Influence of Neonatal Nutrition on Long-Term Outcome

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Over the past 50 years more research has been conducted in preterm infant nutrition than in virtually any other field of neonatal care, yet uncertainty persists in clinical practice. A key factor in this uncertainty has been a lack of knowledge on whether it matters how we feed preterm infants in terms of their long-term health and development.

The concept that early nutrition could have long-term significance, however, raises issues of fundamental biological importance. Is it plausible or, arguing teleologically, evolutionarily likely that such a brief period of life could be a critical one for nutrition? What could be the nature of the triggering mechanisms for these events? Are there indeed convincing data showing that early nutrition influences long-term outcome in animals or man? Since these questions are critical to the thesis that early nutrition could matter in preterm infants, I shall first discuss this area in general biological terms.

THE CONCEPT OF BIOLOGICAL PROGRAMMING

The idea that nutrition in early life could have long-term consequences invokes the more general process that I shall refer to as "programming." As a working definition "programming" occurs when an early stimulus or insult, operating at a critical or sensitive period, results in a permanent or long-term change in the structure or function of the organism (1). An essential component of this concept is the notion of a "sensitive" or "critical" period—a critical window in time—when an early event may operate.

It is now well recognized that a wide variety of hormones, drugs, and sensory inputs have programming effects during critical windows in development. But what is the evidence that nutrition operates in this way?

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Animal studies have shown that nutrition during a sensitive early period may program long-term outcome. Hahn (2) manipulated litter size in rats to produce litters of 4 or 14 pups. In small litters the pups were overfed during the short suckling period. In adulthood, the previously overfed rats developed permanently raised plasma cholesterol and insulin concentrations. Rats weaned onto a high carbohydrate diet also showed lifelong increases in activities of two key enzymes in lipid biosynthetic pathways: fatty acid synthetase and hydroxymethylglutaric acid (HMG) coenzyme A (CoA) reductase (important in fat and cholesterol synthesis). While rats are born immature, it remains to be established whether the rat is a good model for the human preterm infant. Perhaps also relevant to preterm nutrition is the animal evidence that fetal nutrition may have lifelong consequences. For instance, protein deprivation in pregnant rats results in offspring that in adult life have a permanent decrease in pancreatic β cell size, number, and insulin secretory capacity and a permanent reduction in the pancreatic capillary bed (3).

Long-term programming has also been demonstrated in primates. In a study by Lewis and co-workers (4), infant baboons were randomly assigned to one of three formulas for the first 4 months. The formulas provided low, normal, and high energy intakes. After the 4-month period all the animals were fed in the same way. The excess weight gained during infancy in the animals with high energy intakes was soon lost. In female baboons, especially, early overfeeding resulted in a dramatic increase in body weight and fat mass during adolescence and early adult life. In this instance, the effects of the initial “programming” event were not manifested until a much later stage in life, raising the important question as to how the “memory” had been stored in the meantime.

The influence of breast versus formula feeding on later lipid metabolism and vascular disease has also been explored in a series of studies by Mott and co-workers (5) using the baboon model. In these studies random assignments were made to breast or formula feeding during infancy (the first 4 months); after this the animals were fed in the same way. Compared with the formula-fed group, those who were breastfed in infancy had, in adult life, increased cholesterol absorption, reduced cholesterol turnover, and, when placed on a high saturated fat Western-style diet, developed higher plasma levels of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) cholesterol and lower levels of potentially protective high-density lipoprotein (HDL) cholesterol. These lipid abnormalities would be expected to result in an increased risk of atherosclerosis, and indeed at necropsy the breastfed animals showed a significantly greater area of atherosclerotic plaque than those that had been formula-fed in infancy.

These primate data again raise the possibility that preterm infants (who are exposed to much greater extremes of nutrition than the term infant and at a more rapid and perhaps more vulnerable stage of development) could be programmed by early nutrition for later morbidity.

