Nucleotide supplementation and the growth of term small for gestational age infants

M Cosgrove, D P Davies, H R Jenkins

Abstract
A double blind randomised controlled trial in small for gestational age (SGA) infants, whose intestinal mucosa was shown to be functionally impaired as a result of intrauterine undernutrition, was carried out to investigate the hypothesis that nucleotide supplementation of a milk formula could improve catchup growth. Anthropometric data were collected on 74 infants, 39 randomly assigned to the nucleotide supplemented group (group N) and 35 to a standard formula group (group S). From study entry to 2 months of age, infants in group N had significantly higher mean rates of weight gain (106.3 compared with 94.7 g/kg baseline weight/week) and length gain (21.8 v 19.7 mm/m baseline length/week). Over the whole six months for which the trial formula was provided group N had significantly higher mean rates of gain of weight (80.1 compared with 71.8 g/kg baseline weight/week), length (16.2 compared with 15.0 mm/m baseline length/week), and head circumference (11.8 compared with 10.8 mm/m baseline head circumference/week).

Catchup growth in SGA infants is therefore improved by nucleotide supplementation of infant formula.

Keywords: nucleotides, small for gestational age, catchup growth.

Intrauterine growth retardation (IUGR) is identifiable clinically as babies born small for gestational age (SGA); supply-line failure fetal undernutrition is the main contributor.

It is important for SGA infants to recover their growth deficit in the early months after birth. First, infants who show good catchup in the first few months of postnatal life continue to grow better than infants who do not. Second, there is a correlation between early somatic growth and later neurodevelopmental performance. Third, epidemiological studies of early developmental influences on adult disease have shown the importance of good growth in the first year of postnatal life to lessen risks of early onset cardiovascular disease.

Fetal growth retardation adversely affects the intestinal mucosa and the exocrine pancreas which may hinder digestion, absorption, and utilisation of ingested nutrients, thereby restricting catchup growth.

Studies in rats, pigs, and sheep, who are growth retarded in utero, have shown that the structure of the small intestinal mucosa is impaired. In SGA infants intestinal absorptive function measured by xylose absorption is impaired compared with those normally grown and the physiological maturation decrease in intestinal permeability to macromolecules is similarly delayed. IUGR is also associated with impairment of exocrine pancreatic function in rats, pigs, and human infants. Catchup growth might be enhanced perhaps if these effects of intrauterine undernutrition could be lessened. One possibility is to add substances known to have growth promoting properties to the milk diet of SGA infants.

Nucleotides, low molecular weight intracellular compounds, and monomeric building blocks of DNA and RNA, are such substances. They are prominent in the non-protein nitrogen component of human milk but virtually absent from infant formulas. There are two pathways for the biosynthesis of nucleotides; de novo from amino acid precursors and a less energy costly salvage pathway from the degradation products of nucleic acids and nucleotides.

Cells of the intestinal mucosa have a limited capacity for de novo synthesis. Under conditions of rapid growth an exogenous supply would become essential for optimal function especially if the mucosa has already been damaged. The biological effects of dietary nucleotides on the morphology and function of intestinal mucosa have been reported in several studies. Weanling rats fed a diet supplemented with nucleoside (the residual moiety when the phosphate groups are removed from a nucleotide) developed taller villi and increased mucosal protein, DNA, and maltase activity in the proximal intestine. After experimentally induced diarrhoea dietary nucleotide supplementation enhanced gut mucosal recovery, with increased DNA concentration and di-saccharidase enzyme activities, in rats. The intestinal histology and ultrastructure were also closer to normal compared with a nucleotide-free diet.

Our study investigated the hypothesis that SGA infants, whose intestinal mucosa are functionally impaired by fetal undernutrition, might benefit from nucleotide supplementation of milk formulas with improved catchup growth.

Methods
A double blind randomised controlled trial was designed. Infants were recruited to the trial from four maternity units in South and Mid...
Glamorgan over 18 months from September 1992 to March 1994. Singleton infants delivered after 37 weeks gestation were considered eligible for the study if their birthweight was less than the 5th centile for gestation using the standards of Tanner and Thomson: these allow for sex, birth order, and maternal height. Infants with a major congenital or chromosomal anomaly, or of uncertain gestational age and who could have been less than 37 weeks, were not included.

