

## ORIGINAL ARTICLES

## Randomised controlled trial of vitamin D supplementation on bone density and biochemical indices in preterm infants

M C Backström, R Mäki, A-L Kuusela, H Sievänen, A-M Koivisto, R S Ikonen, T Kouri, M Mäki

### Abstract

**Aims**—To test the hypothesis that a vitamin D dose of 200 IU/kg, maximum 400 IU/day, given to preterm infants will maintain normal vitamin D status and will result in as high a bone mineral density as that attained with the recommended dose of 960 IU/day.

**Methods**—Thirty nine infants of fewer than 33 weeks of gestational age were randomly allocated to receive vitamin D 200 IU/kg of body weight/day up to a maximum of 400 IU/day or 960 IU/day until 3 months old. Vitamin D metabolites, bone mineral content and density were determined by dual energy x-ray absorptiometry, and plasma ionised calcium, plasma alkaline phosphatase, and intact parathyroid hormone measurements were used to evaluate outcomes.

**Results**—The 25 hydroxy vitamin D concentrations tended to be higher in infants receiving 960 IU/day, but the differences did not reach significance at any age. There was no difference between the infants receiving low or high vitamin D dose in bone mineral content nor in bone mineral density at 3 and 6 months corrected age, even after taking potential risk factors into account.

**Conclusions**—A vitamin D dose of 200 IU/kg of body weight/day up to a maximum of 400 IU/day maintains normal vitamin D status and as good a bone mineral accretion as the previously recommended higher dose of 960 IU/day. Vitamin D is a potent hormone which affects organs other than bone and should not be given in excess to preterm infants.

(Arch Dis Child Fetal Neonatal Ed 1999;80:F161-F166)

Keywords: vitamin D; preterm infant; bone mineral density

Metabolic bone disease of prematurity is a common clinical problem in preterm infants.<sup>1</sup> The main aetiological factor is insufficient mineral intake,<sup>2-3</sup> and not vitamin D deficiency. Despite this, the accepted European recommendation is to prescribe preterm infants a

very high vitamin D dose in relation to their body weight.<sup>4</sup> The recommendations for vitamin D supply are different in Europe and the USA. The European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) recommends a dose of 800–1600 IU/day,<sup>4</sup> while the American Academy of Pediatrics (AAP) recommends 400 IU/day.<sup>5</sup> Several studies indicate that a daily vitamin D dose of less than 400 IU/day maintains normal vitamin D status and activity,<sup>6-8</sup> while supplementation at 2000 IU/day for five days gives 1,25 dihydroxy vitamin D (1,25(OH)<sub>2</sub>D) concentrations well above the normal range.<sup>9</sup> Hypervitaminosis D involves a risk of hypercalcaemia, with subsequent complications.<sup>10</sup>

Studies comparing the effects of small and large vitamin D doses on bone mineral content have not been reported before, as far as we are aware. We tested the hypothesis that a vitamin D intake related to the individual's body weight and that is lower than any of these recommendations maintains normal vitamin D status and results in as high a bone mineral content as that attained with the high vitamin D dose recommended by ESPGAN.

### Methods

Forty three preterm infants were enrolled in the study from May 1994 to January 1996. The inclusion criteria were a gestational age under 33 weeks and appropriate weight for gestational age. The only exclusion criterion was major congenital malformation, and the withdrawal criterion was failure to supplement vitamin D, according to protocol. The parents of four enrolled infants failed to do so. Consequently the study was completed in 39 infants. Gestational age was determined from the history of the mother's last menstrual period or ultrasound scan and confirmed with a Ballard examination. Within 48 hours of birth the preterm infants were stratified according to 300 g birthweight ranges and then randomly assigned to receive a vitamin D supplement of 200 IU/kg of body weight/day up to a maximum of 400 IU/day (group A) or 960 IU/day (group B) from the time they tolerated full enteral nutrition until they were 3 months old. The randomisation was concealed from those per-

Department of Paediatrics, Tampere University Hospital, Tampere, Finland  
M C Backström  
R Mäki  
A-L Kuusela  
R S Ikonen

Department of Clinical Chemistry  
T Kouri

UKK Institute, Tampere, Finland  
H Sievänen

Institute of Medical Technology, University of Tampere  
M Mäki

Tampere School of Public Health, University of Tampere, Tampere  
A-M Koivisto

Correspondence to:  
Dr Maria Backström  
Karlebyvägen 1457  
66530, Kvevlax  
Finland.  
Email: maria.backstrom@pp.qnet.fi