In addition to the work on early diet and later metabolism and morbidity, a large
body of animal data, principally from studies on rats, has indicated that the quality of nutrition at a vulnerable period of early brain development could have permanent consequences for brain size, brain cell number, and performance (6,7). These and other animal studies provide convincing evidence that nutrition in early life may influence a wide variety of metabolic, developmental, and pathological processes in adulthood.

DIETARY PROGRAMMING IN MAN: PREVIOUS STUDIES

Unfortunately the great majority of investigations on the consequences of early nutrition in man have been retrospective and flawed by problems with study design. There has been much interest in the relation of early protein-energy malnutrition to later achievement. Given the enormous investment in these studies, it is disappointing that firm conclusions cannot yet be made (8). These largely retrospective studies are seriously confounded by the poverty, poor social circumstances, and lack of stimulation that generally accompany malnutrition.

The effects of individual nutrients on later brain development, however, are receiving increasing attention. Iron deficiency in infancy, common both in the West (9) and in developing countries, has been shown to be related to poor developmental performance. Some evidence suggests that subsequent iron supplementation may not prevent later poor cognitive ability at 5 years and that a brief period of relatively mild deficiency could have long-lasting consequences for cognitive and motor development, behavior, and school performance (10,11). Irreversible long-term consequences of early iron deficiency have also been shown in rats (11,12).

The possibility that inadequate long-chain n-3 fatty acids in the diets of formula-fed preterm infants might impair cerebral and retinal development has caused concern. Developing brain (notably cerebral cortex) and retina accumulate large quantities of docosahexaenoic acids (22:6n3). Such long-chain lipids are not present in significant amounts in many formulas, which often have low contents of the precursor linolenic acid (18:3n3). Compelling primate data (13) now show that insufficiency of these fatty acids at a critical stage of retinal development results in long-term irreversible impairment of retinal function. Corresponding studies in humans at least suggest medium-term effects on retinal function (14).

Several investigations have focused on early diet in relation to diseases found in affluent countries. Earlier studies, for example those relating early lipid intake to later vascular disease or early salt intake to later hypertension, provided inconclusive data. The more recent epidemiological data of Barker and Hales and co-workers (15–21), however, show that weight at birth and at 1 year (potential markers of early nutrition), are highly related to later death from ischemic heart disease, blood lipids, insulin secretion and diabetes, body fatness, clotting factors, atherosclerosis, and hypertension. These data raise the possibility that an adverse environment, including poor nutrition, in the fetus and infant could program the individual for an increased risk of adult degenerative disease.
Collectively, the evidence supporting the general concept of programming, the specific evidence for nutritional programming in animals, and the more recent epidemiological evidence in man now make it biologically plausible that early nutrition could have lifelong effects. This adds another dimension to the importance of preterm infant nutritional research.

EARLY DIET AND OUTCOME IN PRETERM INFANTS

The lack of prospective long-term outcome studies on diet in preterm infants is related to the unattractiveness of this type of work to many investigators, unwillingness of funding bodies to support it, disbelief that early influences could have lasting effects, and the inherent difficulties in mounting formal randomized longitudinal studies. Nevertheless, I would contend that outcome data are not only essential as a basis for clinical management (as in any area of therapeutics), but the preterm infant also provides us with a model for exploring the wider question of whether perinatal nutrition influences long-term outcome in man.

Between 1982 and 1985 nearly 1000 preterm infants were assigned to different diets, studied intensively in the newborn period, and are now being followed up indefinitely (22). The babies were randomized at birth, in four parallel trials, either to a preterm formula (enriched in protein, energy, macrominerals, and trace nutrients to meet the calculated increased requirements of preterm infants) versus donor breast milk, or to preterm formula versus a standard formula; for each comparison these feeds were used as sole diets or as supplements to mother's own milk. The infants remained on the assigned diets for an average of 1 month; after that there was no influence on dietary management. Follow-up data are available to 18 months corrected age; a 7 to 8 year follow-up is in progress. The principal medium-term outcome response chosen was neurodevelopment, but several other key outcomes have been explored.