Mothers who were breast feeding, or intending to breast feed their baby who would otherwise have been eligible, were not initially involved in the study but were asked if they would like to participate in a study of postnatal growth in breast fed SGA infants. If breast feeding was discontinued within four weeks of delivery, recruitment to the main study was then invited. Written informed consent was obtained from a parent of each infant recruited.

A detailed clinical examination of each infant was undertaken (MC) to exclude major congenital anomalies and evidence of conditions known to affect fetal and neonatal growth. Infants seen in the first 72 hours of life had their gestational age clinically assessed using the scoring system of Dubowitz et al.20 to confirm the estimate of gestational age obtained from the menstrual data and ultrasound examination.

ANTHROPOMETRY
Anthropometry was performed on study entry, with measurement of weight (Seca 724 electronic scales, accurate to 20 g), supine length (Neonatometer, Holtain Ltd), and head and mid arm circumference (Raven Babytape, Child Growth Foundation). All measurements were made in duplicate using standard anthropometric techniques21 by a single observer (MC) with the definitive measurement being the mean of the two readings.

RANDOMISATION AND PROVISION OF FORMULAS
On recruitment each infant was consecutively allocated a number which corresponded to cases of infant formula prepared and numbered by SMA Nutrition. The number code was known only to personnel in the manufacturing plant and not to anyone involved in the measurements or analysis of data. The two formulas used were a standard whey dominant infant formula (SMA Goldcap, SMA Nutrition) and the same formula supplemented with the five nucleotide monophosphates in the concentrations shown in Table 1. These concentrations were close to those reported in human milk where the monophosphates are the most abundant form of nucleotide.13 22 The two formulas were identical other than in nucleotide content. The milk was provided for each infant for six months as a ready-to-feed formulation, to prevent the different techniques of reconstituting powdered formulas, which have been suggested to have an important effect on postnatal weight gain, from having any influence.23

Parents who requested feeding advice were instructed to feed their infant ad libitum. No attempt was made to control the age at which weaning was introduced, but if advice was requested this was given in accordance with Department of Health guidelines.24

FOLLOW UP
Infants were assessed at 1, 2, 4, and 6 months of age. Limits imposed were ± four days at one month, ± seven days at two months, and ± 14 days at four and six months. For three days before each visit parents were asked to keep a diary of all milk feeds consumed by the infant, to give an estimate of the mean daily milk intake at each time point. At each visit parents were also asked to report any illness which their infant had had since the previous visit. Anthropometry was repeated on each occasion. Weekly growth rates were obtained by dividing the growth increment over a given time period by the number of days in that period and multiplying by 7, and expressing it per unit baseline weight, length, and head circumference.

On completing the study at six months a full clinical examination was performed. For any individual infant the study was stopped before six months if any of the following events occurred:

- the infant was discovered to have a condition known or suspected to affect growth
- the infant was taken off the trial formula by parental choice.
- the infant was taken off the trial formula on medical advice because of suspected adverse effects
- the infant was lost to follow up.

In the latter three instances data were included for analysis until the last review at which the study protocol was still being followed. If the protocol violation occurred in the first month of life the infant was not included in analysis.

STATISTICAL ANALYSIS
Using data from a previous study of catchup growth in SGA infants,25 it was estimated that a sample size of 40 infants in each group would give 90% power of detecting a difference of 5% in growth rates between the two groups at a significance level of 5%. Results were analysed using Student’s t-test, χ² test and analysis of variance on the SPSS for windows statistical package.

Ethical approval was obtained for the study from the district ethical committees of
Initially breast fed

Baseline head circumference (cm):
Mean (SD)
46.5 (1.8)
46.8 (2.0)
NS

Baseline length (cm):
Mean (SD)
2390 (277)
2468 (408)
NS

Age at study entry (days):
Median (range)
4 (2-28)
5 (2-28)
NS

Birth weight (g):
Mean (SD)
2304 (255)
2364 (269)
NS

Table 2 Characteristics of both groups of infants at study entry

<table>
<thead>
<tr>
<th>Nucleotide supplemented group (N) (n=39)</th>
<th>Standard group (S) (n=35)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birthweight (g): Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2304 (255)</td>
<td>2364 (269)</td>
<td>NS</td>
</tr>
<tr>
<td>Age at study entry (days): Median (range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (2-28)</td>
<td>5 (2-28)</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline weight (g): Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2390 (277)</td>
<td>2468 (408)</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline length (cm): Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>46.5 (1.8)</td>
<td>46.8 (2.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline head circumference (cm): Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33.0 (1.0)</td>
<td>33.0 (1.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Initially breast fed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (15%)</td>
<td>9 (26%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

South Glamorgan and Mid Glamorgan Health Authorities.