Accepted 27 October 1998

forming bone densitometry and determination of serum vitamin D metabolites. The dose in the first group was increased up to a maximum of 400 IU/day as the baby grew, while the dose in group B was kept constant. At 3 months of age the infants in both groups received 400 IU/day. In Finland, infant formulas are enriched in vitamin D. At hospital discharge all parents received written instructions on how to lower vitamin D dose according to the amount of formula used in order to maintain the constancy of the dose. Plasma ionised calcium, serum inorganic phosphate, and alkaline phosphatase were measured every other week during inpatient stay and at 3 and 6 ( $\pm 1$ ) months corrected age (corrected age in weeks = chronological age in weeks - (40 - gestational age in weeks)). Blood samples for parathyroid hormone (PTH) analysis were drawn at week 4. Serum 25 hydroxy vitamin D (25(OH)D) and 1,25(OH)<sub>2</sub>D concentrations were measured from cord blood, at 6 and 12 weeks, and at 3 and 6 ( $\pm 1$ ) months corrected age. Bone densitometry by dual energy x-ray absorptiometry (DXA) was performed at distal and shaft sites of the left forearm at 3 and 6 ( $\pm 1$ ) months of corrected age.

The weight and length of the infants were obtained from clinical charts.

Vitamin D metabolites were measured from 1 ml serum specimens to which tritiated vitamin D<sub>3</sub> derivatives [3H]25(OH)D<sub>3</sub> and [3H]1,25(OH)<sub>2</sub>D<sub>3</sub> were added to monitor recovery throughout the assay. The protein was removed from the samples which were purified using the acetonitrile-C<sub>18</sub> Sep-Pak method.<sup>11</sup> Thereafter the metabolites were further purified and separated using high performance liquid chromatography (HPLC). A LiChrosorb Si 60 (5  $\mu$ m) column (E Merck, Darmstadt, Germany) eluted with hexane-dichloromethane-methanol-isopropanol (76:16:5:3) was used. 25(OH)D was measured using a method based on binding to the competing protein,<sup>12</sup> with serum from a pregnant woman diluted 1 in 20 000 in barbital acetate buffer, pH 8.6, and [3H]25(OH)D. Non-radioactive 25(OH)D served as standard. 1,25(OH)<sub>2</sub>D was measured using a radioreceptor method.<sup>13</sup> Inter- and intra-assay coefficients of variation for each of the metabolites ranged from 11.7 to 14.5%. Samples exceeding a 25(OH)D concentration of 100 nmol/l were diluted to achieve higher precision. The paediatric reference value for 25(OH)D in the laboratory was 30–130 nmol/l, with a lower limit for winter of 17.5 nmol/l.<sup>14</sup> The healthy 95% reference interval used for 1,25(OH)<sub>2</sub>D in the laboratory was 50–215 pmol/l.

Duplicate bone mineral content (mg) and areal bone mineral density measurements (mg/cm<sup>2</sup>) were carried out at the left distal forearm and forearm shaft using DXA (Norland XR-26, Norland Corp., WI) at 3 and 6 ( $\pm 1$ ) months corrected age. The measurement and analysis procedures have been described in detail elsewhere.<sup>15</sup> The DXA analyses were made blind. All scans were taken using general scan software (version 2.2.2) at a scan speed of 10 mm/s and with pixel size 1.0  $\times$  1.0 mm<sup>2</sup>. The

scan width was 5 cm and the total effective dose remained < 1  $\mu$ Sv. The DXA scanner was calibrated daily and its performance continuously followed by our quality assurance protocol.<sup>16</sup> A special software (XRVT, Norland Corp. WI, USA), allowing free adjustment of the bone detection threshold, was used for the analyses. A threshold of 0.040 g/cm<sup>2</sup> was applied in every case, as this was found to be the most appropriate for paediatric DXA measurements in our recent study.<sup>15</sup> To reduce the effect of potential movement artefacts inherent in paediatric measurements, the mean of the duplicated DXA measurements was used, except where one of the measurements was clearly more distorted by movement artefacts than the other; then only the measurement with the best quality was used. For bone mineral content measurements this approach provided an in vivo precision (root mean square coefficient of variation) of 4.9% for the distal forearm and 3.8% for the forearm shaft. For bone mineral density measurements the respective precision was 3.4% and 3.0%.

The DXA scans were taken with the baby lying on its back on the scanner table, the left arm being abducted and in full supination. The hand and elbow were held still by one of the investigators. No sedation was used. Between the repeated measurements, made within 15 minutes, the baby was allowed free movement. The starting point of the scanning was located immediately distal from the radiocarpal joint line to permit detection of the distal endplate of the ulna. The end point was located about 6 cm proximal from the start point to allow the cortical shafts to be measured. The soft tissue point was located in the medial soft tissue region of the forearm.

