								
Control	2,05 ± 0,19	2,15 ± 0,14	2,17 ± 0,11	2,18 ± 0,1	2,16 ± 0,09	2,26 ± 0,2	2,48 ± 0,22	2,54 ± 0,26
Study	2,04 ± 0,15	2,11 ± 0,24	2,19 ± 0,14	2,21 ± 0,15	2,23 ± 0,14	2,35 ± 0,09	2,42 ± 0,08	2,45 ± 0,07
Phosphorus (mmol/l)								
Control	2,23 ± 0,43	2,19 ± 0,31	1,93 ± 0,24	1,75 ± 0,26	1,59 ± 0,36	2,01 ± 0,44	2,0 ± 0,36	2,02 ± 0,36
Study	1,96 ± 0,41	2,09 ± 0,35	1,93 ± 0,22	1,79 ± 0,37	1,58 ± 0,21	2 ± 0,39	2,1 ± 0,44	2,03 ± 0,32
Alkaline phosphatase (IU/l)								
Control	426 ± 152	610 ± 316	744 ± 322	881 ± 435	1 198 ± 486	755 ± 428	510 ± 285	367 ± 169
Study	457 ± 150	486 ± 190	559 ± 275	620 ± 368	755 ± 428*	640 ± 507	570 ± 504	472 ± 491
25-OHD (ng/ml)								
Control	21,7 ± 9,8	ND	ND	ND	20,6 ± 6,6	ND	ND	32,8 ± 11,3
Study	16,9 ± 6,7‡	ND	ND	ND	26,3 ± 9,1†	ND	ND	33,5 ± 8,1
$1,25\text{-}(OH)_2D$ (pg/ml)								
Control	54,8 ± 37,4	ND	ND	ND	121,5 ± 49,9	ND	ND	107,7 ± 45,2
Study	47,9 ± 30,4	ND	ND	ND	128 ± 42,2	ND	ND	108,8 ± 43,3

* Mean value is significantly different from mean in controls, $P < 0,001$.
† Mean value is significantly greater from mean in controls, $P < 0,01$.
‡ Mean value is significantly different from mean in controls, $P < 0,05$ (Student's *t*-test).
ND = not done; FU = follow-up; figures in brackets = number in each group.

TABLE IV.
Comparison of energy, calcium and phosphorus intakes in both groups during hospitalisation

	Weeks post-delivery									
Group	1	2	3	4	5	6	7	8		
Energy intake (kcal/kg/d)										
Control	47 ± 9	101 ± 20	116 ± 30	119 ± 30	129 ± 30	131 ± 11	129 ± 7	129 ± 10	130 ± 7	130 ± 8
	(24)	(24)	(24)	(24)	(24)	(23)	(19)	(15)	(9)	(8)
Study	47 ± 9	102 ± 19	127 ± 20	130 ± 25	132 ±15	137 ± 22	136 ± 23	135 ± 18	145 ± 14	145 ± 4
	(29)	(29)	(29)	(29)	(29)	(29)	(17)	(18)	(8)	(9)
Calcium intake (mg/kg/d)										
Control	19 ± 6	40 ± 14	44 ± 15	47 ± 13	50 ± 9	52 ± 5	51 ± 2	51 ± 4	51 ± 2	51 ± 3
Study	20 ± 4	40 ± 9	69 ± 16*	74 ± 18*	77 ± 12*	79 ± 15*	80 ± 14*	79 ± 13*	81 ± 5*	84 ± 3*
Phosphorus intake (mg/kg/d)										
Control	9 ± 4	28 ± 7	40 ± 5	32 ± 8	34 ± 5	34 ± 3	34 ± 2	34 ± 3	34 ± 2	34 ± 2
Study	9 ± 2	28 ± 5	43 ± 8	46 ± 10*	47 ± 6*	48 ± 8*	48 ± 8*	47 ± 7*	49 ± 3*	51 ± 2*

* Mean value is significantly different from mean in controls, $P < 0,01$ (Student's *t*-test).
Figures in brackets = number in each group.

Daily energy intakes in the 2 groups were similar, but calcium and phosphorus intakes were significantly greater in the study group from week 3 until hospital discharge (Table IV). Intakes of both minerals rose sharply from birth concomitant with an increase in feeding volumes until they reached a plateau at 3 weeks post-delivery.

Serum albumin levels were initially low in both groups and fell further in the control group from 6 - 10 weeks while remaining constant in the study group ($P < 0,01$). After discharge, values in both groups rose progressively to reach normal paediatric values. Serum levels of calcium (total and ionised) and phosphorus did not reflect the greater intakes in the study group. Serum ionised calcium levels in both groups were lowest at 2 weeks of age (approximately 2,05 mEq/l) and increased gradually until 8 weeks (2,2 mEq/l). Following discharge, mean values in both groups increased to normal paediatric values. Serum phosphorus concentrations in both groups decreased post-delivery to their lowest values in 10 weeks (from 2,1 ± 0,4 mmol/l to 1,58 ± 0,3 mmol/l). Levels rose again after discharge and remained constant (2,0 ± 0,4 mmol/l). Serum AP levels increased progressively in both groups, reaching peak values at 10 weeks. The control group tended to have higher levels throughout the period of hospitalisation, but this was only statistically significant at the time of discharge from hospital ($t = 4,3$; $P < 0,0001$). With a value greater than 750 IU/1 taken to be highly suggestive of rickets, the controls had a greater number of infants with values in this range (15/24 compared with 15/29 in study group; $\chi^2 = 6,51$; $P = 0,01$) during the period of hospitalisation.

