Increased bone mineral content of preterm infants fed with a nutrient enriched formula after discharge from hospital

N J Bishop, F J King, A Lucas

Abstract
Bone disease with persistent reduced bone mineralisation is common in premature infants. To test the hypothesis that enhancement of nutritional intake after discharge from hospital improves bone mineralisation, 31 formula fed preterm infants were randomly assigned to receive standard or multinutrient enriched milk from the time of discharge. The calcium and phosphorus contents of the enriched milk were 70 and 35 mg/100 ml v 35 and 29 mg/100 ml for the standard formula. Bone mineral content was measured before discharge from hospital in 21 of the infants; there was no difference in the bone mineral content between the groups at that time (35 mg/cm for the two groups). There was a significant increase in bone mineral content for those infants receiving the enriched v standard formula at 3 and 5 months corrected postnatal age; at 3 months the bone mineral content was 83 v 63 mg/cm and at 9 months 115 v 95 mg/cm. The difference between the groups was thus maintained although not increased at a corrected age of 9 months, when the bone mineral content of infants fed the enriched but not the standard formula was no longer significantly different from that of normal infants after adjusting for body size. The difference was not explained by the larger body size in infants fed the enriched formula. The results suggest that the use of a special nutrient enriched postdischarge formula has a significant positive effect on bone growth and mineralisation during a period of rapid skeletal development.

Subjects and methods
We recruited 31 preterm infants before discharge home from the neonatal intensive care unit. Inclusion criteria included weight less than 1850 g at birth and less than 300 g at entry to the study, age less than 100 days at discharge, freedom from major congenital malformations and diseases likely to influence growth and development, and formula rather than human milk fed during their stay in hospital. Ethical approval for the study was given by the Cambridge Health Authority and the Medical Research Council Dunn Nutrition Unit ethics committees.

Infants were randomised, after informed parental consent, to receive either a standard term formula (Farley's Ostermilk, Crookes Health Care), or a specially designed 'follow on preterm formula' (Farley's Premcare, manufactured to our specifications by Crookes Health Care). Full compositional details have been published previously;14; potentially relevant differences are shown in table 1. The largest difference (in percentage terms) is in the mineral content of the two milks, but protein and energy are also increased in the enriched formula.

The randomisation was stratified by sex. The infants had all been in the neonatal intensive care unit of the Rosie Maternity Hospital, Cambridge before randomisation, and had had extensive data collection undertaken from birth. The infants were fed with the formula milk while still in hospital. They were visited at home fortnightly by a research nursing sister.

| Table 1 Key nutrients (per 100 ml) in standard and enriched formula milks |
|------------------------|---------|-------------|
| Factor                | Standard formula | Enriched formula |
| Energy (kcal)*        | 67       | 72          |
| Protein (g)           | 1.45     | 1.85        |
| Calcium (mg)          | 35       | 70          |
| Phosphorus (mg)       | 29       | 35          |
| Magnesium (mg)        | 1.2      | 5.2         |
| Vitamin D (µg)        | 1.0      | 1.2         |

*1 kcal = 4.18 kJ.
Table 2  Demographic data for the infants divided by diet after discharge. Results given as mean (SD) unless stated otherwise

<table>
<thead>
<tr>
<th></th>
<th>Standard formula (n=15)</th>
<th>Enriched formula (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation (weeks)</td>
<td>31.7 (1.9)</td>
<td>30.7 (1.7)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1436 (227)</td>
<td>1513 (173)</td>
</tr>
<tr>
<td>Boys/girls</td>
<td>8.7</td>
<td>7.9</td>
</tr>
<tr>
<td>No of infants ventilated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1 day</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>&gt;7 days</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>No of days in intravenous feeding (median, interquartile range)*</td>
<td>4 (2-8)</td>
<td>5 (3-10)</td>
</tr>
<tr>
<td>Postmenstrual age at trial entry (weeks)</td>
<td>37 (2)</td>
<td>37 (2)</td>
</tr>
<tr>
<td>Feed volume (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-3 months</td>
<td>73.4 (13.5)</td>
<td>71.6 (14.9)</td>
</tr>
<tr>
<td>&gt;3-6 months</td>
<td>69.4 (14.5)</td>
<td>68.8 (12.2)</td>
</tr>
<tr>
<td>&gt;6-9 months</td>
<td>75.1 (32.0)</td>
<td>65.9 (23.9)</td>
</tr>
</tbody>
</table>

*Partial or complete.