In this chapter, three examples of outcome data have been selected that show the importance of early nutrition in preterm infants and support the concept of programming.

Early Diet and Later Allergy

The effect of early diet on later allergy has been much debated. A major problem in the interpretation of results from many studies arises from the lack of random assignment to diet. Clearly, random assignment to breast feeding versus formula feeding would be unethical in healthy infants, and yet social and demographic differences found between these two groups confound comparative analyses. In preterm infants, random allocation to human milk or formula is ethical and feasible. In one limb of our trial we compared infants randomly assigned to banked donor breast milk as sole diet or supplement to mother's milk (i.e., all received only human milk) versus those fed a preterm formula as sole diet or supplement (i.e., all were exposed to cows' milk formula). Beyond 1 month, on average, trial diets were discontinued and
TABLE 1. Family history of atopy, neonatal diet and allergic reactions at 18 months in infants born preterm

<table>
<thead>
<tr>
<th>Allergic reaction</th>
<th>Family history of atopy</th>
<th>Human milk (n = 38)</th>
<th>Preterm formula (n = 37)</th>
<th>Odds ratio (95% CI)</th>
<th>No family history</th>
<th>Human milk (n = 189)</th>
<th>Preterm formula (n = 182)</th>
<th>Odds ratio (95% CI)</th>
<th>Interaction between family history and diet (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eczema</td>
<td></td>
<td>16% (6)</td>
<td>41% (15)</td>
<td>3.6* (1.2, 11)</td>
<td>21% (40)</td>
<td>16% (29)</td>
<td>0.7 (0.4, 1.2)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Reactions to:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow’s milk</td>
<td></td>
<td>3% (1)</td>
<td>5% (2)</td>
<td>2.1 (0.2, 25)</td>
<td>5% (9)</td>
<td>3% (5)</td>
<td>0.6 (0.2, 1.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All foods</td>
<td></td>
<td>11% (4)</td>
<td>22% (8)</td>
<td>2.3 (0.6, 8.3)</td>
<td>10% (18)</td>
<td>9% (16)</td>
<td>0.9 (0.5, 1.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drugs</td>
<td></td>
<td>3% (1)</td>
<td>16% (6)</td>
<td>7.1 (0.8, 50)</td>
<td>7% (14)</td>
<td>5% (10)</td>
<td>0.7 (0.3, 1.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheezing or asthma</td>
<td></td>
<td>21% (8)</td>
<td>30% (11)</td>
<td>1.6 (0.6, 1.4)</td>
<td>21% (40)</td>
<td>22% (40)</td>
<td>0.9 (0.6, 1.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any of the above</td>
<td></td>
<td>34% (13)</td>
<td>65% (24)</td>
<td>3.6* (1.4, 9.1)</td>
<td>46% (86)</td>
<td>42% (76)</td>
<td>0.9 (0.6, 1.3)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Infants were divided into two groups according to whether or not there was a family history of atopy. Within each group the percentage incidence of allergic reactions (number of subjects) is compared in infants randomly assigned to donor milk or preterm formula as sole diets or supplements to mother’s milk (thus the donor-milk-fed group received only human milk). The odds ratio (95% confidence interval, CI) is recorded for the incidence of allergy on preterm formula versus human milk. Corresponding odds ratios in the two family history groups are also compared, to test for an interaction between family history and diet.

* p < 0.05.

there was no significant difference in dietary management between groups. At 18 months the pattern of response depended on whether or not the child had a family history of allergy (23). In infants with no such history, interestingly, those fed previously on cows’ milk formula had a small (nonsignificant) reduction in the incidence of reactions to cows’ milk, other food or drugs, and in eczema and wheezing. In contrast, in the smaller subgroup with a positive family history of allergy, babies given cows’ milk formula rather than breast milk in the neonatal period had a dramatic increase in these allergic responses, notably, in eczema and reactions to food or drugs (Table 1). These data show that in genetically susceptible individuals a brief period of dietary manipulation programs the infant to develop a wide range of allergic or atopic manifestations.

