Early Nutrition and Later Outcome

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The scientific studies of the eighteenth and nineteenth centuries that underpinned the development of more recent pediatric nutritional practice occurred long before the formal development of pediatrics. Franz Simon established a rational, scientific basis for infant feeding with his landmark work in 1838 (1), when the word *pediatrics* had not even entered the English language. This was 20 years before the first chair of pediatrics in the United States and a century before pediatric chairs were created in Britain (in the 1930s). Paradoxically, however, despite the intensity of work over the past two centuries, pediatric nutrition has never emerged as an independent specialty. As a result, education and training in pediatric nutrition are poorly developed, and most pediatricians receive at most a few hours of instruction on nutrition throughout their entire formal training period. Not surprisingly, consistent standards of practice in the field have been slow to emerge, and the massive body of research has, until recently, been inadequately focused on the key questions posed by modern practice.

However, there has now been a paradigm shift in thinking about nutrition that I believe provides a compelling basis for more formal development of the field. Until recent years, the major preoccupation in nutrition was in meeting nutrient needs and preventing nutritional deficiency or excess. Although this, of course, remains important, the current focus has shifted to the rapidly increasing evidence for biological effects of nutrition on *health outcomes*. Thus key questions are now being posed and addressed that would have received much less attention 20 years ago. Among such questions are whether nutritional management matters in terms of the patients response to their disease and, perhaps of greatest biological interest, whether early nutrition matters for long-term health and development.

These issues are particularly pertinent to the relatively new population of infants surviving at low gestation following the rapid emergence of neonatal intensive care during the past 25 years. This field emphasizes the need for specialized nutrition training and provides powerful examples of the biological importance of nutrition for both short- and long-term health outcomes. In this introductory review article, I shall try to create a backdrop for the research and clinical practice issues in the nutrition of the low birthweight infant that will emerge in this timely workshop.
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**The “Does It Matter?” Questions**

Feeding the very low birthweight preterm infant is not a natural physiological process. The cessation of placental nutrition up to 4 months too soon in biological terms and the need for rapid adaptation to postnatal life at an unphysiological stage in development create unique and challenging circumstances. In parallel with the development of neonatology as a whole, preterm nutrition is passing through the evolutionary stages of scientific research generally seen in new areas of clinical practice. It is useful to define these stages to identify the key issues for current research.

Broadly, I see research in a new clinical field as a three-stage process (2). In stage I, anecdotal observations raise the question, “Is this worth pursuing?” In stage II, epidemiological and physiological research provide descriptive and mechanistic data that raise testable hypotheses concerning the potential impact of intervention. Finally, in stage III, formal intervention experiments (generally randomized trials) test the efficacy and safety of clinical or public health practice. Thus stage III research shows whether the intervention tested matters in terms of improving one or more targeted health outcomes (efficacy) with acceptable risk (safety). An example would be the demonstration that the treatment of high blood pressure matters in terms of reducing significant morbidity without unacceptable side effects in the patient.

Until recent years, nutritional research in low-birthweight infants was largely in stage II. Research generally focused on collection of physiological and epidemiological data on growth, nutritional status, metabolic response to feeding, energy metabolism, nutrient absorption and retention, prevalence of nutrition-related disorders, and so on, which are factors of importance to our understanding of the biology and demography of infant nutrition but of uncertain relevance to outcome. It is clear, as in other areas of clinical medicine, that clinical practice in preterm nutrition can only be placed on a secure scientific footing using what I have termed stage III research.

What, then, are the stage III “Does it matter?” questions that are key to preterm nutrition? In the short term, such questions include whether nutritional practice has a major impact on morbidity (e.g., whether it influences critical disease processes such as necrotizing enterocolitis, infection, or mortality risk); in the long term, they include whether the way we feed low-birthweight infants has a critical impact on their health and development in later life.

Of these issues, the one that has commanded most recent attention is the idea that early nutrition could influence long-term effects on neurodevelopment or major diseases in adult life. Indeed, if the way very low-birthweight infants are fed changes their lifetime health, the implications for clinical practice are considerable. I have given this matter most attention in this chapter. I shall start by considering the broader concept of programming and its biological plausibility in the context of nutrition.

**The Concept of Programming**

Generally, events in early life might influence long-term outcome in three ways:

1. Direct damage (e.g., the loss of a limb);
2. Induction (i.e., deletion or impaired development of a somatic structure resulting from a stimulus or insult during a critical period); or
3. Physiological setting by an early stimulus or insult at a critical period, with long-term consequences for function.

Lucas (3,4) suggests that the term *programming* be applied to the latter two processes in which the programming stimulus only exerts long-term effects when applied at a critical or sensitive period.