Bone mineral content (mg) and areal bone mineral density (mg/cm<sup>2</sup>) were determined from two regions of interest: the distal forearm and forearm shaft, which exhibit distinct differences in cortical to trabecular bone ratios. The distal forearm was defined as a box whose length (L<sub>ROI</sub>) was 10% of forearm length (measured with a ruler from the styloid process of the ulna to the tip of the olecranon), and which contained distal regions of both radius and ulna. The distal side of the ROI was parallel to the distal endplate of the ulna. The forearm shaft ROI was also defined as a box with the same L<sub>ROI</sub> located 30% of the forearm length proximal from the distal endplate of the ulna, and containing both the radial and ulnar shafts. The rationale of using the bone length adjusted ROIs was to provide the individual analyses with anatomically comparable bone regions of different-sized bones.

Plasma ionised calcium was analysed using two Ciba Corning Ca<sup>++</sup> Analysers (Halstead, England) adjusted to give similar ionic concentrations. Specimens were obtained in capillary tubes made by the manufacturer.

Serum inorganic phosphate was measured using Vitros 700XR Analysers (Johnson & Johnson Clinical Diagnostics, Rochester, NY, USA) according to the manufacturer's instructions.

Table 1 Group characteristics and anthropometric results in preterm infants receiving different vitamin D doses

|   | Group A 200 IU/kg of body weight/<br>day up to 400 IU/day (n=21) |           | Group B 960 IU/day (n=18) |           | p Value |
|---|--|-----------|---------------------------|-----------|---------|
|   | Median   | Range     | Median                    | Range     |         |
| Gestational age (weeks)                   | 30.3   | 26.7–32.6 | 30.6                      | 24.7–32.9 | 0.19    |
| Duration of parenteral nutrition (days)   | 0  | 0–14      | 0                         | 0–16      | 0.21    |
| Age at full enteral nutrition (days)      | 9  | 4–20      | 7.5                       | 5–15      | 0.12    |
| Duration of assisted ventilation (days)   | 4  | 0–50      | 0                         | 0–60      | 0.01    |
| Duration of oxygen supplementation (days) | 14   | 0–105     | 2                         | 0–115     | 0.06    |
| Infants with chronic lung disease (n)     | 4  |           | 3                         |           |         |
| Weight (g) at birth                       | 1365   | 755–2190  | 1510                      | 735–2250  | 0.45    |
| 3 months corrected age                    | 5955   | 3830–6968 | 5797                      | 4520–7020 | 0.87    |
| 6 months corrected age                    | 7545   | 5400–9090 | 7600                      | 6350–9340 | 0.23    |
| Length (cm) at birth                      | 39.0   | 35.0–45.0 | 40.0                      | 32.0–47.0 | 0.65    |
| 3 months corrected age                    | 60.0   | 52.3–65.0 | 58.7                      | 56.0–62.0 | 0.09    |
| 6 months corrected age                    | 66.3   | 60.6–71.5 | 67.4                      | 62.3–76.0 | 0.41    |
| Head circumference (cm) at birth          | 28.2   | 24.0–32.5 | 29.0                      | 24.5–31.0 | 0.67    |
| 3 months corrected age                    | 41.0   | 38.0–44.0 | 41.0                      | 38.5–43.0 | 0.48    |
| 6 months corrected age                    | 43.4   | 38.5–46.7 | 44.5                      | 42.8–46.5 | 0.05    |

Serum alkaline phosphatase activity was measured at 37°C using a Hitachi 704 Analyser (Boehringer-Mannheim, Mannheim, Germany) according to the recommendation of the Scandinavian Society for Clinical Chemistry 1974.

Serum intact PTH was determined from EDTA plasma by two site immunoradiometric assay (INTACT PTH Parathyroid Hormone Kit, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Blood samples were placed on ice and plasma was separated at +4°C and kept frozen at –20 °C until assayed. Our 5–95% healthy reference interval for intact PTH is 1.0–6.8 pmol/l.

When tolerated, the infants received enteral nutrition according to current ESPGAN recommendations.<sup>4</sup> Unsupplemented breast milk was used when birthweight exceeded 2000 g. For smaller infants the breast milk was supplemented with preterm breast milk fortifiers FM 85 (Nestle, Vevey, Switzerland) or Presemp (Semper, Stockholm, Sweden). Hypocalcaemia or hypophosphataemia was corrected with peroral phosphorus or calcium supplements. The amount of perorally received calcium and phosphorus was calculated once weekly from week 3 onwards during hospital stay.