Results
Eighth nine infants were initially recruited with 44 randomised to the nucleotide supplementation group (N) and 45 to the standard group (S). Fifteen infants were excluded from analysis; eight had conditions which could have interfered with growth (three central nervous system malformations, one cystic fibrosis, one congenital cytomegalovirus, one chromosomal abnormality (karyotype 47 XXX), one protracted diarrhoea of infancy, one traumatic brain injury); four failed to return for follow up – three from group S and one from group N; and three were taken off the trial formula in the first month of life – two from group S (one became constipated, one was unsettled) and one from group N (vomiting and loose stools).

Baseline details of the remaining 74 infants (39 N, 35 S) are shown in table 2. There was a non-significant excess of boys in group N (24/39) compared with 14/35 in group S. There were no significant differences in birthweight and baseline measurements of weight, length, and head circumference. The infants in group N had a median gestational age of 38 compared with 39 weeks in group S. The proportions of infants whose mothers smoked during pregnancy and who were firstborn were similar. Fifteen infants (6 N, 9 S) were initially breast fed, the duration of breast feeding being less than two weeks in nine (3 N, 6 S), more than two but less than three weeks in five (2 N, 3 S) and three weeks in one (group N). In four infants (3 N, 1 S) the initial anthropometry was performed so close to the age of 1 month that the next measurements were taken at the age of 2 months. The volume of milk ingested per kilogram of body weight was similar in the two groups (table 3). Reasons for infants not completing the study are given in table 4. Suspected adverse effects were equally represented in the two groups and there was no difference in the rates of illness reported by the parents at each visit.

The rates of growth from study entry to two months (the time period of purest comparison between the two formulas, because no infant was being weaned by two months; many were by four months) differed significantly. Group N infants had significantly higher mean values for weight gain (106.3 compared with 94.7 g/kg baseline weight/week, 95% confidence intervals for difference 0.5 to 22.8), and length gain (21.8 compared with 19.7 mm/m baseline length/week, 95% confidence intervals for difference 0.8 to 3.6) (table 5). The difference for head circumference gain tended towards significance (19.0 compared with 17.3 mm/m baseline head circumference/week, 95% confidence interval for difference −0.2 to +3.6). Over the whole six months, the benefits to growth for group N persisted: weight gain 80.1 compared with 71.8 g/kg baseline weight/week (95% confidence intervals for difference −0.1 to +16.7), length gain 16.2 compared with 15.0 mm/m baseline length/week (95% confidence intervals for difference −0.2 to 2.1), and head circumference gain 11.8 compared with 10.8 mm/m baseline head circumference/week (95% confidence interval for difference −0.1 to 2.0).

Analysis of variance showed that sex, initial breast feeding, and maternal smoking had no significant effects on the analysis. A further analysis of variance, to investigate the effects of age in days at study entry, showed that when this was entered as a covariate the effects of feed on growth rates remained significant.

Discussion
Nucleotide supplementation of infant formula has already been introduced in Japan, Spain, and the United States. No deleterious effects have been reported, but neither have there been beneficial effects shown on growth in normally grown infants born at or before term.27 This study supports the hypothesis that SGA infants would benefit from nucleotide supplementation and has shown that the differences between the overall rates of growth in the first two months are sustained over the next four months. These differences are considerable; the difference in growth rates attributable to nucleotide supplementation is of the order of 10%.

Babies are born SGA for a variety of reasons. Our inclusion criteria were designed to exclude infants with congenital anomalies, including chromosomal abnormalities. Infants whose small size might be explained by maternal short stature were unlikely to be included.