Evidence for programming, outside the context of nutrition, is considerable (3,4). Examples are cited here. Early imprinting of behavior in birds has been recognized for centuries (5). *Hormonal* signals operating during critical windows have numerous programming effects. Thus in rats testosterone secreted by the fetal testis at a critical period programs the brain for male sexual behavior; a single dose of testosterone given at this time to a female fetus will permanently induce *male* sexual behavior (6). Teratogenic drugs, recognized since the 1920s, have powerful programming effects on somatic development. But postnatal programming by drugs may also occur: a single dose of phenobarbitone given to a neonatal rat may induce lifelong change in the activity of a key enzyme, cytochrome P450-dependent mono-oxygenase. Normal visual inputs are essential for the development of the visual pathway, hence squint amblyopia. The programming window is usually early fetal life or infancy, but, arguably, in the case of antigen-induced programming of the immune system, sensitivity may be lifelong. Whether there are other windows for programming at critical stages in life such as adolescence or menopause requires exploration.

These examples indicate that programming in fetal or postnatal life may result in initiation of normal development processes resulting from endogenous or exogenous physiological signaling during critical periods of development or that it may cause a long-term response to an environmental stimulus. Teleologically, the ability to respond to early environment to induce a lifetime change in structure or function could have evolved as a mechanism that allowed the organism to fine-tune its machinery in an adaptive way according to its early milieu. It seems likely, however, that some environmental insults could have programming effects of a nonadaptive, adverse nature.

**NUTRITIONAL PROGRAMMING IN ANIMALS**

Does early nutrition operate in this programming manner? McCance (7) provided key evidence for this in animals. He manipulated litter size in rats so that rats from large litters received less milk than those from small litters during the 21-day suckling period, by which time rats from large litters were substantially smaller than those from smaller litters. At this point both groups were fed normally, but the smaller animals (from large litters) continued to diverge in body size from the larger animals. Thus 3 weeks of dietary manipulation had resulted in a lifetime “programming” of growth trajectory.

McCance then showed that it was only the early weeks that constituted a critical period for such effects. Equivalent dietary manipulation for a 3-week period a few weeks later had no lasting impact. The underfed animals showed catch-up growth when they were refed; the critical window for growth programming by early nutrition had passed.
Following these key studies in the 1960s, many animal experiments on nutritional programming confirmed the wide variety of lifetime programming effects on metabolism, blood pressure, diabetes, obesity, atherosclerosis, behavior, and learning (see later); these would be of considerable public health importance were they to apply to humans.

Extensive animal data, largely on rats, show that nutrition at a vulnerable period of brain development may have permanent effects on brain size, brain cell number, behavior, learning, and memory (8,9). In Smart's review of 165 animal studies on early undernutrition on later learning (9), the number of studies in which undernourished animals fared worse than controls greatly outweighed those that favored the controls. The extent to which these animal data have relevance to human cognitive development is, however, uncertain.

With regard to health outcomes, experimental studies on fetal nutrition have shown, for instance, that protein-undernourished fetuses had long-term reduction in pancreatic cells and insulin secretion (10). Hahn (11) manipulated litter size in neonatal rats so that rats from small litters were temporarily overfed during the brief suckling period and found that in adulthood these animals had permanent elevation of plasma insulin and cholesterol. Weaning these animals onto a high-carbohydrate diet further induced lifelong elevation in the activities of HMG-CoA reductase and fatty acid synthetase (key enzymes for cholesterol and fat synthesis).

In primates (baboons), overfeeding during infancy in the female resulted in obesity that was not manifested until early adult life (12), raising the question of where the “memory” of the early event had been stored in the intervening period. In further studies (13), baboons were randomly assigned to breastfeeding or formula; then both groups were placed on a “Western-style” high-saturated-fat diet. The previously breastfed group had, in adult life, higher concentrations of plasma low-density and very-low-density lipoprotein (LDL, VLDL) cholesterol, lower protective high-density lipoprotein (HDL) cholesterol, and increased cholesterol absorption, perhaps relating to the permanent change in bile acid secretion. These data imply that breastfeeding may program these primates to be conservative with cholesterol but that this might be disadvantageous to lipid metabolism if they were subsequently placed, unphysiologically, on a high-saturated-fat diet. Indeed, at necropsy the previously breastfed baboons had significantly more atherosclerosis than those fed formula.

Our own studies in rats (14,15) showed that both pre- and postnatal nutrition may influence adult outcomes but that the critical period for programming depends on the outcome studied. Lifetime effects on body size were only seen in relation to postnatal nutritional manipulation; thus animals fed by mothers given a low-protein diet during lactation were permanently smaller, whereas prenatal low-protein diet given to the mother had no long-term effect on the size of the offspring. In contrast, lifetime changes in hepatic glucose metabolism (glucokinase and phosphoenol pyruvate carboxykinase [PEPCK] activities) were induced only by prenatal dietary manipulation in the mother. For some outcomes, however, the critical window for programming was longer. Thus Lucas et al. found that either prenatal or postnatal (during lactation) low-protein diet given to the mother followed by a nutrient-enriched diet
given to the offspring resulted in a programmed reduction in plasma triglycerides, HDL and total cholesterol, and systolic blood pressure in these offspring when they reached adult life (15). For some outcomes, the direction of response may depend on timing of the programming stimulus. We found lifespan was significantly decreased in animals born to mothers that had a low-protein diet in pregnancy but were then suckled by mothers on a normal protein diet, whereas the converse (offspring of mothers fed normally during pregnancy but suckled by mothers fed a low-protein diet) resulted in a significant increase in lifespan (16).

