Archives of Disease in Childhood, 1982, **57**, 447–452

Skeletal changes in preterm infants

W W K KOO, J M GUPTA, V V NAYANAR, M WILKINSON, AND S POSEN

Prince of Wales Children's Hospital, University of New South Wales; Sydney Hospital, University of Sydney, Australia

SUMMARY The skeletal changes in 19 very low birthweight infants (less than 1500 g) were observed from birth to 10 weeks, by means of clinical, biochemical, and radiological techniques. All infants were receiving a supplement of 800 IU vitamin D a day from age 2 weeks. None of the infants showed any specific physical sign of rickets during the period of study. Six infants showed radiological evidence of skeletal demineralisation; 1 of these had severe changes of rickets and 1 had both rickets and fractures. These 6 infants were of shorter gestational periods and lower birthweights than the infants not showing radiological changes. They tended to have more clinical problems and to reach a predetermined volume of feeds (160 ml/kg a day) later than the unaffected infants. Serum alkaline phosphatase values were significantly higher at 5 weeks in the infants with abnormal radiographs than in those without. There were no significant differences between the two groups in relation to serum calcium, inorganic phosphate, 25 hydroxyvitamin D, and immunoreactive parathyroid hormone. The pathogenesis of the skeletal lesions of very low birthweight infants remains unknown.

Radiological changes interpreted to show skeletal demineralisation or rickets have been observed in preterm infants for many years.[1 2] In some patients this has been associated with fractures and respiratory distress.[3–6] Deficiencies of vitamin D,[3 4] calcium,[7 8] and phosphorus,[9 10] and chronic diuretic therapy[11] have been aetiologically implicated. There are insufficient data to determine whether vitamin D deficiency is related to the production of the skeletal changes, and if so, the appropriate amount of vitamin D required to prevent this. This study reports clinical, biochemical, and radiological observations in a group of very low birthweight (VLBW) infants given a uniform, empirical supplement of ergocalciferol (800 IU/day) from age 2 weeks.

Patients and methods

Nineteen VLBW infants were studied. A complete physical examination was carried out by one of us (W K) on at least 2 occasions. The birthweight of these infants ranged from 740 to 1295 g (median 1100). All but one of the infants were of appropriate weight for gestational age (range 26–31 weeks) according to standard intrauterine growth charts.[12] One baby was growth retarded with a birthweight of 1270 g at 36 weeks' gestation. The various clinical problems are listed in Table 1. Ethical approval and informed parental co-operation was obtained for this study.

Table 1 *Clinical assessment of the patients (most patients had more than one problem)*

Problems	*Number*
Respiratory distress requiring	
Increased F_IO_2 for > 24 hours	12 (6)
Mechanical ventilatory support	9 (5)
'Chronic lung disease'	5 (3)
Apnoea	14 (6)
Intraventricular haemorrhage	4 (3)
Patent ductus arteriosus	6 (4)
Retinopathy of prematurity	3 (1)
Anaemia (Hb < 8 g/dl)	9 (5)

Figures in parentheses denote the number of babies with radiological skeletal demineralisation.

Fourteen babies were fed on pooled expressed breast milk for at least a week, 7 of whom were fully breast fed at discharge from hospital. A commercially prepared modified cows' milk formula (Nan, Nestlé Company, Australia) was used if necessary. This had a stated energy content of 3000 kJ/l with calcium, phosphorus, and vitamin D contents of 510 mg, 360 mg, and 520 IU/l respectively. Whenever possible, the infants were fed within 4 hours of birth and given 60 ml/kg a day at hourly intervals for the first 24 hours. The intake of milk was increased and feeding frequency decreased as the infants tolerated the feeds. An intake of at least 160 ml/kg a day was achieved by

day 8 in 11 babies and by day 14 in three. The remaining 5 babies achieved an intake of at least 120 ml/kg a day by day 14 but did not achieve an intake of 160 ml/kg a day until aged 20–34 days. Thereafter, all infants had feeds increased to about 200 ml/kg a day as tolerated.