Influence of Early Bone Disease and Early Diet on Future Growth and Bone Development

Early bone disease is extremely common in human-milk–fed infants. Peak alkaline phosphatase activity, taken as a measure of bone disease in infants, has often been
found to be high, above 1000 to 1200 IU/liter and in populations correlates with bone
disease detected on radiograph. Many infants in our study left the hospital with
extremely high alkaline phosphatase activity and significant bone disease seen on
radiograph. None of the infants was readmitted because of it. The important question
is whether this clinically silent disease influenced later outcome.

To explore the hypothesis that bone disease in the newborn period affected future
stature, regression analyses were performed (24) on all factors related to stature at
18 months of age. Five factors were found to be independently related to the height
of the infant at age 18 months. Three were related to a 1-cm deficit in height: being
a twin, being fed human milk, or being female. Being small for gestational age was
associated with a greater reduction in height, but evidence of bone disease in the
newborn period was most highly correlated to a substantial reduction in later height
(1.6 cm).

Pilot data (unpublished) on 62 of the children at 5 years of age showed that those
who had been randomly assigned preterm formula were 3.1 cm taller and 1.8 kg
heavier than those fed human milk during the first month of life. If this difference is
confirmed in the larger data set of nearly 800 children at 7½ to 8 years of age, we
shall be able to conclude that a very brief period of dietary manipulation in the
perinatal period could have long-term effects on growth.

Equally interesting was our observation (unpublished findings in collaboration with
N. J. Bishop) that early human milk or preterm formula feeding had a profound
influence on bone mineral content, determined by photon-absorptiometry, at 5 years.
These data raise the possibility that early nutrition (perhaps supply of bone minerals)
could have a programming effect on the development of bone as a tissue.

It seems reasonable to suggest that early nutrition has a programming effect on long-
term linear growth, and our preliminary studies later in childhood have continued to
support that view. One could speculate, teleologically, that it makes good sense from
an evolutionary point of view for an infant to monitor its environment after birth and
set growth projections according to nutrient availability.

Early Diet and Later Development

Our preterm infant feeding trial has provided the first opportunity to study the
effect of a brief period of dietary manipulation on later brain development in a large
strictly randomized prospective trial in man. Studies at the 18 months follow-up, for
example (25), show that infants randomly assigned to a standard term formula rather
than a nutrient-enriched “preterm” formula for on average the first 4 weeks subse-
sequently had a major deficit in developmental scores. This was particularly marked
for motor development, where the deficit was of the order of one standard deviation
(15 points). As in animal studies, the greatest effects of diet were seen in small-for-
gestation infants, who were already nutritionally deprived at birth; such infants fed
term formula had deficits in mental and motor developing of 16 and 23 points
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(Fig. 1). It is remarkable that such a brief period of early dietary manipulation had such large effects on outcome.

Whether these long-term dietary effects on development relate to the early differences in brain growth observed between diet groups (22) or to the lack of a specific critical nutrient or signal for cerebral development now requires intensive investigation. Our cohort is currently being assessed at 7½ to 8 years.

Studies in full-term infants suggest that human milk may be beneficial for cognitive development (though this is debated). Our data support this thesis. As noted previously, psychomotor development at 18 months of age was 15 points higher in the infants fed a preterm formula, compared with those fed a term formula. However, in a parallel clinical trial in which the infants were randomly assigned to either the same preterm formula or banked breast milk, the slight advantage in psychomotor development seen at age 9 months in the preterm formula-fed infants disappeared by 18 months of age. These (unpublished) data show that banked breast milk, despite its poorer nutrient content, is associated with significantly greater psychomotor development scores than those seen with term formula. These two randomized trials suggest that breast milk could promote neurodevelopment.