The data were analysed using the SPSS for Windows statistical software package version 6.1 (SPSS Inc, Chicago, IL). Median and range are given as descriptive statistics for the group characteristics and mineral intakes due to apparently skewed distributions. Intergroup

differences in these were tested using the non-parametric Mann-Whitney test and, when appropriate, cross tabulation and  $\chi^2$  test. Mean and standard deviation (SD) are given as descriptive statistics for the serum concentrations of vitamin D metabolites, bone mineral content and bone mineral density, and the intergroup differences were first tested by unpaired *t* test, the 95% confidence limit (95% CI) being used as the primary statistics. If significant differences in risk factors for developing metabolic bone disease (duration of assisted ventilation and lactation, use of diuretics, sedatives and cortisone, prevalence of respiratory and metabolic acidosis, oral mineral intake, presence of respiratory distress syndrome (RDS), bronchopulmonary dysplasia, sepsis or asphyxia) were found, an analysis of covariance and, when appropriate, two way analysis of variance was used for evaluating the effect on bone mineral data. Correlations between the 25(OH)D concentrations *vs* vitamin D dose, 25(OH)D concentrations and 1,25(OH)<sub>2</sub>D concentrations *vs* bone mineral content and bone mineral density were determined using Pearson's correlation coefficients (*r*). The  $\alpha$ -level was set at 0.05.

As no forearm DXA data on preterm infants were available during the course of this study, we considered an intergroup difference corresponding to one standard deviation a significant difference. Then the total sample size of about 40 infants randomised into two groups

Table 2 Calculated peroral phosphorus and calcium intakes (mg/kg of body weight/day) of preterm infants at different ages receiving either high or low vitamin D doses

|                           | Group A 200 IU/kg of body weight/ up to<br>400 IU/day |        |        | Group B 960 IU/day |        |        | p Value |
|---------------------------|---|--------|--------|--------------------|--------|--------|---------|
|                           | n   | Median | Range  | n                  | Median | Range  |         |
| Phosphorus at week 3      | 21  | 30     | 23–91  | 18                 | 46     | 26–105 | 0.15    |
| 4                         | 16  | 73     | 11–95  | 12                 | 82     | 25–95  | 0.22    |
| 5                         | 14  | 42     | 0–86   | 11                 | 86     | 25–128 | 0.02    |
| 6                         | 14  | 30     | 0–95   | 8                  | 78     | 28–86  | 0.16    |
| 7                         | 11  | 30     | 0–89   | 6                  | 60     | 15–93  | 0.65    |
| Average phosphorus intake | 10  | 46     | 7–87   | 6                  | 75     | 41–86  | 0.13    |
| Calcium at week 3         | 21  | 62     | 20–136 | 18                 | 69     | 54–156 | 0.11    |
| 4                         | 16  | 85     | 13–142 | 12                 | 122    | 53–170 | 0.16    |
| 5                         | 14  | 76     | 0–128  | 11                 | 128    | 53–157 | 0.02    |
| 6                         | 14  | 59     | 35–141 | 8                  | 124    | 58–147 | 0.09    |
| 7                         | 11  | 60     | 23–133 | 6                  | 105    | 31–138 | 0.51    |
| Average calcium intake    | 10  | 82     | 25–131 | 6                  | 114    | 75–128 | 0.16    |

Table 3 Serum 25(OH)D and 1,25(OH)<sub>2</sub>D concentrations in preterm infants at different ages receiving either high or low vitamin D doses

|   | Group A 200 IU/kg of body weight/day up to 400 IU/day |              | Group B 960 IU/day |              |                          |
|---|---|--------------|--------------------|--------------|--------------------------|
|   | n   | Mean (SD)    | n                  | Mean (SD)    | Mean difference (95% CI) |
| 25(OH)D from umbilical cord                 | 6   | 29.8 (10.0)  | 8                  | 29.2 (11.8)  | 0.6 (-12.5 to 13.7)      |
| chronological age 6 weeks                   | 15  | 45.7 (18.4)  | 11                 | 66.7 (30.3)  | -21.0 (-40.7 to -1.3)    |
| chronological age 12 weeks                  | 10  | 79.1 (30.0)  | 6                  | 113.5 (37.7) | -34.4 (-70.8 to 2.1)     |
| corrected age 3 months                      | 9   | 87.0 (18.1)  | 16                 | 93.4 (36.4)  | -6.4 (-25.7 to 12.8)     |
| corrected age 6 months                      | 19  | 86.4 (23.9)  | 16                 | 90.2 (26.8)  | -3.8 (-21.3 to 13.6)     |
| 1,25(OH) <sub>2</sub> D from umbilical cord | 6   | 43.8 (21.5)  | 8                  | 69.2 (25.6)  | -25.8 (-53.6 to 2.8)     |
| chronological age 6 weeks                   | 13  | 120.5 (64.7) | 11                 | 138.8 (62.0) | -18.3 (-72.3 to 35.6)    |
| chronological age 12 weeks                  | 9   | 178.0 (66.8) | 6                  | 160.2 (79.8) | 17.8 (-64.3 to 100.0)    |
| corrected age 3 months                      | 16  | 150.1 (46.6) | 15                 | 135.6 (55.9) | 14.4 (-23.2 to 52.2)     |
| corrected age 6 months                      | 19  | 110.2 (28.2) | 15                 | 162.3 (39.6) | -52.2 (-75.7 to -28.4)   |

provided 90% statistical power to detect the aforementioned difference at the  $\alpha$ -level of 0.05.