Table 3 Milk intake recorded by parents in feeding diary (ml/kg/day)

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Group N</th>
<th>Group S</th>
<th>95% Confidence intervals for difference (group N-group S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>216</td>
<td>209</td>
<td>−15 to 28</td>
</tr>
<tr>
<td>2</td>
<td>194</td>
<td>198</td>
<td>−22 to 15</td>
</tr>
<tr>
<td>4</td>
<td>153</td>
<td>144</td>
<td>−15 to 32</td>
</tr>
<tr>
<td>6</td>
<td>112</td>
<td>93</td>
<td>0 to 38</td>
</tr>
</tbody>
</table>

Table 4 Reasons for failure to complete study

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant taken off trial formula before 6 months</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>'Not satisfied'</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Intercurrent illness</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Problem with delivery</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Failed to return</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Reviewed outside time limits</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>
Table 5. Mean rates of growth of weight, length, and head circumference at two and six months from entry to study

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group N</th>
<th>Group S</th>
<th>95% Confidence interval for group N (group N-group S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g/kg baseline/week)</td>
<td>106.3</td>
<td>94.7</td>
<td>0.5 to 22.8 P = 0.04</td>
</tr>
<tr>
<td>Length (mm/m baseline/week)</td>
<td>21.9</td>
<td>19.7</td>
<td>0.8 to 3.6 P = 0.03</td>
</tr>
<tr>
<td>Head circumference (mm/m baseline/week)</td>
<td>19.0</td>
<td>17.3</td>
<td>0.2 to 3.6 P = 0.07</td>
</tr>
<tr>
<td>Weight (g/kg baseline/week)</td>
<td>80.1</td>
<td>71.8</td>
<td>0.1 to 16.7 P = 0.05</td>
</tr>
<tr>
<td>Length (mm/m baseline/week)</td>
<td>15.0</td>
<td>12.2</td>
<td>0.2 to 2.1 P = 0.01</td>
</tr>
<tr>
<td>Head circumference (mm/m baseline/week)</td>
<td>11.8</td>
<td>10.8</td>
<td>0.1 to 2.0 P = 0.02</td>
</tr>
</tbody>
</table>

because of Tanner and Thomson's standards. Upward crossing of the centiles in both groups was not as great in the SGA infants as that for the group size of the SGA infants was largely attributable to fetal undernutrition.

For obvious reasons we could not subject these otherwise healthy infants to small intestinal biopsy to study directly the effects on the mucosa. The improved growth associated with nucleotide supplementation might have been unrelated to effects on the intestinal mucosa. Perhaps the improved growth was improved by adding nucleotides, possibly because of the effects on taste, but a study from Japan found no difference in the volume of milk intake and acceptability in groups of infants who did or did not receive a nucleotide supplement to their cow's milk based formula. In our study there was no difference in the estimated milk volumes taken in the two groups, as recorded by their parents.

The addition of the five nucleotide monophosphates contributed extra nitrogen and phosphate to the formula and this could conceivably have contributed a positive growth effect. However, the maximum concentration of nucleotide was about 4 mg/100 ml and the phosphate component of this was small compared with the concentration of phosphorus in the standard formula which ranged from 29-39 mg/100 ml. Furthermore, nucleotides are non-protein nitrogen and so do not contribute directly to the protein pool. The beneficial effects of nucleotides, therefore, are unlikely to be due to the extra nitrogen and phosphate.

Several studies have reported the theoretically beneficial effects of nucleotides on the immune system, including an increase in the concentration of natural killer cells in infants fed on a nucleotide supplemented formula. During our study, data was collected on the illnesses observed by parents; there was no excess of reported events in the S group, which might have suggested enhanced protection against infection as a possible explanation for the improved growth seen in the S group.

The improved growth seen in the nucleotide supplemented group of SGA infants is likely to have been due to trophic effects of nucleotides on the intestinal mucosa previously damaged by intrauterine malnutrition. We appreciate that human milk is rich in nucleotides and that where possible SGA infants should be breastfed. When this is not possible, and in view of the increasing recognition of the importance of catchup growth for SGA infants, we suggest that nucleotides be seriously considered as a supplement to the milk diet of these infants in the early months of postnatal life.

We thank Mrs Debbie Savory and Mrs Maggie Stuart for their invaluable help as research nurses for the study; Mr Frank Dunstan of the Department of Medical Computing and Statistics, University of Wales College of Medicine for his assistance with the statistical analysis, and SMA Nutrition of the Wyeth group, especially Mr Dave Goldstraw and Mr Shouma Vincent, for their generous support and advice throughout the study.

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