In this regard, we looked at our study data to determine whether infants given their own mother's milk (instead of banked breast milk) have better neurocognitive development (26). Early preterm breast milk has a much higher protein and energy content than banked breast milk. A screening regression model analysis of the data collected at the 7½ to 8 years of age follow-up in the first 300 children in our study showed that five factors were independently related to the children’s intelligence quotient (IQ) scores. The most important factor was the early feeding of the mother’s milk to the infant, which was strongly related to later IQ \( p < 0.0001 \), followed by social class, mother’s education, sex, and the amount of ventilation support required during the perinatal period.

Two important factors were explored further, providing evidence that the mother’s milk feeding, and not other ancillary factors, might have explained our findings. First, infants of mothers who chose to provide breast milk but failed to do so had the same lower IQ as infants whose mothers did not elect to provide their milk. This could imply that the high IQ in breast-milk–fed infants was not simply a marker for the “health aware” mother who might confer developmental advantage in her infant for non-nutritional reasons. Second, most of these infants received their mother's milk through a nasogastric tube and were not subsequently breastfed. The infants in our study who were fed only their mother’s milk by tube while in hospital still had an 8-point advantage in their cognitive development, and a dose-response relationship was seen—the greater the proportion of mother’s milk the infant received, the higher the IQ. It still remains possible, however, that our findings could be explained by social, parenting, or genetic differences between groups.

Even if causality cannot be proven here, the possibility that human milk feeding could confer neurocognitive advantage in these infants requires that we examine the biological plausibility of the hypothesis. Breast milk contains long-chain lipids not found in formula, so formula-fed preterm infants theoretically might not achieve the
FIG. 1. Mental and motor development indices (Bayley scales) at 18 months in infants randomly assigned to a standard "term" formula (TF) or a special nutrient-enriched "preterm" formula (PTF) during the early weeks postpartum, in babies born small (above) and appropriate for gestation (below). Bars represent mean (SE) neurodevelopmental scores.
optimum long-chain lipid composition of the brain. A variety of hormones and growth factors is also found in human milk; some of these, such as thyroxine, have known effects on brain development. The possibility that human milk could affect neurocognitive development has important implications for human nutrition and must be explored further.

CLINICAL IMPLICATIONS

From the findings presented it would appear that the preterm infant may be highly "sensitive" to its early nutrition in terms of later outcome. Our data suggest that meeting the preterm infant's calculated increased requirement for in utero nutrient accretion may confer long-term benefits. These requirements may be met either by using preterm formula or fortified breast milk or a combination. The important question is whether these options are equivalent. Our findings strongly indicate (though do not prove) that for neurocognitive development the presence of human milk in the diet may confer additional benefit, above that achievable with a preterm formula. Our previous studies show that breast milk may also achieve important short-term outcomes including a reduction in necrotizing enterocolitis (27) (and systemic infection, unpublished data). In my view, therefore, it seems prudent to actively encourage mothers, on medical grounds, to provide at least some breast milk for their infants, even if it is not their intention to breast feed eventually. Any short fall in maternal milk supply can be made up by using a preterm formula. This suggested policy is different from current policy on most units, where mothers are asked if they wish to provide their milk rather than being positively advised to do so.

From a biological point of view, our study provides support for the thesis that nutritional programming during a sensitive period in early life may influence long-term health and development.

REFERENCES


**DISCUSSION**

*Dr. Evrard:* I am puzzled about your population. If all your babies were premature how could you decide to have a group fed on a term formula?

*Dr. Lucas:* This is an important question and I’m glad you asked it. This was a study of babies randomly assigned to diet between 1982 and 1985. At that time it was common for premature babies to be fed on standard “full-term” formulas rather than preterm formulas. In fact all we did in our study was to assign babies randomly to diets that were currently available and used for premature infant feeding. You are probably right to imply that it would not now be considered ethical, particularly in the light of the data I have presented, to use a full-term formula for feeding premature infants.

*Dr. Evrard:* Have you any idea about how the duration of breast feeding affects the IQ outcome?