Table 3  Anthropometric data at trial entry and at corrected postnatal ages of 3 and 9 months grouped by dietary assignment. Results given as mean (SD)

<table>
<thead>
<tr>
<th></th>
<th>Standard formula (n=15)</th>
<th>Enriched formula (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial entry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>2383 (221)</td>
<td>2401 (343)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>46 (0.14)</td>
<td>46.3 (0.22)</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>33.5 (0.10)</td>
<td>33.2 (0.12)</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>5531 (223)</td>
<td>5819 (200)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>58.9 (0.61)</td>
<td>60.4 (0.49)</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 months</td>
<td>40.9 (0.37)</td>
<td>41.5 (0.41)</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>7584 (400)</td>
<td>8208 (389)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>69.6 (0.81)</td>
<td>70.7 (0.94)</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(FJK) who recorded anthropometric and clinical details. Length was measured to the next succeeding 1 mm using a horizontal stadiometer and weight to the nearest 10 g using Sartorius electronic balance scales. Every two weeks supplies of milk were delivered to the home in preweighed crates of ready-to-feed bottles. The mother was instructed to use a teat directly on the bottle during a feed, and afterwards to remove the teat, recap the empty or partially empty bottle, and replace it in the crate. At the end of each two week period the entire crate was reweighed. The volume intake of formula milk was calculated as the difference in weight of the crate at the beginning and end of the two week period. The infants received the assigned diet, either alone or in conjunction with other foods, after weaning, up to a corrected postnatal age of 9 months.

Measurement of bone width and mineral content was undertaken using single photon absorptiometry (Lunar SP2, Lunar Radiation). A collimated beam of photons from an iodine-125 source passed across the arm to a photomultiplier detector. The source and detector moved in tandem across the arm, and the bone width and mineral content were calculated from the attenuation of the photon beam. Each infant was placed supine with the left arm extended. The forearm was enfolded in a tissue equivalent bag made from dialysis tubing filled with warm water. With the arm held perpendicular to the beam path, two scans across the forearm were undertaken along the same track. Where the difference between scans exceeded 5% the scan was repeated. The surface radiation dose for each scan would be 20 μSv with a new source; approximately 10% of that from

a forearm radiograph, and well within the fluctuations of normal daily background radiation.

Measurements were undertaken at the one third distal site – that is, the position corresponding to one third of the distance from the tip of the olecranon to the ulnar styloid process, measured distally from the styloid process. The radius at this point approximates a cylinder over a two to three centimetre distance and thus provides a geometrically stable measuring area. Twenty of the 31 infants studied had their bone width and mineral content measured before discharge from hospital; the remainder had returned to peripheral referring units before such a measurement could be made. All infants returned to our unit for measurements at corrected postnatal ages of 3 and 9 months.

The analysis of results was restricted initially to those infants for whom a baseline measurement of bone mineral content had been performed. These were then compared with the results obtained when the complete groups were analysed.

All infants were included in the regression analyses; the results from the initial group comparisons suggest that it is appropriate to do this, and in addition it was thought unlikely that the measurement of bone mineral content in hospital would of itself influence later bone growth or mineralisation.

All the analyses were undertaken using the DataDesk package for the Apple Macintosh computer.

Results
Tables 2 and 3 show the demographic and anthropometric data at trial entry and corrected ages of 3 and 9 months separated by diet group. The groups were well matched for gestational age, birth weight, and severity of initial respiratory illness. There were no significant differences in body weight, length, head circumference, or skinfold thickness at trial entry. Enriched formula consumption was similar between the two groups. Details for the infants as a combined group have been published previously14; table 3 includes a precise of the volume intake on each diet. We have reported previously that the infants receiving the nutrient enriched formula became longer and heavier than those fed the standard term formula.14

The radial bone width and mineral content estimations for each group before discharge and at corrected ages of 3 and 9 months are shown in table 4 for the infants measured initially while in hospital. There were significant differences in bone mineral content between the diet groups at both postdischarge time periods (difference in means (95% confidence interval) of enriched and standard formula groups 20 (6 to 34) mg/cm, and 27 (13 to 40) mg/cm at each age respectively).