Such animal data have importance in suggesting human interventions and in defining underlying programming mechanisms (see later). However, public health policy for early nutrition in humans must ultimately depend on human studies.

NUTRITIONAL PROGRAMMING IN HUMANS

Given the evidence for programming in general and the evidence for nutritional programming in animals, nutritional programming in humans might be predicted. This has not been easy to prove, largely because most studies have not had an experimental design but have documented retrospective epidemiological associations often subject to alternative explanation. Collectively, the human epidemiological data are extensive, and illustrative studies are discussed here.

Early Nutrition and Later Cognitive Function: Epidemiological Evidence

Numerous investigators have attempted to test the hypothesis, using epidemiological models, that suboptimal nutrition at a vulnerable stage in brain development has permanent effects on cognitive function. Epidemiological associations found between malnutrition and reduced cognitive performance, however, might not be causal. Malnutrition, principally studied in developing countries, is inextricably associated with poverty and poor social circumstances that might explain the adverse outcomes. Prospective randomized or satisfactorily controlled studies are rare and most do not provide unequivocal data.

Several studies suggest that breastfeeding promotes long-term neurodevelopment, in some cases even after attempts to adjust for confounding factors (17), though whether or not these effects reflect residual confounding by educational and parenting differences between groups is uncertain. Our evidence that human milk may promote neurodevelopment and IQ in infants born preterm is stronger (17,18), with implications for practice. Human milk contains numerous factors that could influence neurodevelopment.

Nutrition and Later Disease: Epidemiological Studies

Most studies on early nutrition and later health have been epidemiological and inconclusive. Unlike the studies in baboons mentioned earlier, breastfed and bottle-fed infants have not been shown to differ in later total plasma cholesterol at 8 years (19) or
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total cholesterol, LDL, or HDL at up to 16 years (A Lucas et al., unpublished). Early salt intake has been associated with later high blood pressure in some studies, but we and others have failed to show a causal link in preterm or normal individuals (20).

Breastfed and formula-fed babies may have potentially important differences in later health outcomes. Breastfeeding has been associated with a protective effect against insulin-dependent diabetes (21) (not observed by all) and a reduced incidence of lymphoma (22). In one epidemiological study prolonged breastfeeding in males was associated with a greater incidence of atherosclerosis in late adult life (23). Whether these associations are causal or confounded by the major demographic differences between groups needs further exploration.

Recent studies, notably by Barker and coworkers, have shown relations between anthropometric indices at birth and at one year (possible markers of early nutrition) with cardiovascular disease and its risk factors (23,24). Low body weight, head circumference, and ponderal index at birth and low weight at 1 year relate to increased risk of later cardiovascular disease. Small size at birth and up to 1 year has also been associated with higher blood pressure and adverse changes in plasma concentrations of glucose, insulin, fibrinogen, factor VII, and apolipoprotein B; abdominal circumference at birth is inversely associated with raised serum concentration of total cholesterol, LDL cholesterol, and apolipoprotein B. These provoking and important observations have been interpreted by the investigators as supporting the hypothesis that poor fetal nutrition, perhaps secondary to poor maternal nutrition, adversely programs the individual for later cardiovascular disease, hypertension, and diabetes. Their suggestion is that improvement of fetal nutrition might be an important public health measure (24). Supporting this is the evidence that early nutrition in animals has been shown to program corresponding outcomes. There are inconsistencies, however: in rats, overnutrition rather than undernutrition may be associated with later elevation of blood cholesterol (11), and chronic undernutrition has been associated with longevity (25). A key issue for debate is the proposed nutritional interpretation. Poor intrauterine growth might be associated with other, nonnutritional derangements that could be responsible for long-term programming.

AN EXPERIMENTAL APPROACH TO NUTRITIONAL PROGRAMMING IN HUMANS

The epidemiological data discussed earlier raise two critical issues: first, although such studies generate hypotheses, they do not prove nutritional cause; and second, it is speculative to use these findings to underpin public health or clinical interventions in human nutrition.

Clearly, public health and clinical policy would be most soundly based on experimental rather than on epidemiological studies. Therefore 17 years ago I elected to devote major attention of my research group to developing the use of the infant nutrition intervention experiments in a formal way, to explore the concept of nutritional programming in humans and to underpin nutritional practice. The elements of this program, which collectively were novel at that time, included the following in each
clinical trial:

1. Formal randomized nutritional intervention in infancy with planned long-term follow-up.
2. Carefully calculated size to detect differences between groups for a key targeted health or developmental outcome ("efficacy") with adequate power; and trials large enough to detect differences in adverse outcome ("safety") between groups.
3. Trials conducted in a similar manner to a pharmaceutical intervention trial employing what are now termed good clinical practice guidelines.
4. Cohort details documented to facilitate long-term (or lifetime) follow-up.