The study was approved by the ethics committee of Tampere University Hospital and written informed consent was obtained from the parents.

### Results

The group characteristics and anthropometric results of the infants are given in table 1. The groups were matched for birthweight and gestational age. There were no differences in weight and length at 3 and 6 months of corrected age. The infants receiving vitamin D 200 IU/kg/day seemed to have smaller head circumference at 6 months.

The infants receiving vitamin D 200 IU/kg/day had more episodes of RDS and thus needed longer assisted ventilation (table 1), received longer morphine treatment, and had respiratory acidosis more often (data not shown). This also accounts for the smaller mineral intake in the preterm infants receiving the smaller vitamin D dose (table 2). There were no differences between the study groups for serum inorganic phosphate, plasma ionised calcium, serum alkaline phosphatase concentrations or serum intact parathyroid hormone (data not shown).

#### SERUM VITAMIN D METABOLITE CONCENTRATIONS

The serum 25OHD concentrations tended to be higher in infants receiving vitamin D 960 IU/day, but the differences reached significance only at the age of 6 weeks (table 3). Altogether, six preterm infants had serum 25OHD concentrations above the upper reference level and

five of these represented the group receiving the higher vitamin D dose. At birth, three out of 14 infants had a serum 25OHD concentration below the lower reference level. When vitamin D substitution was started these values had returned to normal even in the group with lower vitamin D intakes. The vitamin D dose of 200 IU/kg/day was evidently adequate as none of the serum 25OHD concentrations was below the lower reference limit at any age after birth.

The mean serum 1,25(OH)<sub>2</sub>D concentrations at birth, at 6 and 12 weeks, 3 and 6 months were within the reference limits in both groups (table 3). In the group receiving the lower vitamin D intake one out of 13 infants at week 6 had 1,25(OH)<sub>2</sub>D concentrations below the lower reference limit. In the higher vitamin D intake group, two out of 11 infants at week 6 and one out of six infants at week 12 had 1,25(OH)<sub>2</sub>D concentrations below the lower reference limit. At every time point two to three infants had serum 1,25(OH)<sub>2</sub>D concentrations above the upper reference limit in both groups. There was no correlation between 25OHD and 1,25(OH)<sub>2</sub>D at any time.

#### BONE MINERAL CONTENT AND DENSITY

The results of the bone mineral content and density measurements at 3 and 6 months of corrected age are shown in table 4. There were no significant intergroup differences in these measurements at forearm shaft or distal forearm at any age. There was no significant correlation at any time point between serum 25OHD or 1,25(OH)<sub>2</sub>D concentrations and bone mineral density. In contrast, there was a positive effect of a high mean calcium and phosphorus intake on forearm shaft bone mineral density at 3 months of age ( $r = 0.36$ ,  $p = 0.03$  for calcium and  $r = 0.34$ ,  $p = 0.03$  for phosphorus). Despite the fact that the preterm infants in the lower vitamin D dose group seemed to have more episodes of RDS, required longer assisted ventilation, and longer morphine treatment, had respiratory acidosis more often and a smaller mineral intake in general, these infants had as high bone mineral content and bone mineral density as the preterm infants on the higher ESPGAN recommended vitamin D dose even after controlling for the above variables.

### Discussion

ESPGAN recommends a vitamin D dose of 800–1600 IU/day for preterm infants,<sup>4</sup> based

Table 4 Bone mineral content (BMC) (mg) and bone mineral density (BMD) (mg/cm<sup>2</sup>) at 3 and 6 months corrected age measured by dual energy x-ray absorptiometry at forearm shaft and distal forearm in preterm infants receiving different vitamin D doses

|                                 | Group A 200 IU/kg of body weight/day up to 400 IU/day (n=21) | Group B 960 IU/day (n=18) |                          |
|---------------------------------|--|---------------------------|--------------------------|
|                                 | Mean (SD)  | Mean (SD)                 | Mean difference (95% CI) |
| Forearm shaft: BMC at 3 months  | 255 (49)   | 258 (39)                  | -3 (-32 to 27)           |
| 6 months                        | 326 (67)   | 328 (56)                  | -2 (-42 to 39)           |
| BMD at 3 months                 | 135 (16)   | 139 (9)                   | -4 (-12 to 5)            |
| 6 months                        | 150 (17)   | 152 (18)                  | -2 (-14 to 9)            |
| Distal forearm: BMC at 3 months | 201 (37)   | 208 (33)                  | -7 (-29 to 16)           |
| 6 months                        | 274 (50)   | 272 (46)                  | -1 (-29 to 33)           |
| BMD at 3 months                 | 109 (14)   | 113 (9)                   | -4 (-12 to 4)            |
| 6 months                        | 120 (13)   | 124 (11)                  | -4 (-11 to 4)            |

