Plasma active vitamin D concentration in low birthweight infants with rickets and its response to vitamin D treatment

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SUMMARY Impairment of 25-hydroxylation may be a cause of rickets in infants of low birthweight, weighing between 2000 and 2500 g. In addition there may be impairment of 1α -hydroxylation in infants weighing less than 2000 g. Our data show that a supplementary dose of vitamin D₂ of at least 500 IU daily is a reasonable regimen for infants who weighed between 2000 and 2500 g at birth. However for infants who weighed less than 2000 g with a gestation of under 38 weeks, administration of 1α -OHD₃ may be more effective in preventing rickets.

Infantile rickets is often found in low birthweight infants¹ and low concentrations of plasma 25-hydroxyvitamin D (25-OHD) have been reported². Lack of vitamin D due to a decreased supply of nutrients transported across the placenta^{3,4} and to poor intestinal absorption^{5,6} are contributory factors. Such infants moreover, have a greater requirement for vitamin D because of more rapid bone growth.^{7,8} Furthermore, it is likely that impairment of 25-hydroxylation in the liver^{6,9} and of 1α -hydroxylation in the kidney contribute to the presence of rickets in infants of low birthweight. In this study of the pathogenesis of rickets in low birthweight infants we consider whether the administration of vitamin D₂ or its analogue 1α -OHD₃ can prevent rickets.

Materials and methods

A study was made of 147 infants of low birthweight (<2500 g at birth), whose gestation was between 25 and 42 weeks, and for whom parental permission for the study was obtained. All infants were fed a formula comprising the following: calcium 14.35 mmol/l, phosphorus 12.09 mmol/l, and vitamin D₂ 560 IU/l. Each infant was placed in one of three groups: group A infants received no daily supplement of vitamin D apart from that supplied by the formula. Group B infants received 500 IU a day of vitamin D₂ in addition to that supplied by the formula, starting after the first week of life. Group C infants received 0.1-0.15 µg/kg a day of 1α -OHD₃ in

addition to the basic formula, starting after the first week of life. The diagnosis of rickets was made radiographically between ages 3 and 5 months, using the following standard criteria:¹⁰

(1) Roentgenographical evidence of enlargement, cupping, and fraying of epiphyseal line.

(2) Rarefaction and irregular fraying of the zone of provisional calcification of the radius or ulna, with some splaying of the metaphyses.

Samples of blood were taken after a 3-hour fast during autumn, winter, and spring. None of the mothers had hypocalcaemia, hypophosphataemia, or bone disease. Plasma 25-OHD¹¹ and 1,25-dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}$)¹² were measured by using competitive protein binding assay. Plasma (3 ml) was assayed for $1,25(\text{OH})_2\text{D}$ by competitive protein binding assay and intestinal cytosol preparations from rachitic chicks were used as the binding protein. The method involves extraction with dichloromethane chromatography on separated LH-20 (Pharmacia Fine Chemical Company) followed by separation on silicic acid columns with the use of high pressure liquid chromatography (Waters Associates, Milford, Mass). The assays were performed with a known internal standard to determine recovery from the plasma samples. The standard curve incubations were carried out in quadruplicate and the sample incubations were performed in triplicate. With this method the mean concentration for the 9 term infants (1 week-6 months) was 72.0 ± 27.3 (mean $\pm$ SD)¹² pg/ml. Serum calcium, phosphorus, and

alkaline phosphate were measured with an autoanalyser.

Results

Vitamin D intake in each group was measured. The infants in group A had received 50–400 IU of vitamin D₂ a day until aged 3 months and then they received 400–650 IU a day. The vitamin D₂ intake in group B had averaged 50 IU a day until the end of the second week of life, and then was 550–1150 IU a day. The 1 α -OHD₃ intake in group C had been 0.1–0.3 μ g a day since the second week of life, in addition to an average of 470 IU vitamin D₂ a day.

Plasma 25-OHD concentrations at 3–5 months and the incidence of rickets in infants weighing 2000–2500 g at birth are shown in Fig. 1.

Rickets occurred in 14 (37%) of the 38 infants in group A and in 4 (17%) of the 24 infants in group B; no rickets was present in group C. The mean plasma 25-OHD concentration in group B infants was significantly greater than that in group A infants, the mean plasma 25-OHD concentration in group A infants without rickets was significantly greater than that in group A infants with rickets, and the mean plasma 25-OHD concentration in group B infants without rickets was significantly greater than that in infants with rickets, and markedly greater than that in group A infants with rickets.