For all the infants at corrected ages of 3 and 9 months the results are shown in table 5. The differences in byrne mineral content between the diet groups at both discharge time periods

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Enhanced bone mineralisation with enriched nutrient formula

Table 4  Bone width and mineral content of the radius before discharge from hospital and at 3 and 9 months corrected postnatal age grouped by dietary assignment (data are mean (SD) and 95% confidence interval of difference of group means); analysis restricted to those infants first measured in hospital

<table>
<thead>
<tr>
<th>Bone width (cm)</th>
<th>Enriched formula (n=11)</th>
<th>Standard formula (n=9)</th>
<th>Difference of means</th>
<th>95% Confidence interval</th>
</tr>
</thead>
</table>

Before discharge
- Bone width 0.366 (0.043) 0.360 (0.030) 0.006 -0.023 to 0.039
- Bone mineral content 0.684 (0.071) 0.610 (0.078) 0.074 0.004 to 0.141

3 months
- Bone width 0.603 (0.085) 0.562 (0.084) 0.041 -0.039 to 0.121
- Bone mineral content 0.645 (0.102) 0.610 (0.078) 0.035 0.004 to 0.141

9 months
- Bone width 0.581 (0.021) 0.536 (0.024) 0.046 -0.046 to 0.078
- Bone mineral content 0.684 (0.071) 0.610 (0.078) 0.074 0.004 to 0.141

Table 5  Bone width and mineral content of the radius at 3 and 9 months corrected postnatal age grouped by dietary assignment (data are mean (SE) and 95% confidence interval of difference of group means); all infants

<table>
<thead>
<tr>
<th>Bone width (cm)</th>
<th>Enriched formula (n=16)</th>
<th>Standard formula (n=20)</th>
<th>Difference of means</th>
<th>95% Confidence interval</th>
</tr>
</thead>
</table>

3 months
- Bone width 0.669 (0.022) 0.619 (0.022) 0.050 -0.011 to 0.111
- Bone mineral content 83.6 (4.5) 79.1 (3.6) 4.5 9.1 to 31.5

9 months
- Bone width 115.3 (5.4) 94.7 (3.7) 20.6 7.4 to 33.8
- Bone mineral content 95.3 (11.1) 76.2 (9.3) 19.1 20.2 to 30.0

we observed that bone mineral content was similar in all infants fed this diet, and this was true whether or not infants were born at term. The data show that by 9 months the bone mineral content in infants fed the enriched diet was about two standard errors lower than in normal controls (figure). After adjusting for body size the bone mineral content in the enriched formula group was even closer to that of the normal controls. The adjustment for body size was performed by plotting the mean value for bone mineral content at the mean expected age for body weight for each group, as weight rather than length was independently associated with the bone mineral content.

We considered that the effect of the enriched formula on mineralisation might be explained by the greater growth rate and body size of infants fed this diet; it would be reasonable to expect that larger infants would have bigger bones and therefore more bone mineral. To explore this, we undertook multiple regression analysis with bone mineral content as the dependent variable and body weight, height, bone width, sex, and diet type (standard or enriched formula) as independent factors. The diet after discharge was the factor most strongly associated with bone mineral content at 3 months ($r=3.85; p<0.001$), exceeding the...
Discuss

Discussion

We have shown in a prospective randomised double blind controlled study that preterm infants fed a special nutrient enriched formula as opposed to standard milk formula after discharge from hospital had a higher bone mineral content at 3 and 9 months corrected postnatal age. This effect was independent of body size. After adjusting for a range of antenatal, neonatal, and anthropometric factors, diet after discharge from hospital was the factor which was most strongly related to bone mineral content at an age of 3 months. The difference in mineral accretion velocity was only significantly different between the groups over this first period, but the difference in bone mineral content between the standard and enriched formula fed groups was still maintained at 9 months. By a corrected postnatal age of 9 months the infants receiving the enriched but not the standard formula had a bone mineral content no longer significantly different from that expected for age, after adjusting for body size.

The effect of dietary mineral supplementation on the bone mineralisation in preterm infants during the period in hospital has been studied extensively. A large number of studies support the view that an inadequate intake of bone mineral substrate (rather than vitamin D deficiency) is the principal factor in the aetiology of bone disease of prematurity.1 4 11-13 There have, however, been relatively few studies of the longer term follow up of infants born prematurely in terms of skeletal growth and bone accretion. In a previous study of the effect of early diet on later growth and development4 we showed that bone disease, as indicated by increased plasma alkaline phosphatase activity, was the factor most strongly associated with reduced body length at 18 months corrected postnatal age, with early diet (formula v unsupplemented human milk) also exerting a substantial independent effect.