*Dr. Lucas:* Since we eliminated babies who went home breast feeding and still found an 8-point advantage we can say that receiving human milk for an average of 1 month (which was the mean length of time the babies were on their assigned diet) was enough to account for the later IQ advantage. Whether 2 weeks’ worth, or 4, or 6 or 10 weeks’ worth makes a
difference is something we shall analyze further when the whole cohort has come through and we have the largest possible sample for this type of subgroup analysis.

**Dr. Di Toro:** Have you any information about infants suffering from various disorders that might require a modification of energy or protein intakes?

**Dr. Lucas:** The great problem with our present stage of nutritional research is to obtain any proof that early diet matters at all in any group of premature babies. Now that we are obtaining evidence that it does indeed matter we have two important areas to examine. The first is, Which are the nutrients that matter? The second is (and this is the basis of your question), Are there particular high-risk groups that need special nutritional management? Our subgroup analyses to date suggest that males and small-for-gestation (SGA) infants require special nutritional attention. It seems particularly important for these babies that we at least meet the *in utero* requirements. But whether they have different protein-energy needs from appropriately grown infants I cannot say. Professor Heird said that for the average SGA infant there is no major difference in needs. I believe that babies with bronchopulmonary dysplasia form a high-risk group and we are looking at these at the moment. However, I think your question needs another 10 years to answer.

**Dr. Heird:** I have to clarify a point relating to small-for-gestation infants. Our data addressed how they used nutrients. Thus what I meant to say was that on the same nitrogen intake SGA infants have the same retention of nitrogen as appropriate-for-gestational-age (AGA) infants. Obviously SGA infants will need more nutrients in order to catch up in the same time as AGA infants.

**Dr. Putet:** The differences in the results between term formula and preterm formula or breast milk seem to suggest that quality rather than quantity of nutrients has the main effect. What was the difference between the fat content of the term formula and the preterm formula? Was it the same mixture?

**Dr. Lucas:** I’m not certain that I fully agree that it is a question of quality rather than quantity. In our term formula versus preterm formula comparison the diets differed only in nutrient quantity. The fat quality was the same, as was the protein quality. They differed only in terms of amount, so that, for example, the preterm formula provided 2 g protein per dl and 80 kcal (which I think we might agree now is too much from the point of view of body fat deposition), while the term formula provided 1.45 g protein and 68 kcal per dl. I think that where quality comes into play is where we are comparing a formula with human milk, and then it may be either the special nutrient content that gives an additional advantage for human milk or the non-nutritional contents. I am suggesting that in order to meet the special requirements of the preterm baby we ideally need to give them some human milk as well as preterm formula or else human milk with a commercial fortifier.

**Dr. Orzalesi:** Could you speculate on why males are at such a disadvantage? Just as with our previous discussions about vitamin K and malignancies, we find good data but no sensible explanation.

**Dr. Lucas:** This is certainly a great mystery. I am sure you are aware from animal data that early malnutrition influences brain development more in males than females. All we can say at present is that brain structure seems to be different in males and females but we are nowhere near establishing a mechanism for this phenomenon.

**Dr. Koldovsky:** About 30 years ago I was involved in some experiments with rats that were prematurely weaned and were compared to normally weaned litter mates. Prematurely weaned males lost their spermatogenesis before normally weaned males, so the trouble lies not only in Woody Allen’s second organ, but in his first, too!

**Dr. Orzalesi:** My other query is related to the concept of the interaction between the
genetic potential of the individual and the environment. I really think your data need to be extended to full-term infants because the major variables in preterm infants are not related only to the fact that they are at a different stage of brain development and so on, but also to the kind of population that provides low birthweight infants in the first place. This population is socioculturally and in many other ways different from the population that provides full-term babies.

**Dr. Lucas:** I’m glad you raised this because I have been worried that anyone might extrapolate our findings to full-term babies. The evidence that full-term babies are affected by early nutritional management in terms of their long-term outcome is simply not there. It is certainly a priority from a research point of view to look at nutritional programming in the full-term infant.

**Dr. Agostoni:** Did you relate sodium or potassium intake to blood pressure values or correct for familial incidence of hypertension?