Serum 25-OHD and 1,25-$(OH)_2$D levels measured at 2 weeks, on hospital discharge and on discharge from the study, are listed in Table III. 25-OHD levels remained constant in the control group during hospitalisation, but rose significantly in the study group. After discharge, values rose in both groups. Throughout the study mean concentrations were within the normal range of 10 - 40 ng/ml. Three of the 24 control infants (12,5%) and 3/29 (10%) infants in the study group had values of less than 10 ng/ml on entry into the study, which are suggestive of a vitamin D-depleted state. Mean 1,25-$(OH)_2$D values rose some 2,5 times during hospitalisation and then remained at that level during follow-up. No differences were noted between the two groups of infants.

The data from the three time points (i.e. 2 weeks of age, hospital discharge and final follow-up) were combined; serum AP levels were found to be inversely related to those of serum phosphate in both groups ($r = -0,5$; $P < 0,001$). A negative correlation was also found between 1,25-$(OH)_2$D and serum phosphate, although it only reached statistical significance in the study group ($r = 0,25$; $P = 0,026$ v. $r = 0,21$; $P = 0,12$ in the control group). There was no correlation between serum AP levels and serum ionised calcium levels in either group (control group: $r = -0,05$; $P = 0,68$; study group: $r = 0,004$; $P = 0,97$). A positive correlation was found between serum AP levels and 1,25-$(OH)_2$D which reached significance in the study group ($r = 0,25$; $P = 0,026$). There was also a positive correlation between 1,25-$(OH)_2$D and serum ionised calcium (control group: $r = 0,28$; $P = 0,03$; study group: $r = 0,37$; $P < 0,01$).

Radiological findings were similar in both groups (Table V). The prevalence of periosteal reactions, which were present in about one-third of patients' radiographs at the time of hospital discharge, had decreased markedly by the last follow-up visit. The prevalence of osteopenia was similar in both groups and remained unchanged throughout the study. Radiological rickets was an infrequent finding and in all cases was mild with minimal loss of metaphyseal plate and slight splaying and irregularity of the metaphysis. There was no difference in AP values in infants with or without evidence of osteopenia (734 ± 386 IU/l and 781 ± 256 IU/l respectively: $t = 0,41$; $P = 0,34$). Those infants with periosteal reactions tended to have higher AP levels (822 ± 432 IU/l) than those without periosteal reactions (677 ± 242 IU/1: $t = 1,31$; $P = 0,098$). Those infants with radiological rickets had significantly higher AP values than those without (1 153 ± 668 IU/l and 709 ± 286 IU/l respectively: $t = 2,73$; $P = 0,005$).

TABLE V.
Prevalence of radiological findings in control and study groups on discharge from hospital and at final follow-up visit

	OMM		MF	
	Discharge	Follow-up	Discharge	Follow-up
Periosteal	9/23	2/15	11/26	0/18
reaction	39%	13%	42%	0%
Osteopenia	6/23	4/15	8/26	5/18
	26%	27%	31%	28%
Rickets	3/23	1/15	0/26	1/18
	13%	7%	0%	6%

OMM = own mother's milk; MF = mixed feed.

Discussion

Metabolic bone disease is a well-recognised problem in premature infants and in particular in very-low-birth-weight infants who are fed breast-milk.[8,9] Radiological rickets, however, is an uncommon finding, although osteopenia and biochemical evidence of MBD (hypophosphataemia and elevated AP levels) are frequently noted in the first 3 months of life.[1,10] The findings in this study confirm that overt rickets is not a major problem in breast-fed very-low-birth-weight infants born at Baragwanath Hospital, although biochemical (raised AP levels) and radiological evidence (osteopenia and periosteal reactions) of MBD occurred frequently. AP is an indicator of osteoblastic activity and bone turnover and levels 5 - 6-fold above normal reflect impaired bone mineralisation and MBD.[11,12] In this study, AP levels were significantly higher in those infants with radiological rickets and were also higher, although not significantly so, in those with periosteal reactions. These findings are suggestive of biochemical and radiological evidence of MBD, although they must be interpreted with reservation in view of the small number of infants affected and the wide standard deviation. The diagnosis of osteopenia is a subjective assessment and is not as reliable a sign of MBD as are periosteal reactions and rickets. The failure to demonstrate a correlation between AP values and the presence of osteopenia is thus not unexpected.