There are several windows in which infant nutritional intervention experiments are feasible and ethical.

1. *Preterm* infants can be randomized to diet to test the importance of the perinatal period as a window for nutritional programming.
2. Formula-fed full-term infants could be randomly assigned to formulas of different nutrient content to test ways in which early infancy might be critical for nutrition. These interventions can also be targeted to infants growth-retarded at birth, who have been shown epidemiologically to be at long-term risk for growth and neurodevelopmental deficits and for ischemic heart disease and its antecedents. A key question is whether early nutritional intervention could reprogram these infants following poor intrauterine growth and ameliorate risk.
3. Infants can be randomly assigned to different weaning foods to test whether nutritional sensitivity extends into infancy or beyond.

In around 20 major outcome studies, testing a range of key hypotheses, we have more than 5,000 infants and children in all these categories, in various stages from the intervention period to long-term follow-up, the oldest subjects followed prospectively now to 16 years.

**TRIALS ON THE LOW-BIRTHWEIGHT, PRETERM INFANT**

Of our trials, the most long-standing (now running for 16 years) has been a five-center study on 926 infants born preterm (mean gestation of 31 weeks) (18). Such infants were considered to be valuable for nutritional programming studies for the following reasons:

1. Since it was unknown (16 years ago) which were the optimal diets for this population, it was ethical to randomly assign available diets during hospital study to address the question as to which diet was associated with a better long-term outcomes.
2. Since preterm infants are a "captive" population, intensive nutritional, physiological, biochemical, and clinical monitoring was feasible during the intervention period.
3. It might be predicted that preterm infants, born during a stage of rapid development, would be particularly sensitive to programming stimuli.
Illustrative results from this work, comprising two parallel nutritional intervention studies, are presented in this chapter. The study design has been presented elsewhere (18), and only brief details are given here. The 926 infants weighing less than 1850 g at birth represented an unselected cohort from the five centers recruited between 1982 and 1985. No parent refused consent.

In study 1 (26), conducted in three centers that had a human milk bank, subjects were randomly allocated to banked donated breast milk (from unrelated donors) or a special nutrient-enriched preterm infant formula, designed by us to meet the nutrient needs of the fast-growing immature preterm infant. Donor human milk was unsupplemented, as frequently given in the early 1980s. When mothers failed to provide their own breast milk, the infants received donor milk or preterm formula as sole diets \( n = 159 \). When mothers did provide their expressed milk, donor milk and preterm formula were randomly assigned a supplement to mother’s milk \( n = 343 \) in volumes according to the mothers’ success in providing their own milk (mean, close to 50% of intake).

In study 2 (27), the random allocation was to standard full-term formula (suitable for full-term infants, used often in the 1980s) or preterm formula, with 160 in the sole diet group and 264 in the supplement to mother’s milk group. The protein \( \text{g/100 ml} \), energy \( \text{kcal/100 ml} \), and calcium \( \text{mg/100 ml} \) contents of the four diets were, respectively, as follows: preterm formula—2.0, 80, and 70; standard formula—1.5, 68, and 35; banked breast milk—1.3, <50, and 30; and mothers’ expressed milk—1.5, 62, and 30. (Values for the latter are mean values for 6,000 pooled 24-hour samples.) The breast milk was donated by breastfeeding mothers in the community who collected milk that dripped from the contralateral breast when feeding their own infants. Many of the infants required initial parenteral nutrition, and the median number of days to attain full enteral feeds was 7 days in study 1 and 9 days in study 2. The assigned diet was given (for a median of 4 weeks) until the baby attained a weight of 2,000 g or was discharged from the neonatal unit, whichever was the sooner. After discharge from the neonatal unit, mothers fed their babies as they and their advisors chose. Follow-up staff were blind to the original dietary assignment.

Neurodevelopment

At long-term follow-up of this cohort, the principal targeted outcome was neurodevelopment. Within each study, calculated sample size was for one-third of a standard deviation (5 quotient points) for trials with randomized diet as sole diet (trial A) or breast milk supplement (trial B) combined, and half a standard deviation (8 quotient points) for the sole diet trial (trial A) alone. The subjects were seen at 18 months corrected age and at 7.5 years. Data from the 18-month follow-up only are published so far.

In study 2, babies in trial A fed standard formula had a 6-point lower mental development index and a 15-point lower psychomotor score \( p < 0.001 \); and in trials A + B \( n = 310 \) a balanced addition, preserving randomization), a 6-point lower psychomotor score \( p < 0.01 \), despite the blunting effect of mother’s milk usage...
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(trial B) in both randomized groups. The effect size was substantially greater in males (27). Recently, we confirmed that the significant developmental disadvantage, seen principally in male preterm babies fed a standard term formula (which we now recognize does not meet the nutrient needs of this group), was also seen at 7.5 years, when IQ (notably, verbal IQ) was significantly depressed (unpublished).