**Dr. Lucas:** There were major differences in sodium, chloride, and potassium intake between the groups, particularly sodium and chloride. However, the preterm formula also had substantially more protein, energy, and other nutrients than the other feeds and it will be difficult to isolate those. We now have some evidence to suggest that early sodium intake could have an impact on blood pressure at 7 to 8 years of age, but it is too early as yet to give a firm view on this.

**Dr. Agostoni:** My other question is, Was there a difference in weaning age between the groups?

**Dr. Lucas:** There was no difference in weaning practices between the groups. Obviously there was a wide range of variation in the time at which weaning foods were introduced but there were no overall differences in the randomized groups.

**Dr. Simmer:** I have some data from a small pilot study on term infants looking at the influence of diet. It addresses Dr. Lucas’s question about the quality of diet versus the quantity. The study was a prospective randomized trial of infant formulas with different fatty acid composition and included a breastfed group. The subjects were healthy term infants. At 5 months of age we carried out measures of neuronal development including the assessment of visual acuity by evoked potentials, using the minimum angle of resolution (the smaller the angle of resolution, the better the acuity). In an admittedly small number of infants matched for age, social class, and maternal education, we found that breastfed infants had better visual acuity. They also had higher erythrocyte levels of docosahexaenoic acid (DHA), which is present in breast milk but not in formula, and much lower levels of erythrocyte linoleic acid. Visual acuity was directly related to the DHA levels and negatively related to the levels of linoleic acid.

**Dr. Lucas:** These are exciting data that obviously need to be pursued.

**Dr. Salle:** You did not mention skeletal mineralization. What differences did you find at 5 years of age between the various feeding groups?

**Dr. Lucas:** We did a pilot study on 64 infants from our trial at 5 years of age in which we compared a group on banked breast milk with a group on preterm formula. We were interested to see whether the extremes of early nutrition might be reflected in differences in bone mineralization at this relatively late stage. To our great surprise we found that the babies who had received human milk in the neonatal period had an increase in bone mineral content of one standard deviation above our normal standards for babies in the Cambridge area, while babies fed on the preterm formula had values slightly below the normal standard. The polarization of the results was statistically highly significant. So we have the paradox that although we would expect babies fed on human milk during the neonatal period to have lower
bone mineral content than babies fed on preterm formula, the opposite was true. Somewhere between the neonatal period and 5 years of age the situation was reversed, so that babies fed on human milk ended up with denser bones. One possible explanation for this paradox could be that a low intake of calcium and phosphorus in early life programmed these babies to be frugal with these minerals, so that when later on they were put onto an increased calcium and phosphorus intake they developed denser than normal bones. It is of interest to me that the populations most at risk of osteoporosis in later life are those with the highest intakes of calcium and phosphorus in early life, i.e., the Western populations. In developing countries, where there are much lower intakes, osteoporosis seems to be much less of a problem.

Dr. Donzelli: Perhaps we should take into account not only the care but also the caregivers in assessing the longer-term outcome. The infants in your study were not only subject to different types of feed but they were also nursed in different units. How comparable was the care given in the units? One may have been less invasive than another.

Dr. Lucas: There are of course many factors that influence IQ. The purpose of our randomization procedure was to try to extricate the effects of feeding from the many other social and biological factors that influence IQ or any of the other things that we have measured. I accept that the type of care given may have an impact on later outcome but this would have to be examined in a study specifically designed to look at this issue.

Dr. Boyd: Accepting that you have really been studying a group of infants who should be in utero, have you gone back to the randomized studies of nutritional supplementation in pregnancy and asked the questions you have now formulated?

Dr. Lucas: No, but this is an extremely valuable suggestion. There would be much to be gained by reexamining those studies.