Hillman *et al.*[10] have defined four factors which appear to be important in mineral homeostasis in the very-low-birth-weight infant: birth weight, mineral availability, vitamin D status and post-conceptional age. The premature infant has increased mineral requirements[13] and it is generally accepted in First-World countries that very-low-birth-weight infants should be fed premature milk formula which has been specifically modified to cater for these needs.[8] The policy at Baragwanath Hospital, which serves mainly a disadvantaged population, is to establish breast-feeding in order to minimise long-term nutritional problems. In this study, infants fed breast-milk only, achieved similar weight gain and growth to those fed a combination of premature infant formula and breast-milk. The increased mineral and energy content of the premature formula was effectively diluted by its being combined with breast-milk, and this might explain the lack of marked differences in results between the two groups.

Calcium and phosphorus intakes in both groups were suboptimal compared with recommended intakes of 200 mg/kg/d calcium and 100 mg/kg/d phosphate,[14] although the study group attained higher intakes (95 mg/kg/d calcium; 56 mg/kg/d phosphate) than the control group (50 mg/kg/d calcium; 35 mg/kg/d phosphate). The differences in mineral intake were not reflected in the serum levels of calcium and phosphate. All infants showed a rise in serum levels of both minerals following discharge, which suggests that they were deficient in calcium and phosphate during the period of hospitalisation and that serum values were subnormal, although the normal ranges for very-low-birth-weight infants are uncertain. Further evidence that phosphate deficiency played a role in the pathogenesis of MBD is suggested by the negative correlation between both AP and serum 1,25-$(OH)_2D$, and serum phosphorus. The lack of correlation between serum calcium and AP suggests that calcium deficiency did not play a role in the development of MBD in this study. The finding of a significant positive correlation between serum calcium and 1,25-$(OH)_2D$ suggests that a relative calcium excess existed in the face of hypophosphataemia; this further supports the hypothesis that primary phosphate and not calcium deficiency is the major aetiological factor in the progression of MBD. Furthermore, the biochemical findings of hypophosphataemia, normocalcaemia and raised 1,25-$(OH)_2D$, as well as those of hypophosphaturia, hypercalciuria and normal parathyroid hormone levels which were not measured in this study are evidence of a primary phosphate deficiency.[15]

It was not possible fully to assess the effect of calcium and phosphorus supplementation on the development of MBD in very low-birth-weight infants as neither feed in this study supplied the amount of calcium and phosphorus required to achieve *in utero* accretion rates. However, the study group did have lower alkaline phosphatase values than the control group, which suggests that the additional calcium and phosphorus in the mixed feed did reduce the severity of the perturbations in mineral homeostasis.

As reported by Hillman *et al.*,[10] serum concentrations of 25-OHD are also a determining factor in the development of MBD. 25-OHD is the major circulating form of vitamin D and is thus a reflection of vitamin D status.[16] The premature infant has increased vitamin D requirements (800 IU/d), although no definitive evidence of immaturity in the metabolic pathway of vitamin D has been documented,[17-19] and this probably does not play a major role in the pathogenesis of MBD provided that vitamin D intake is adequate. All infants in this study received 800 IU vitamin D from 2 weeks of age. The mixed feed group had a significant increase in serum 25-OHD levels prior to discharge from hospital, while in the control group the increase only occurred after hospital discharge. The earlier rise in 25-OHD concentrations in the study group could be accounted for by the extra vitamin D content provided in the premature formula (approximately 100 IU/d). The lack of correlation between 1,25-$(OH)_2D$ and 25-OHD levels in both groups at birth and at the end of the study is in keeping with vitamin D repletion.

Decreasing gestational age and birth weight,[3,10] and the presence of systemic disease and acidosis have also been shown to be predisposing factors in the development of MBD. The majority of infants enrolled in and completing this study were small for gestational age, and had minimal postnatal complications considering their birthweights. The infants who were appropriate for gestational age and who weighed under 1 000 g at birth, in whom a higher prevalence of MBD would have been expected, were excluded by virtue of the Baragwanath Neonatal Unit policy of not ventilating neonates with a birthweight of under 1 000 g. These factors might explain the relatively low prevalence of radiological rickets in the population studied.

In conclusion, overt rickets is not a major problem in very-low-birth-weight infants born at Baragwanath Hospital, although biochemical evidence (raised serum AP levels) and radiological evidence (periosteal reactions and osteopenia) of MBD occur frequently. The combination of breast-milk with a specialised premature formula (containing 550 mg calcium and 300 mg phosphorus) showed no major advantage from the point of view of growth or the development of MBD in these infants. Thus, it is recommended that the use of own mother's milk be encouraged with the aim of establishing breast-feeding in very-low-birth-weight infants at Baragwanath Hospital. However, biochemical monitoring, especially of AP, should be performed regularly during the first 3 months of life and those infants with levels greater than 750 IU/l should be further investigated and supplemented with calcium and phosphate if necessary. The benefits of a milk formula with higher calcium and phosphate content were not tested in this study.

The authors wish to thank G. Moodley, M. Cavaleros and D. Zachen for their technical assistance and M. Sefuba for helping with patient follow-up.

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