Thus we showed that a brief period of dietary manipulation in the neonatal period (4 weeks on average), using a nutrient-enriched rather than a standard formula, significantly influenced neurodevelopment at 18 months. Our further follow-up at 7.5 years (unpublished), when IQ is more predictive of that in adults, indicates that the disadvantage for the standard-formula-fed group could therefore represent a permanent effect. These data provide some of the only evidence from a large long-term randomized trial that early diet, during a “critical” or vulnerable period, could “program” neurodevelopment.

Surprisingly, in study 1, despite the poor nutrient content of donor breast milk, the outcome of those individuals fed on it in the neonatal period was no worse from that seen with preterm formula. We have suggested (18) this may be due to ameliorating, beneficial factors in donor milk. Indeed, infants fed solely on donor milk (in study 1) had a substantial psychomotor advantage over those fed solely on standard formula (study 2); in this (nonrandomized) comparison, both diets were similarly low in nutrient content, yet the donor-milk-fed group had a 9-point advantage in psychomotor scores.

Health Outcomes: Bone Mineralization

These preterm studies have provided an important opportunity to test whether a period of nutritional intervention during early life could affect the propensity to disease in later life. This is currently being investigated, particularly with respect to two key endpoints: cardiovascular disease (and its markers) and bone health. Data on the latter are discussed briefly here.

Adult degenerative bone disease (osteoporosis), a major public health problem in the West, has been linked to peak bone mass attained in young adult life (28). Following attainment of peak bone mass, bone mineral content falls and may descend below the safety level for clinical disease. Most interventions to reduce the incidence of clinical disease have been in middle life. Little attention has been given until recently to the possibility that early factors could influence bone mineralization in childhood and hence peak bone mass. We have tested the hypothesis that diet in the neonatal period in preterm infants could have a long-term impact on bone mineral content and bone metabolism, of potential relevance to the propensity to bone disease in adulthood. At a 5-year follow-up we found that bone mineral content (adjusted appropriately for body size) was higher in children previously assigned randomly to human milk than in those assigned to formula (29). These data raised the possibility that either factors in human milk or, alternatively, a diet suboptimal for preterm infants (e.g., in calcium content), as human milk is, could program greater bone mineralization later in life. Our unpublished data (MF Fewtrell et al.) at 9- to 12-year follow-up
now indicate that children fed suboptimally in the neonatal period for just 1 month on average have an increase in plasma osteocalcin (a marker for bone formation) by early adolescence. Clearly, this example of nutritional programming, determined in a strictly experimental context in humans, now needs further investigation in view of its potential implications for long-term bone health.

Programming Mechanisms

Nutritional programming has been convincingly demonstrated in animals, including primates, and there is now compelling evidence from experimental studies that this process operates in humans. More recently, attention has turned to the mechanism (3). Some programming events might have immediate effects on structural development (e.g., on dendritic arborization or glial cell growth in the brain), with long-term consequences. However, nutritional programming here might not simply reflect failure to fuel a growth process. Nutrients might be critical signals acting directly or through coupling mechanisms on “receptors” in sensitive tissues. With regard to the programming or “setting” of later function (e.g., of a key metabolic pathway), the question is how the “memory” of an early event is “stored” throughout life despite continuous cellular replication and replacement. Proposed mechanisms include adaptive effects on gene expression transmitted to the progeny of the originally programmed cells. Alternatively, the early nutritional milieu may stimulate adaptive clonal selection or differential cell proliferation so that the quantity or proportion of cell populations in a tissue is permanently affected. Indeed, in collaboration with Hales, we have obtained indirect evidence of the latter mechanism in relation to the programming of metabolically significant cell populations in the liver (14). The exploration of such fundamental processes is critical to an understanding of the biology of early nutrition.

Negative Findings

Although the foregoing examples emphasize the sensitivity of preterm infants to their early nutritional environment, not all interventions have had the hypothesized longer-term effects. We have been unable to show that early nutrition in preterm infants during the neonatal period influences long-term growth (unpublished) or blood pressure (20); and human milk fortification did not significantly promote neurodevelopment (30), though a small effect cannot be excluded and a larger study is needed.

Safety

In pharmaceutical trials of a new agent, safety is generally as important as efficacy. Although this chapter principally considers key aspects of nutritional “efficacy” (i.e., long-term benefit), our own and others’ data show that safety is a surprisingly important and often neglected aspect of nutrition. Nutritional management choices may have a major influence on potentially life-threatening disease processes, including
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necrotizing enterocolitis and systemic sepsis (30, 31; unpublished observations). For this reason regulatory bodies are now paying particular attention to safety testing of novel nutritional interventions. Interestingly, the addition of long-chain polyunsaturated fatty acids (LCPUFA) to infant formula (32) represents a watershed in the history of infant nutrition research in this respect. The standards of safety and efficacy testing of LCPUFA being required by regulatory and government bodies in the United States, Canada, and the United Kingdom are unprecedented and herald a new era in which infant nutritional research is being viewed in a pharmaceutical context. As we look back, we see that numerous new advances in infant nutrition (including the addition of LCPUFA to European low-birthweight formulas) were introduced without adequate human safety (or efficacy) trials—a situation that would have been unacceptable in the introduction of a new drug.