In infants with low volumetric intake, the caloric content of the milk formula was increased by the addition of a glucose polymer and medium chain triglyceride oil. Supplementary or total parenteral nutrition was administered to 3 patients for 2, 5, and 22 days using a standard regimen.[13] The vitamin D content of the parenteral nutrition fluid was 100 IU/100 ml. All the infants were given a multivitamin preparation containing 800 IU vitamin D (ergocalciferol) from age 14 days. Iron supplementation (6 mg elemental iron/kg a day) was begun at age 6 weeks in all babies. Frusemide (1 mg/kg) was given to 1 patient on 3 occasions and to 3 patients once. No patient had chronic diuretic therapy.

Cord serum from 11 inborn babies was assayed for calcium, 25 hydroxyvitamin D (25 OHD), and immunoreactive parathyroid hormone (iPTH). Blood was collected immediately before a 'morning' feed at ages 5 weeks (n = 17) and 10 weeks (n = 18) for the measurement of serum calcium, inorganic phosphate, alkaline phosphatase (SAP), total protein, 25 OHD, and iPTH. Obvious haemolysis was not observed in any serum sample. Serum calcium, inorganic phosphate, and alkaline phosphatase levels were measured by commercial kits.* Serum protein was measured by the Biuret method.

Serum 25 OHD concentrations were measured by a competitive protein binding assay[14] which does not distinguish between 25 OHD_2 and 25 OHD_3 and which has intra-assay and interassay coefficients of variation of 10.9% and 12.5% respectively. The normal adult range for 25 OHD determined by this assay is between 30 and 220 nmol/l and the range is similar for normal children.

Serum iPTH was determined with a guinea-pig antiserum (Burroughs Wellcome antiserum 211/32) and bovine PTH standards.[15] This antiserum measures predominantly the C terminal end of the PTH molecule although the N terminal portion of the hormone is also detected, with measurable serum iPTH in 60% of normal adults. The minimum detectable concentration is 0·2 μg/l; normal adult values are less than 0·5 μg/l. Normal values for children did not differ greatly from those of normal adults. The intra-assay and interassay coefficients of variation were 6·2% and 9·5% respectively.

Single view radiographs of wrists and ankles including the distal portions of associated long bones were taken at 5 and 10 weeks postnatally and were graded as follows:

Normal. Normal density of bony cortex along shaft with normal dense white line at metaphyses and normal band of lucency in submetaphyseal region (Fig. 1a).

Grade 1. Loss of dense white line at metaphyses, increased submetaphyseal lucency, and thinning of cortex (Fig. 1b).

Grade 2. Changes of grade 1 plus irregularity and fraying of metaphyses, with splaying and cupping—that is changes of rickets (Fig. 1c).

Grade 3. Changes of rickets with evidence of fractures (Fig. 1d).

The assessments were made by two radiologists who had no knowledge of the clinical and biochemical details.

Statistical analysis included standard and paired *t* tests; with respect to serum iPTH, this was carried out only in samples with measurable concentrations.

Results

Radiographical examination showed abnormalities in 6 patients: 4 with grade 1, one with grade 2, and one with grade 3 changes. The baby with grade 3 changes had fractures of the distal right radius and the lower left tibia. All radiological abnormalities were noted only on the radiograph taken 10 weeks postnatally except for 1 infant who had grade 1 changes at both 5 and 10 weeks.

The various clinical problems experienced by the infants are listed in Table 1. All infants had some degree of craniotabes at some time during the period of study but none showed classical features of rickets.[16] Other clinical parameters of the infants with and without radiological abnormalities are shown in Table 2.

The results of the investigations at birth, 5 weeks, and 10 weeks postnatally are shown in Table 3. There was only 1 statistically significant biochemical difference between the infants with normal and those with abnormal radiographs—namely mean SAP in the 5-week specimens was higher in infants with radiological changes. Both patients with radiological rickets and 2 patients without radiological changes had SAP values greater than 400 IU/l at 30°C.

There was no statistical difference between the serum 25 OHD levels of the two groups of patients at birth, at 5 weeks, or at 10 weeks postnatally. However, the postnatal changes in serum 25 OHD levels were different in each group. At 5 weeks,

* Calcium Rapid Stat Kit, Pierce Chemical Company, Illinois, USA; 'Spin Chem' phosphorus (manual) procedure, Smith Kline Instruments Inc. USA; Calbiochem—Behring alkaline phosphatase—SVR, Calbiochem—Behring Corp La Jolla Ca 92037 USA.