Plasma 25-OHD concentrations at 3–5 months

Fig. 1 Plasma 25-OHD concentrations (mean $\pm$ SD) at 3–5 months and the incidence of rickets in infants weighing 2000–2500 g at birth.

Fig. 2 Plasma 25-OHD concentrations (mean $\pm$ SD) at 3–5 months and the incidence of rickets in infants weighing less than 2000 g at birth.

Fig. 3 Plasma $1,25(\text{OH})_2\text{D}$ concentrations (mean $\pm$ SD) and the incidence of rickets in infants weighing less than 2000 g at birth.

and the incidence of rickets in infants weighing less than 2000 g at birth are shown in Fig. 2.

Rickets occurred in 17 (55%) of the 31 infants in group A and in 12 (36%) of the 33 infants in group B, but it was present in only 2 of the 13 infants in

Fig. 4 Birthweight and gestational age of infants¹⁷ treated prophylactically with vitamin D₂ and $1\alpha\text{-OHD}_3$ (normal range; Seino et al.¹²).

Table 1 Serum calcium, phosphorus, and alkaline phosphatase levels (mean $\pm$ SD) in infants weighing 2000–2500 g at birth

Rickets	No supplements			Vitamin D ₂ (500 IU/day)			$1\alpha\text{-OHD}_3$ (0.1–0.15 $\mu\text{g/kg}$ a day)		
	Calcium (mmol/l)	Phosphorus (mmol/l)	Alkaline phosphatase (KA units)	Calcium (mmol/l)	Phosphorus (mmol/l)	Alkaline phosphatase (KA units)	Calcium (mmol/l)	Phosphorus (mmol/l)	Alkaline phosphatase (KA units)
Absent	2.45 $\pm$ 0.25 (n=14)	2.21 $\pm$ 0.29 (n=14)	23.6 $\pm$ 8.4 (n=14)	2.55 $\pm$ 0.15 (n=14)	1.98 $\pm$ 0.29 (n=14)	30.8 $\pm$ 11.1 (n=14)	2.60 $\pm$ 0.10 (n=10)	2.02 $\pm$ 0.26 (n=10)	32.6 $\pm$ 21.5 (n=10)
Present	2.35 $\pm$ 0.15 (n=16)	1.98 $\pm$ 0.32 (n=16)	33.7 $\pm$ 0.6 (n=16)	2.55 $\pm$ 0.2 (n=4)	1.98 $\pm$ 0.32 (n=4)	31.1 $\pm$ 3.6 (n=4)	—	—	—
Normal range	2.25–2.75	1.44–2.20	18.8–36.0						

Table 2 Serum calcium, phosphorus, and alkaline phosphatase levels (mean $\pm$ SD) in infants weighing less than 2000 g at birth

Rickets	No supplements			Vitamin D ₂ (500 IU/day)			$1\alpha\text{-OHD}_3$ (0.1–0.15 $\mu\text{g/kg}$ a day)		
	Calcium (mmol/l)	Phosphorus (mmol/l)	Alkaline phosphatase (KA units)	Calcium (mmol/l)	Phosphorus (mmol/l)	Alkaline phosphatase (KA units)	Calcium (mmol/l)	Phosphorus (mmol/l)	Alkaline phosphatase (KA units)
Absent	2.43 $\pm$ 0.25 (n=9)	2.11 $\pm$ 0.32 (n=9)	31.0 $\pm$ 11.0 (n=9)	2.50 $\pm$ 0.15 (n=16)	2.08 $\pm$ 0.19 (n=16)	31.2 $\pm$ 11.5 (n=16)	2.55 $\pm$ 0.20 (n=8)	1.89 $\pm$ 0.22 (n=8)	30.2 $\pm$ 19.0 (n=8)
Present	2.38 $\pm$ 0.25 (n=18)	1.95 $\pm$ 0.42 (n=18)	44.3 $\pm$ 17.6 (n=18)	2.48 $\pm$ 0.10 (n=9)	1.89 $\pm$ 0.13 (n=9)	40.7 $\pm$ 19.6 (n=9)	2.50 (n=3)	1.73 (n=3)	31.9 (n=3)
							2.05	1.57	31.0

group C. Although the mean plasma 25-OHD concentration in group B infants was significantly greater than that in group A infants and the mean plasma 25-OHD concentration in group B infants without rickets was significantly greater than that in group A and group B infants with rickets, the mean plasma 25-OHD concentration in group A and group B infants with rickets was not significantly lower than that in group A infants without rickets.

Plasma $1,25(\text{OH})_2\text{D}$ concentrations and the incidence of rickets in infants weighing less than 2000 g at birth are shown in Fig. 3.