IMPLICATIONS

In clinical terms it is increasingly clear that nutritional management of very low-birthweight infants should be based on stage III efficacy and safety trials. Data from such trials should take precedence over stage I and II data of a more theoretical nature. Since the nutritional scientific literature is dominated by stage II data, clinicians must be selective in citing studies that influence their practice. For instance, since stage III data show that relatively brief suboptimal nutrition at a critical stage of rapid early brain development has potentially lifelong consequences for neurocognitive function, this must be weighed against stage II data on theoretically adverse metabolic consequences of meeting the very low-birthweight infants’ needs in the early weeks. For instance, since meta-analyses of randomized trials indicate that transpyloric feeding may induce a higher death rate than intragastric feeding (33), this must take precedence over any more theoretical perceived benefit for the transpyloric tube feeding.

Research implications of the new approach to early nutrition are many. Two important issues are raised in this chapter. First, future clinical trials of early nutrition must be large enough to incorporate targeted clinical safety testing if this is indicated. Second, if nutrition influences long-term outcome, then it is critical that “intermediate” endpoints in clinical trials are identified to avoid the near impossibility of lifelong follow-up. Our own center is committed to exploring and using such endpoints. Cognitive function is a good example of an outcome that can be predictive of adult outcome when measured in mid-childhood. We are currently investigating whether magnetic resonance imaging and spectroscopy of the brain, when combined with functional neuropsychological testing in children, will permit noninvasive exploration of more detailed permanent effects of nutrition on the brain. With regard to health outcomes, it is important to identify tools that can detect the early origins of adult disease. Currently, we are using noninvasive vascular ultrasound to examine endothelial dysfunction dynamically as a probable measure of early atherosclerosis, with promising early results showing that growth retardation in utero may be associated with impaired endothelial function at age 9 to 10 years (34) and adulthood (CPM
Leeson et al., unpublished). Endothelial function is currently being measured by us in a large follow-up study of preterm infants randomized to their early diet, to test the hypotheses that early suboptimal nutrition and prematurity itself adversely influences this early evidence of atherosclerotic disease.

In more general terms, the studies performed to date on preterm infants form an important part of the current experimental evidence for nutritional programming in humans, an area of major biological significance with important implications for practice.

REFERENCES


DISCUSSION

Prof. Haschke: When comparing the two formulas, there were a lot of confounders. Would you like to comment?

Prof. Lucas: You may need to be more specific. This was certainly a randomized blinded study of two formulas, as far as we could engineer it. They were similar in their ingredients, differing only in the amounts of those ingredients. They had the same protein mix, but they differed in the amounts of protein; and they had the same fat blend, but they differed in the amounts of fat. The formulas were blinded to the investigators and were given code numbers, so the investigators or the nurses in the unit did not know which they were giving. Confounding factors may creep into any randomized trial. If you feed babies on diets which differ in nutrient content, then of course they will produce different growth rates, which then produce a different response in the attending staff. Babies who grew less well on the term formula tended to have been given slightly larger volumes than those fed on the preterm formula. However, the increase in volume was never enough to cancel out the difference in nutrient intake between the two groups. Perhaps you have some specific aspects of confounding in mind?

Prof. Haschke: You presented similar data last year, where you described three groups. One group was fed the term formula, one was fed the preterm formula, and a third was fed breast milk. As far as I remember, there was no difference between the preterm formula and the breast milk groups, whereas there was a large difference between the term formula, on the one hand, and the preterm formula and the breast milk groups, on the other. So I am confused that the data are now presented in a different way.

Prof. Lucas: We have two quite separate randomized trials, one comparing term formula with preterm formula, which is the one I presented, and another comparing banked breast milk with preterm formula, which I did not have time to present today. Both were strictly randomized trials. Thus we have two parallel independent clinical trials done in different centers, comparing two diets. However, because they have the same preterm formula in common, it has allowed us to cross-compare the two trials. The data I presented today are from a pure clinical trial conducted in two centers comparing two randomized diets.
Dr. Micheli: You opened an important window on the relation between structure and function by telling us that the size of the hippocampus could be related to memory. Do we have any means of measuring this at the bedside in extremely low-birthweight infants?

Prof. Lucas: You are looking at the question the other way around from us. What we are interested in doing, since magnetic resonance imaging is extremely expensive and difficult, particularly in very low-birthweight babies, is to find psychometric tests that relate to particular parts of the brain, so that we can do psychometric studies that have significance for what is happening in the brain.

Some people have managed to do imaging studies in the neonatal period (e.g., Edward’s group in England), but you really need to have committed machinery for that. But I would not want you to come away from this meeting thinking that I said that nutrition is the reason for the hippocampus being smaller in low-birthweight babies. That is a hypothesis, and we now need to test it prospectively. We are looking for parts of the brain where we can relate structure to function, and then we can look at the impact of nutrition on the functional outcome as a proxy for an effect on structure.