on, among others, the studies of Glorieux *et al*<sup>6</sup> and Salle *et al*.<sup>17</sup> They suggested that a vitamin D dose of 400 IU/day is insufficient and that vitamin D requirements in preterm infants are at least three times higher. However, the control group in their studies received no vitamin D at all, while the study group received 2100 IU/day of vitamin D. The infants received a minimal phosphorus enrichment (60 mg of phosphorus/100 ml human milk). In our present study we questioned the ESPGAN recommended high vitamin D dose, as it seems to be merely a recommendation and not an established requirement based on results from clinical intervention trials.

On the other hand, the American Academy of Paediatrics recommends a vitamin D dose of 400 IU/day for preterm infants.<sup>5</sup> A daily dose of 400 IU of vitamin D maintains adequate serum 25OHD concentrations.<sup>6,7</sup> A recent American study has shown that even as little as 160 IU/day of vitamin D for 24–29 days maintains normal and stable vitamin D status provided the preterm infants receive adequate mineral intake.<sup>8</sup> The effect of the small vitamin D dose on bone mineral density was not investigated, as DXA measurements were not available when their study started. This latest American low dose study prompted us to elucidate the effects of such a small vitamin D intake on vitamin D status and bone mineral content and density, as determined by DXA, while currently recommended mineral supplementation was used to ensure optimal bone mineral accretion.

Maternal nutritional influences are potentially important in the extrapolation of these data to other populations. European studies<sup>9–18</sup> show low concentrations of serum 25OHD concentrations from cord blood compared with findings in studies conducted in the USA.<sup>19–20</sup> Dairy products are supplemented with vitamin D in the USA, but not in Europe. This probably accounts also for different vitamin D dose recommendations for preterm infants between these regions. However, our study clearly shows that irrespective of low 25-OHD concentrations in cord blood, serum vitamin D is adequately corrected for the small weight related vitamin D dose in this Finnish population.

Pittard and co-workers<sup>6</sup> found no toxic serum 25OHD concentrations in preterm infants receiving 400 IU/day of vitamin D. In our study six preterm infants had serum 25OHD concentrations above the upper reference limit, five of whom belonged to the study group receiving the high ESPGAN recommended vitamin D dose of 960 IU/day. Overall, the serum 25OHD concentrations tended to be higher in infants receiving vitamin D at 960 IU/day.

In our study the serum 1,25-(OH)<sub>2</sub>D concentrations at birth, at 6 and 12 weeks, and at 3 and 6 months were within the reference limits in both groups, indicating sufficient 1-hydroxylation of vitamin D even with a small vitamin D dose. The high serum 1,25-(OH)<sub>2</sub>D concentrations seen in both groups probably reflect a state of relative mineral deficiency, but

### Key points

- The ideal vitamin dose for preterm infants is controversial
- A directly administered vitamin D dose of 200 IU/kg of body weight/day (maximum 400 IU/day) maintains normal vitamin D status and does not endanger bone mineral accretion in preterm infants supplemented with minerals
- Vitamin D administration should be precise and based on evidence based criteria, analogous to those for other potent hormones

the detrimental effects on bone must be kept in mind. Our results clearly show that a vitamin D dose of 200 IU/kg/day is adequate, as none of the 25OHD concentrations was below the lower reference limit at any age after birth and the 1-hydroxylation of vitamin D is comparable with that seen in the group receiving 960 IU/day.

Studies comparing the effects of a small and high vitamin D doses on bone mineral accretion have not been reported before, as far as we are aware. We have shown for the first time that a vitamin D intake that is tailored to individual body weight and lower than any of the recommended doses, results in as high a bone mineral density and content as the high vitamin D dose recommended by ESPGAN. There was no significant difference between the groups for either bone mineral or bone density content at 3 and 6 months of corrected age. Due to the small sample size and relatively wide 95% confidence intervals, however, we cannot preclude the possibility that a real and considerable intergroup difference might still exist. The risk factors for developing metabolic bone disease of prematurity seemed to be more prevalent in preterm infants receiving the low vitamin D dose; nevertheless, these infants had as high a bone mineral and bone density content as the preterm infants receiving the high vitamin D dose.