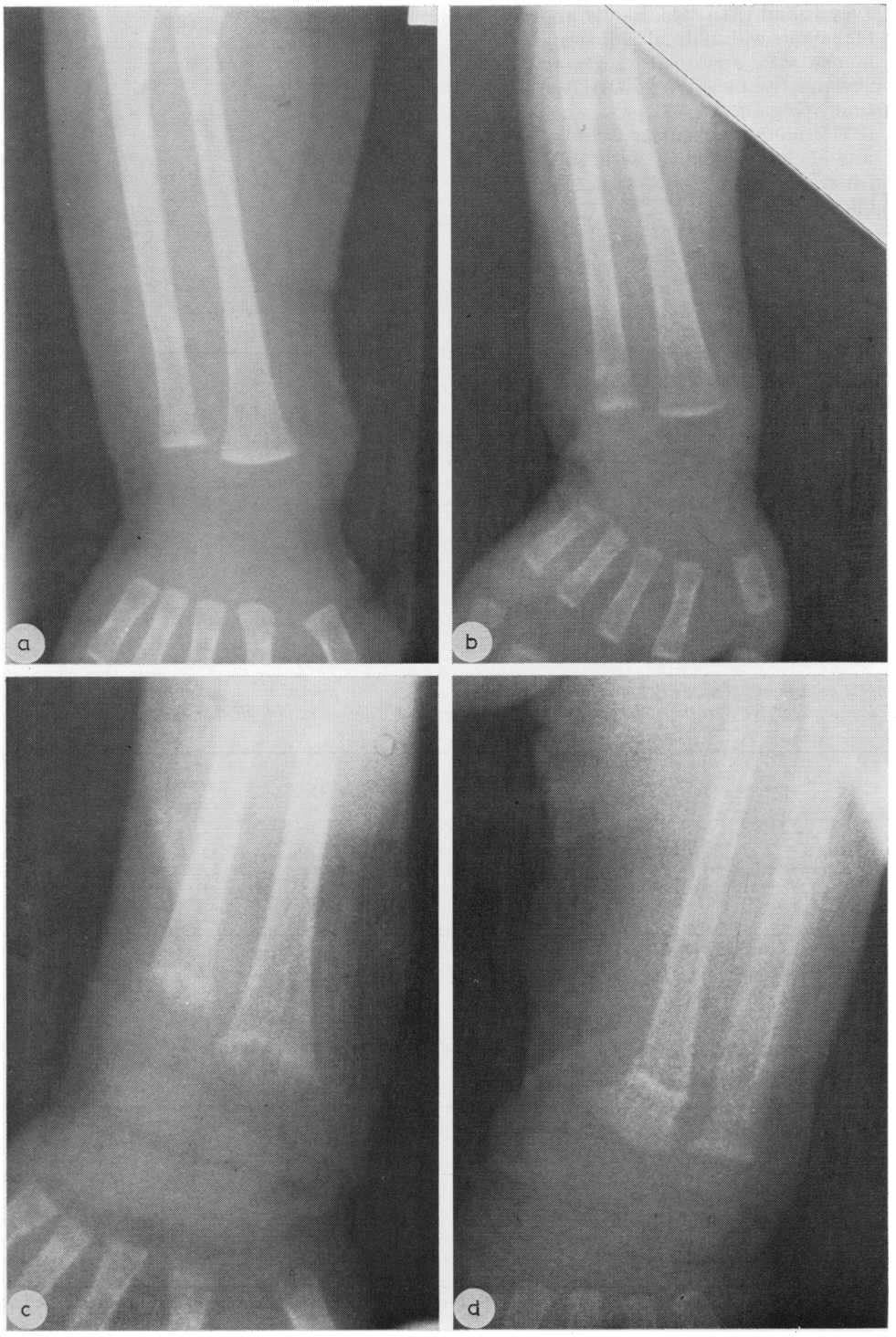


Fig. 1 *Skeletal x-ray changes in VLBW infants receiving vitamin D supplementation: (a) normal, (b) grade 1 changes, (c) grade 2 changes, (d) grade 3 changes.*