Prof. Nowak: In relation to your vascular studies, couldn’t the difference in vascular function between normal and underweight babies have been related to differences in the size of the arteries you analyzed?

Prof. Lucas: You do of course have to adjust for vessel size, based on current weight. We have a number of ways of doing this; it’s a very rapidly developing field. You can adjust for the area of the artery and you can apply various techniques that remove factors that you think might be confounders. Of course you can also adjust for current body size, as well as the size of the blood vessel. All I can say is that the findings I presented are robust in that none of the adjustments that we’ve made to take account of vessel size and body size have removed the relation between birthweight and endothelial function.

Dr. Atkinson: I want to ask you about your bone data, the osteocalcin. We know that osteocalcin fluctuates tremendously during puberty. Were the data you presented on the differences in osteocalcin corrected for pubertal status, which is, I think, rather variable among preterm infants?

Prof. Lucas: We do have detailed information on the pubertal status of these children, though not by direct examination, but by self-examination, which we feel is the most ethical approach here. From the most recent analyses, the results have stood up to adjustment for pubertal status. The actual raw data that I presented you were by randomized group, and since the groups were randomized, any confounding caused by puberty should have been balanced out. We have to take account of the possibility that one diet group put the children into earlier puberty than the other, but I can confirm that that was not so. However, I do agree with you that there is a problem in studying children as they go into adolescence.

Prof. Pohlandt: Your data on the incidence of necrotizing enterocolitis are often cited as evidence that human milk protects against necrotizing enterocolitis (NEC). But in your paper the incidence of NEC was only a secondary outcome criterion, among others. You are not able to do a confirmatory statistic, only an exploratory statistic. Your data only support the hypothesis that human milk is protective, but you always present the data as evidence. I would like you not to do that.

Prof. Lucas: I have always been quite circumspect about those data. First, it was
not a randomized trial; the only randomized component of the trial that would have been suitable for this purpose was the comparison of banked breast milk versus preterm formula, but there were only 150 babies in that limb, which is not enough to show a difference. Having said that, however, there was a threefold difference in the incidence of NEC between those two groups. I would like to emphasize that safety aspects of diet were an important concept from the start of the study. Thus NEC was an important safety issue that we identified early in the trial planning; this is not a post hoc analysis. The importance of the data that we presented in Lancet was that they generated a hypothesis and confirmed previous findings of the protective effect of human milk. Since our publication—which I don’t think would have stood alone—further data have been obtained; I presented the results of a national survey where we looked at every single case of NEC in Britain and showed that there is a relation between disease severity and the amount of human milk used. Thus even if we are not yet at the level of proof, the data are sufficiently suggestive for us to include in our advice to mothers.

Dr. Frank: Do you have any clues as to reason for the female advantage in some of your developmental studies?

Prof. Lucas: The first thing to say is that increased vulnerability of males is quite a general phenomenon. It is seen across many animal species. Nearly all the effects of early diet on neurodevelopment have been seen in male rather than female animals. Apart from neurodevelopment, there are other outcomes that are influenced selectively by gender. For instance, we published data recently in the rat showing that suboptimal nutrition programmed a change in triglycerides and cholesterol in the offspring when they reached adult life, and on close examination of the data we found that these effects only occurred in males, not in females. So we have to ask why, in evolutionary terms, should there be greater vulnerability in males than in females. One general biological argument could be that there is a greater evolutionary investment in females than in males, because of their importance for reproduction. It may be that we are not looking so much at the vulnerability of males as at the greater protection that has evolved in females to ensure their survival.

Dr. Chessex: Rather than a nutritional effect, I think there may be another explanation for gender protection and this relates to antioxidant function, which is different between the sexes. We have data showing that endothelial cells put in an environment in which the peroxide content is similar to that of parenteral nutrition solutions survive better if they are from females. And in cells from tracheal aspirates from term and preterm infants, there is also better survival of female cells. We have shown that the glutathione content of cells from girls is significantly higher than from boys, and that glutathione reductase activity is significantly greater in females than in males.

Prof. Lucas: You are looking at fundamental differences between males and females that could be related to the phenomena I was discussing; that is, a biological underlying protection favoring females. There is certainly a fundamental difference in their metabolism. As far as the nutritional effects are concerned, we are looking at randomized interventions here that produce a difference in outcome within the male group. That does not give you a mechanism. It simply says that if you randomly assign babies to diet A and diet B, there is a difference. You may well be identifying the reason that there are underlying differences, and these come to light when they interact with diet.
Dr. Walker: I was curious about your observations regarding breastfeeding versus formula feeding and the expression of allergy. As you know, there have been several prospective trials in the UK, the United States, and Europe suggesting that breastfeeding may delay the expression of allergy in allergy-prone infants but does not prevent it, because there are so many other factors in the environment that affect the allergic reaction. Do you think the difference in your results is because your studies dealt with preterm infants?