The plasma $1,25(\text{OH})_2\text{D}$ concentrations in group B infants with rickets were significantly lower than those in group B infants without rickets ($P < 0.01$). Although there was a wide variation, the mean plasma $1,25(\text{OH})_2\text{D}$ concentration in group C infants was higher than that in the controls of term infants.

There was not a significant rise of serum calcium in group C infants (Tables 1 and 2).

Birthweights and gestational ages of infants treated prophylactically with vitamin D2 and $1\alpha\text{-OHD}_3$ are shown in Fig. 4. It seems that in preterm infants in whom gestation was less than 38 weeks, $1\alpha\text{-OHD}_3$ prevented rickets better than vitamin D2.

Discussion

It has been suggested that the hydroxylation of vitamin D to 25-OHD, and 25-OHD to $1,25(\text{OH})_2\text{D}$ may be impaired in extremely low birthweight infants.^{6,9} The present study does not prove conclusively that impairment of 25-hydroxylation or poor intestinal absorption of vitamin D exists in infants weighing 2000–2500 g at birth since the administration of vitamin D2 (500 IU/day) significantly increased plasma 25-OHD concentration. However, an oral supplement of 500 IU a day of vitamin D2 did not change the plasma 25-OHD concentration in the infants with rickets. From these results we cannot be sure which is the main cause of rickets—poor intestinal absorption or impairment of 25-hydroxylation—since we did not give intravenous vitamin D supplements to the infants. Hillman *et al.* showed that neither oral nor intravenous vitamin D supplementation increased serum 25-OHD levels in preterm infants,⁹ so we think that a block in hepatic 25-hydroxylation is the more likely alternative. The maternal supply of vitamin D would not seem to be unusual for our subjects; no mother had hypocalcaemia, hypophosphataemia, or bone disease. Nevertheless, in our infants weighing 2000–2500 g at birth the impairment of 25-hydroxylation might have caused rickets.

Although in infants weighing less than 2000 g at birth the administration of 500 IU a day of vitamin D2 significantly increased the plasma 25-OHD concentration, the incidence of rickets in this group was still high (36%). There seems to be an impairment of 25-hydroxylation in these infants, since plasma 25-OHD concentrations were not changed by the administration of vitamin D2. At the same time, higher requirement for vitamin D seems to be causing rickets⁸ in infants weighing less than 2000 g at birth because the mean plasma 25-OHD concentration in group A and group B infants with rickets was not appreciably lower than that in group A infants without rickets. There may be an impairment of 1α -hydroxylation in the rickets of infants weighing less than 2000 g at birth, since plasma $1,25(\text{OH})_2\text{D}$ concentrations were low. It is possible that the conversion of 25-OHD to $1,25(\text{OH})_2\text{D}$ is more depressed in infants weighing less than 2000 g than in infants weighing between 2000 and 2500 g.

$1\alpha\text{-OHD}_3$, which is rapidly converted to $1,25(\text{OH})_2\text{D}_3$,¹³ has been considered a valuable substitute for $1,25(\text{OH})_2\text{D}$.¹⁴ A physiological dose of $1\alpha\text{-OHD}_3$ is considered to be 0.04–0.08 $\mu\text{g/kg}$ a day in children.¹⁵ In vitamin D dependency, which is believed to be the result of deficient 1α -hydroxylation of 25-OHD, treatment with $1\alpha\text{-OHD}_3$ by mouth in small doses, considered to be in the physiological range (0.08–0.1 $\mu\text{g/kg}$ a day), corrected the biochemical and clinical symptoms.¹⁶ In the present study fairly high doses of $1\alpha\text{-OHD}_3$ (0.1–0.15 $\mu\text{g/kg}$ a day) decreased the incidence of rickets in infants weighing less than 2000 g at birth, and increased the plasma $1,25(\text{OH})_2\text{D}$ concentrations. These results support our assumption that an impairment of 1α -hydroxylation as well as of 25-hydroxylation may exist in infants weighing less than 2000 g at birth.

The results of the study suggest that low birthweight infants receiving formula feeds should be maintained on a supplemental vitamin D intake. From these data a supplemental vitamin D2 intake of at least 500 IU a day in addition to that supplied by the typical formula seems to be a reasonable regimen for infants weighing 2000–2500 g at birth. However, for infants weighing less than 2000 g at birth and of gestation below 38 weeks, administration of $1\alpha\text{-OHD}_3$ (0.1 $\mu\text{g/kg}$ a day or more) may be more effective for preventing rickets.

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