Dr. Singh: It worries me that what happens over a period of 1 month is then related to what happens at 18 months or 7 years. During that long period many more things happen. There are many more diet differences, environmental differences, emotional disturbances, genetic effects, and so on, so it becomes difficult to accept the single effect of a short period of early diet. I think it is necessary to repeat the studies and obtain more data to confirm these observations. Secondly, as far as language development is concerned it is well known that genetically females are more advanced than males under normal conditions. So the differences you have shown are probably differences that exist anyway, whatever the nutritional intake. Finally, with regard to the data suggesting that small babies have an increased incidence of coronary artery disease and hypertension, there is a high incidence of low birthweight and fetal growth retardation in developing countries, but epidemiological evidence suggests that the incidence of coronary disease in India is lower than in the West.

Dr. Lucas: The answer to the first two of your questions is randomization. Taking your first question, of course I accept that there are many factors throughout life that influence long-term outcome. It is precisely because of this potential confounding that careful randomization was applied. The two groups differed from an epidemiological point of view only in the way that were fed in the neonatal period. Any systematic difference found on follow-up has to be due to the early feeding differences unless we were extremely unlucky with our randomization. I am not saying that subsequent events cannot interact with the initial intervention but a systematic difference between groups has to be related to the initial randomization.

Secondly, in relation to differences between males and females, such a difference obviously exists but the difference between diet groups in children of the same sex cannot be explained on this basis. The major difference we saw between males fed on preterm formula and males
fed on full-term formula cannot be explained by any inherent difference between the sexes. It has to be an interaction between diet and sex, not sex per se.

Dr. Von Kries: You stress that this was a randomized trial, but such trials are not immune from bias. You have clearly looked carefully at confounders, but was the distribution of confounders similar in the three groups? Another problem with randomized controlled trials is loss to follow-up, which may be related to the outcome of interest. It is important that you give details about follow-up rates. Finally, in your analysis you stressed the differences between boys and girls. The finding may be correct, but the way you presented the data is not the usually accepted way to present the statistical analysis of such subgroup data. You need to run interaction tests rather than just pointing out the $p$ values. Have you done these?

Dr. Lucas: All these points are very important. First, if you have large enough randomization groups you expect to see an equal distribution of confounders. We have checked for this and I can assure you that we have found no differences in the distributions that could explain the results.

Your second point is also crucial. At 7½ to 8 years our follow-up is presently 99% of subjects still in Britain. We lost a few subjects to the United States because they were children of servicemen and the United States Army won't tell us where they are, so we cannot check them. This reduces the follow-up rate to between 96% and 97%. I think you would agree that such rates are reasonable.

As far as interactions are concerned, for verbal IQ, as you might expect and given a whole standard deviation difference between groups, there is a significant interaction between sex and diet.

Dr. Hernell: You suggest that preterm formula would give quantity and breast milk quality. What about exclusively breastfed infants? Contrary to what you might expect, exclusively breastfed infants have better bone mineralization. What about the other outcome variables?

Dr. Lucas: The proportion of babies fed exclusively on breast milk is rather low in our study. We are therefore waiting until the entire cohort has reached 7½ to 8 years before we analyze the remaining data.

Dr. Dufour: Did you take into consideration the time spent by the mother near her child. You said that those mothers who could not breastfeed tended to live quite far away. The presence of the mother near the child could be an important variable as well as whether or not the child was receiving breast milk.

Dr. Lucas: We have not analyzed this though the information is available. This is an important issue and we shall certainly examine it. Thank you for raising it.

Dr. Giovannini: Do you think human milk is the best choice for all situations?

Dr. Lucas: I think that human milk is probably unsuitable on its own for feeding premature babies. There is no reason to use unsupplemented human milk. It was evolved for the needs of full-term infants. Since premature infants historically would not have survived, or very rarely, it is extremely improbable that preterm mothers' milk would have evolved to meet their special needs. We know that babies fed on unsupplemented human milk develop a number of nutritional deficiencies. Having said that it appears that human milk contains factors that are valuable for preterm infants. I think that what we need is a combination of these factors together with the more suitable macronutrient composition of specially designed formulas. It seems likely that the desirable attributes of human milk are biologically complex so it will probably be necessary to provide some human milk in the diet. If breast milk is to be used as the major food it needs fortification.