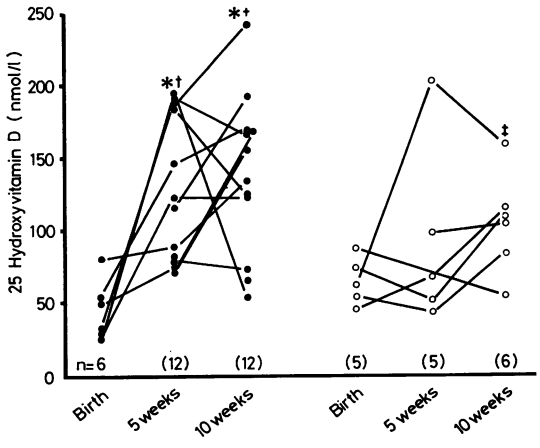
serum 25 OHD levels had risen significantly above cord values in the infants with normal radiographs. In contrast, infants with abnormal radiographs showed no appreciable rise in serum 25 OHD until 10 weeks postnatally (Fig. 2).

Cord serum iPTH values were similar to those of older children and adults. Postnatally, serum iPTH values were widely scattered but tended to be higher than in older children and adults.

Table 2 *Clinical features of VLBW infants without and with radiological skeletal demineralisation (mean ± 1 SD)*

Number of infants	*Birthweight (g)*	*Gestational age (weeks)*	*Age when feeds >160 ml/kg a day (days)*	*Age when birthweight doubles (days)*
Without skeletal demineralisation:				
13	1138±138*	29·2±2·5†	9·6±4·9†	60·6±8·9
With skeletal demineralisation:				
6	893±77	26·8±0·8	19±11·6	56±9·3

* P<0·001 (*t* test), † P<0·05 (*t* test) compared with number of infants with radiological skeletal demineralisation.



● Infants with normal radiograph, ○ infants with abnormal radiograph.

* P<0·001 (*t* test), † P<0·01 (paired *t* test), ‡ P<0·05 (*t* test) compared with values at birth.

Fig. 2. *Postnatal changes in serum 25 hydroxyvitamin D in very low birthweight infants.*

Table 3a *Postnatal changes in serum calcium, inorganic phosphate, alkaline phosphatase, total protein, 25-hydroxyvitamin D, and immunoreactive parathyroid hormone of VLBW infants without radiological skeletal demineralisation (mean ± 1 SD)*

Serum values	*Birth cord blood (n = 6)*	*5 weeks (35·1±2·4 days)† (n = 12)*	*10 weeks (67·8±3·8 days) (n = 12)*
Calcium (mmol/l)	2·35±0·16	2·40±0·09	2·34±0·09
Inorganic phosphate (mmol/l)		1·97±0·24	2·04±0·41
Alkaline phosphatase (IU/l at 30°C)		274±70†	280±116
Total protein (g/l)		45·5±4·4	45·3±4·8
25-OHD (nmol/l)	43·8±20·3	127·9±50·6‡¶	139±55·8‡¶
iPTH* (µg/l)	0·32±0·07	0·64±0·22§	0·73±0·46§

* One serum sample at birth and one at 10 weeks had undetectable iPTH. †P<0·05 (*t* test) compared with patients in Table 3b. ‡ P<0·001 (*t* test), § P<0·05 (*t* test), ¶ P<0·01 (paired *t* test) compared with cord blood.

Table 3b *Postnatal changes in serum calcium, inorganic phosphate, alkaline phosphatase, total protein, 25-hydroxyvitamin D, and immunoreactive parathyroid hormone of VLBW infants with radiological skeletal demineralisation (mean ± 1 SD)*

Serum values	*Birth cord blood (n = 6)*	*5 weeks (37·4±2·2 days) (n = 5)*	*10 weeks (67·2±4 days) (n = 6)*
Calcium (mmol/l)	2·31±0·30	2·34±0·12	2·31±0·09
Inorganic phosphate (mmol/l)		1·93±0·21	1·96±0·23
Alkaline phosphatase (IU/l at 30°C)		373±143	325±58
Total protein (g/l)		42·2±4·7	43·5±3·9
25-OHD (nmol/l)	63±15·9	92±65·3	103·3±35·6†
iPTH* (µg/l)	0·27	0·65±0·22	0·90±0·57

* Four serum samples at birth and one sample at 10 weeks had undetectable iPTH. Five serum samples assayed for iPTH at 10 weeks. † P<0·05 compared with cord blood (*t* test).

Conversion: SI to traditional units—25-OHD 1 nmol/l ≈ 0·4 ng/ml.