Other groups have studied the bone mineral content of preterm infants after discharge from hospital.1 7-19 Congdon et al found a rapid acceleration in the rate of mid-forearm bone mineralisation after discharge from hospital for infants born preterm up to a median corrected age of 10 weeks.17 Chan and Miles found higher values than ours at a corrected postnatal age of 3 months for preterm infants receiving term formula after discharge from hospital (95 mg/cm), but the infants' average bone mineral content at discharge was also substantially higher (52 mg/cm).18 Schanler et al reported the bone mineral content of infants born preterm at intervals up to 2 years of age.19 The infants had received expressed milk from their mothers while in hospital, and then either continued with breast feeding or with a standard formula choice after discharge from hospital. The results obtained from that non-randomised study are broadly similar to our own, with the infants receiving the formula milk having a greater bone mineral content at 6 months and 1 year of age than those receiving milk from their mothers. Bone mineralisation was similar in each group at the age of 2 years.
In this study, infants born at 27–34 weeks’ gestation and fed on mineral enriched preterm formula in hospital showed poor bone mineralisation at discharge with a mean value of around 50% of that expected for their age after conception. Interestingly, we found the infants fed a standard formula after discharge had a markedly decreased bone mineral content even at 9 months after term, when the mean value was only 70% of that expected for age, and 76% after adjusting for body size. The corresponding values at 9 months for infants fed the enriched formula were 86% and 91%.

It was remarkable that at three months after term, the diet after discharge was the only clinical factor identified that related to the bone mineral content. At this age, we found no evidence that infants who were growth retarded at birth or who were immature, sick, or had a higher peak alkaline phosphatase activity were at risk for developing more poorly mineralised bones. It is possible that such effects were missed because our cohort was relatively small and our selection criteria excluded infants with prolonged respiratory disease. Moreover, before discharge none of the infants were fed with unsupplemented human milk or standard formula which would supply low intakes of bone minerals. Nevertheless, it appears that the nature of the diet after discharge is highly influential for bone mineral accretion in infancy.

In this study the mean stay in hospital was only six weeks (a typical period of hospital stay for a very low birthweight preterm infant). In contrast, our infants received their formula after discharge for an average of 43 weeks. It should not be surprising, therefore, if nutrition policy after discharge proved to be at least as influential as hospital management in terms of later nutritional status.

The bone mineral content estimated by single photon absorptiometry correlates well with total body calcium measured by in vivo neutron activation analysis16 and dual energy x-ray absorptiometry20 in adults. Our results therefore suggest an overall increase in the total amount of bone mineral in the skeleton for those infants who received the enriched formula. A number of studies of fractures in childhood have shown that reduced bone mineralisation is a predisposing factor.21–23 We have shown previously that although there was no overall increase in the risk of clinically presenting fractures up to the age of 5 years for infants born prematurely, there was a trend towards earlier presentation (at less than 2 years of age) for those born at less than 33 weeks’ gestation.24 We speculate that the increased bone mineral content shown here for infants receiving the enriched formula might ameliorate that risk.

Bone mineralisation continues throughout childhood and into adolescence with a more rapid accretion of bone mineral during puberty.25–27 Helin et al reported that infants born prematurely had a reduced bone mineral content at follow up to an age of 16 years, but gave no information about early diet;25 there are no similar data available for adults born preterm. This raises concern over the potential long term effect on skeletal development of prolonged undermineralisation during the first year of life. It is possible that inadequate provision of dietary calcium and phosphorus during this period when overall growth is normally at its most rapid might affect future bone growth and mineralisation. Clearly, long term follow up of premature infants randomised to different diets in the neonatal period is required to investigate this.

The home use of a multinutrient enriched rather than standard milk formula has improved skeletal growth and bone mineralisation in a group of preterm infants. Previously we have reported that those infants fed the same enriched formula, had improved weight gain and linear growth velocity without impaired feed tolerance.14 This group of infants appears to have nutritional requirements which exceed the provisions of the routinely supplied standard term formula. We suggest the use of nutrient enriched postdischarge milk should now be considered for each preterm infant as they leave hospital.

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