Vitamin D should be administered directly into the mouth and not added to the milk. Vitamin D is a fat soluble vitamin and might adhere to the surface of feeding bottles. When human milk is administered through a feeding tube, losses of as much as 50% of the fat content can occur.<sup>21,22</sup> There has been discussion as to whether vitamin D should be dosed with a margin of safety.<sup>8</sup> We claim, however, that vitamin D administration should be precise and based on established requirements as are other potent hormones. There are no data to support the belief that preterm infants need a disproportionately high vitamin D dose in relation to their weight. Preterm infants can 1-hydroxylate vitamin D from an early age.<sup>8,9,17,23</sup> 1,25 (OH)<sub>2</sub>D is the most biologically active metabolite of vitamin D. Its main function is stimulation of intestinal calcium and phosphorus absorption. It is also a potent factor in bone resorption.<sup>24,25</sup> Hypervitaminosis D involves a risk of hypercalcaemia, hypercalciuria, polyuria, dehydration, hypertension,

stones in the lower urinary tract and metastatic calcifications.<sup>10</sup> Moreover, it is now recognised that even a wide variety of tissues and cells unrelated to calcium metabolism are target sites for 1,25(OH)<sub>2</sub>D and that the tissues respond in a variety of ways.<sup>26-31</sup> Thus vitamin D affects other organs besides bone, being a steroid type molecule with potent mitogenic effects. This is also one of the reasons why vitamin D should not be given in excess to preterm infants.

In conclusion, we recommend a directly administered vitamin D dose for preterm infants of 200 IU/kg body weight/day up to a maximum of 400 IU/day. This dose does not give too high or too low serum 25OHD concentrations and results in as good a bone mineral accretion as the higher dose of 960 IU/day, recommended by ESPGAN. This is provided that calcium and phosphorus are also given to ensure appropriate bone mineral gain.