Discussion

The vitamin D requirements of preterm infants are not well defined. Early studies[2 17] demonstrated that as little as 100 to 200 IU of vitamin a day prevented biochemical evidence of rickets and sustained normal skeletal growth, but at that time few infants with birthweights of less than 1500 g survived.

This study supports the findings of Glaser *et al.*[2] that physical examination, clinical course, and SAP values are unhelpful in identifying infants with abnormal radiological changes. This study however, suggests that several clinical features support the presence of abnormal radiological features in VLBW infants. Infants with radiological changes were smaller and more preterm at birth and tended to have more problems postnatally. Six of the 8 infants with birthweights less than 1 kg developed abnormal radiographical features whereas none of the 11 infants with birthweights greater than 1 kg did so. In addition, the infants with abnormal radiographical changes reached a minimum daily milk intake of 160 ml/kg a day significantly later than those with normal radiographs.

All infants received the same vitamin D supplementation (800 IU/day) and a similar caloric intake. However, the mineral content of the diet is likely to have been lower in infants with lower milk intake. None of the infants fed exclusively on breast milk was affected radiographically. This may be related to their greater birthweight, fairly uneventful postnatal course, or their ability to achieve a consistently higher daily milk intake. We are unable to comment on the role of parenteral nutrition in the pathogenesis of rickets[18] as only one patient in this study (with normal radiological findings) received prolonged supplementary parenteral nutrition.

The cord serum 25 OHD levels in the present study are similar to those reported by Hillman and Haddad.[19] However, it is difficult to compare the two sets of data since most of the measurements of the 9 VLBW infants reported by Hillman and Haddad[19] were obtained during the first 4 weeks after birth. In addition, Hillman and Haddad[19] used vitamin D supplements ranging from 0 to 400 units a day. In our study serum 25 OHD values of infants with normal radiographs rose rapidly compared with those with abnormal radiographs. These findings suggest that the absorption and 25 hydroxylation of vitamin D are well developed by age 5 weeks in some infants. We are unable to say whether the delayed rise in serum 25 OHD levels in some infants is due to poor absorption or poor hydroxylation of vitamin D2, or whether these low values contribute to the bone changes in these infants.

Extremely low serum 25 OHD and high serum iPTH levels in the presence of rickets in VLBW infants were reported by Hoff *et al.*[20] In contrast, the infants with and without radiological changes in our study have similar serum 25 OHD levels. Our infants began regular vitamin D supplementation earlier than those of Hoff *et al.*[20] The dosage was about double that used by Hoff *et al.*[20] and the daily volume of milk consumption achieved was greater among our patients.

Steichen *et al.*[21] reported raised serum 1,25 dihydroxyvitamin D ($1,25(OH)_2D$) levels in infants with radiological rickets at age 2 months or more. Dihydroxylated vitamin D metabolites assays were not performed in this study but it is well known that serum 25 OHD concentrations correlate poorly with $1,25(OH)_2D$ values.[22]

Difficulties with the technique and interpretation of iPTH assays are well documented.[23] Furthermore, it is difficult to compare our results with those reported by Hoff *et al.*,[20] owing to the fact that the investigations were carried out at different postnatal ages. However, it would appear that serum iPTH increases with age in the postnatal period in VLBW babies. The very high values observed postnatally may reflect a peculiarity of the regulation of peptide hormone secretion[24] or the immaturity of the renal function.[25]

With the use of infant-adapted photon absorptiometry,[21] a more sensitive measure of the degree of bone mineralisation in VLBW infants is now possible and it may ultimately be found that radiographical studies tend to underestimate the extent of the problem. In the study of Steichen *et al.* it has been shown that the addition of calcium and phosphate supplements to the diet can improve the bone mineral content of the VLBW infants.[21] Our data neither support nor refute these findings. It seems likely that the skeletal demineralisation of prematurity is multifactorial, involving vitamin D, minerals, and possibly other factors such as the degree of catabolism associated with initial illnesses the VLBW infants experienced.

We thank Dr K Kozlowski for reviewing the *x*-rays, Ms J Robinson and her staff for carrying out the biochemical assays, and Mrs N Tom for secretarial assistance.

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Correspondence to Dr W Koo, University of Cincinnati Medical Center, Pediatrics: Newborn Division, 231 Bethesda Avenue, Cincinnati, Ohio 45267, USA.

Received 2 November 1981