- Takada M, Shimada M, Hosono S, et al. Trace elements and mineral requirements for very low birth weight infants in rickets of prematurity. *Early Hum Devel* 1992;29:333-8.
- Horsman A, Ryan SW, Truscott JG. Bone mineral content and body size 65 to 100 weeks postconception in preterm and fullterm infants. *Arch Dis Child* 1985;64:1579-86.
- Steichen JJ, Gratton TL, Tsang RC. Osteopenia of prematurity: the cause and possible treatment. *J Pediatr* 1980;96:528-34.
- ESPGAN Committee on nutrition of the preterm infant: Nutrition and feeding of preterm infants. *Acta Paediatr Scand* 1987;suppl 336:1-14.
- AAP Committee on Nutrition. *Pediatric Nutrition Handbook*. Elk Grove Village, Ill: American Academy of Pediatrics, 1985.
- Pittard WBIII, Geddes KM, Husley TC, Hollis WB. How much vitamin D for neonates? *Am J Dis Child* 1991;145:1147-9.
- Cooke R, Hollis B, Conner C, Watson D, Werkman S, Chesney R. Vitamin D and mineral metabolism in the very low birth weight infant receiving 400 iu of vitamin D. *J Pediatr* 1990;116:423-8.
- Koo WW, Krug-Wispe S, Neylan M, Succop P, Oestreich AE, Tsang RC. Effect of three levels of vitamin D intake in preterm infants receiving high-mineral containing milk. *J Ped Gastroenterol Nutr* 1995;21:182-9.
- Glorieux FH, Salle BL, Delvin EE, David L. Vitamin metabolism in preterm infants: Serum calcitriol values during the first five days of life. *J Pediatr* 1981;99:640-3.
- Chesney RW. Requirements and upper limits of vitamin D intake in term neonate, infant and older child. *J Pediatr* 1990;116:159-66.
- Turnbull H, Trafford DJH, Makin HLJ. A rapid and simple method for the measurement of plasma 25-hydroxyvitamin D<sub>2</sub> and 25-hydroxyvitamin D<sub>3</sub> using Sep-Pak<sub>18</sub> cartridges and a single high performance liquid chromatographic step. *Clin Chim Acta* 1982;120:65-76.
- Parviainen MT, Savolainen KE, Korhonen PH, Alhava EM, Visakorpi JK. An improved method for routine determination of vitamin D and its hydroxylated metabolites in serum from children and adults. *Clin Chim Acta* 1981;114:233-47.
- Reinhardt TA, Horst RL, Orf JW, Hollis BW. A microassay for 1,25-dihydroxyvitamin D not requiring high performance liquid chromatography: Application to clinical studies. *J Clin Endocrinol Metab* 1984;58:91-8.
- Ala-Houhala M, Parviainen MT, Pyykkö K, Visakorpi JK. Serum 25-hydroxyvitamin D levels in Finnish children aged 2 to 17 years. *Acta Paediatr Scand* 1984;73:232-6.
- Sievänen H, Backström MC, Kuusela A-L, Ikonen RS, Mäki M. Dual Energy X-ray absorptiometry of the forearm in preterm and term infants: evaluation of the methodology. *Pediatr Res* 1999;45:100-5.
- Sievänen H, Oja P, Vuori I. Scanner-induced variability and quality assurance in longitudinal dual-energy X-ray absorptiometry measurements. *Med Phys* 1994;21:1795-805.
- Salle BL, Glorieux FH, David L, Meunier G. Vitamin metabolism in preterm infants: Serial serum calcitriol values during the first five days of life. *Acta Paediatr Scand* 1983;72:203-6.
- Mawer EB, Stanbury SW, Robinson MJ, James J, Close C. Vitamin D nutrition and vitamin D metabolism in the premature human neonate. *Clin Endocrinol* 1986;25:641-9.
- Moyer-Mileur L, Chan GM, Gill G. Evaluation of liquid or powdered fortification of human milk on growth and bone mineralization status of preterm infants. *J Pediatr Gastroenterol Nutr* 1992;15:370-4.
- Pittard WB, Geddes KM, Husley TC, Hollis BW. Osteocalcin, skeletal alkaline phosphatase and bone mineral content in very low birth weight infants: A longitudinal assessment. *Pediatr Res* 1992;31:181-5.
- Greer FR, McCormick A, Loker J. Changes in fat concentration of human milk during delivery by intermittent bolus and continuous mechanical pump infusion. *J Pediatr* 1984;105:745-9.
- Stocks RJ, Davies DP, Allen F, Sewell D. Loss of breast milk nutrients during tube feeding. *Arch Dis Child* 1985;60:164-6.
- Santerre J, Salle B. Renal aspects of calcium and phosphorus metabolism in preterm infants. *Biol Neonate* 1988;53:220-9.
- Wong GL. Skeletal effects of parathyroid hormone. In: Peck W A, ed. *Bone and mineral research*. Amsterdam: Elsevier, 1988:103-30.
- Sekamoto S, Sekamoto M. Bone collagenase, osteoblasts and cell mediated bone resorption. In: Peck W A, ed. *Bone and mineral research*. Amsterdam: Elsevier, 1986:49-102.
- Yang S, Smith C, Prah J, Luo X, DeLuca HF. Vitamin D deficiency suppresses cell-mediated immunity in vivo. *Arch Biochem Biophys* 1993;303:98-106.
- Hustmyer FG, Girasole G, Manolagas SC. Signal dependent pleiotropic regulation of lymphocyte proliferation and cytokine production by 1,25-dihydroxyvitamin D<sub>3</sub>: potent modulation of the hormonal effect by phorbol esters. *Immunology* 1992;77:520-6.
- Horiuchi H, Nagata I, Takahashi K, Tsuchimoto TM, Komoriya K. 1,25-dihydroxyvitamin D<sub>3</sub> inhibits thromboxane release from activated macrophages. *Res Commun Chem Pathol Pharmacol* 1992;78:235-43.
- Bargman JM, Silvermann ED, Klein MH. Effect of 1,25-dihydroxyvitamin D<sub>3</sub> in vivo on circulating T-lymphocytes. *Miner Electrolyte Metab* 1989;15:359-64.
- O'Connell TD, Simpson RU. Immunochemical identification of the 1,25-dihydroxyvitamin D<sub>3</sub> receptor protein in human heart. *Cell Biol Intern* 1996;20:621-4.
- Puchacz E, Stumpf WE, Stachowiak EK, Stachowiak MK. Vitamin D increases expression of the tyrosine hydroxylase gene in adrenal medullary cells. *Braun Res* 1996;36:193-6.



## Randomised controlled trial of vitamin D supplementation on bone density and biochemical indices in preterm infants

M C Backström, R Mäki, A-L Kuusela, et al.

*Arch Dis Child Fetal Neonatal Ed* 1999 80: F161-F166  
doi: 10.1136/fn.80.3.F161

---

Updated information and services can be found at:  
<http://fn.bmj.com/content/80/3/F161.full.html>

- 
- These include:*
- References** This article cites 28 articles, 3 of which can be accessed free at:  
<http://fn.bmj.com/content/80/3/F161.full.html#ref-list-1>
- Article cited in:  
<http://fn.bmj.com/content/80/3/F161.full.html#related-urls>
- Email alerting service** Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

- 
- Topic Collections** Articles on similar topics can be found in the following collections
- [Child health](#) (992 articles)
  - [Infant health](#) (591 articles)
  - [Neonatal health](#) (636 articles)
  - [Clinical diagnostic tests](#) (437 articles)
  - [Clinical trials \(epidemiology\)](#) (179 articles)
  - [Radiology](#) (420 articles)
  - [Radiology \(diagnostics\)](#) (389 articles)

---

### Notes

---

To request permissions go to:  
<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:  
<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:  
<http://group.bmj.com/subscribe/>