Prof. Lucas: One thing that is different about our observations is that this is the only randomized trial of human milk versus formula that I am aware of. One of the great problems with all breast milk versus formula comparisons is that they are nonrandomized, because it is unethical to tell mothers to breastfeed their babies on a randomized basis. But in preterm infants, you have a unique circumstance in that it is possible to assign babies randomly to banked breast milk from unrelated donors or to formula. The nonrandomized nature of most of the comparative studies of breast milk and formula means that they are highly confounded by the large differences in demographic and social circumstances, indeed by the behavior of families as a whole, with respect to almost every aspect, including feeding practices, that might influence allergy. Thus it is very difficult to compare breast milk and formula cleanly in term infants. In the (albeit premature) babies in our study, the data are unconfounded by those social biological factors, and this represents a pure randomized comparison. However, it is also possible that what you said is true for this population as well, in the sense that we have taken a snapshot at a particular point in time, 18 months. It may well be that by 2 or 3 years of age the groups will have caught up with each other. That remains to be tested. But at 18 months there’s a major difference between these randomized groups in a clean blinded study.

Prof. Koletzko: You implied that your observation of a lower prevalence of allergic manifestations in infants previously born preterm who were fed banked human milk gives us conclusive evidence that breastfeeding, at least in preterm infants, protects against allergic manifestations. I have the same concerns as Dr. Pohlandt about the post hoc analysis of various secondary outcomes of your studies. I also believe that feeding banked human milk may not necessarily be the same as feeding own mother’s milk. The data from a large study reported by Saarinen and coworkers from Finland (1), who followed some 6,200 term infants prospectively from birth for 18 months, showed that infants who were supplemented with banked human milk during the first days after birth in hospital had a lower rate of cow’s milk protein allergy than those who were fed their own mother’s milk only. So the question arises as to whether factors in banked human milk, such as the multitude of foreign proteins or a difference in anti-inflammatory or immunological mediators, could have different effects from unsupplemented own mother’s breast milk.

Prof. Lucas: I want to emphasize that the data I presented were in babies with a positive family history of allergy. If you look at those with no family history of allergy, the effect is not shown. So we seem to have an interaction between genes and the environment.

The most serious issue you raise is the question of whether it is possible to look for multiple outcomes in one study. We had a limited number of main outcomes, and in our publications we always make it clear that our study is being used in this way. It would obviously be impossible and unethical to repeat such very large studies, so we must get the most we can out of this one. Clearly, you have to examine the data in
terms of the significance of the results in relation to multiple outcomes, and you can apply your own Bonferroni procedures or whatever to the results obtained. In fact, in the case of allergy the differences between groups are really highly significant. Nevertheless, I would accept that any single randomized trial, whether you are looking at multiple outcomes or even a single outcome, simply generates a hypothesis for further testing. In the meantime, we have patients to manage and we have to base our management decisions on the best-quality data that we have at any time. I do agree that it is extremely important that further studies are done on the long-term immunological effects of diet in newborn infants, though I would emphasize again that there are no other data comparing human milk and formula in a randomized way. We must therefore regard these as potentially important data to build on in future randomized trials.

Dr. Sedaghatian: I thought there were many cells in human milk, lymphocytes and so on, that could cause an immunogenic response in babies given human milk supplements. Though you showed us a reduced allergic response at 18 months, other participants obviously feel that this may not be permanent. I think it would be wise to use mother’s own milk and compare it with banked milk and premature formula in terms of the long-term outcome for allergy.

Prof. Lucas: Obviously, we can analyze the data in that way. We have got babies fed on banked breast milk, on mother’s own milk, and on formula. It is quite possible to do that three-way analysis. The problem here is one of randomization. We can produce epidemiological data comparing mother’s own milk with formula, but we can produce much more robust randomized data if we use banked breast milk, while recognizing the difference. I want to emphasize that in our original study both groups received their own mothers’ milk. They were randomly assigned to formula as a sole diet or as a supplement to mothers’ milk, or banked breast milk as a sole diet or supplement to mothers’ milk. So own mother’s milk is common to both groups, and what you are looking at is the difference relating to the component of the diet that was either formula or human milk. You are looking at the difference between having cow’s milk protein in the diet and not having it.

Dr. Rigo: There are studies suggesting that weight gain composition is different between males and females (2). We have data showing that the increase in lean body mass in low-birthweight infants is greater in boys than in girls but that fat mass deposition is greater in girls. Do you think this suggests that the nutrient requirements of boys and girls are different during the neonatal period?

Prof. Lucas: Yes, that is possible. Ruth Morley is quite specifically looking at body composition in relation to neurodevelopment in males and females in the post-discharge period. This is so close to her topic, perhaps she would like to answer.

Dr. Morley: The data on fatness in female infants at birth are intriguing. I don’t believe it is yet possible to conclude that the female infant has more fat, though I would be very interested to know whether female infants lay down fat earlier than male infants during gestation. If so, that may be one of the factors that protects female infants, because they may have greater nutrient reserves.

Prof. Lucas: That is interesting and important. One of the great problems is the difficulty in measuring body fatness in very young babies. We published two studies showing that the two ways in which you would be most likely to measure body fatness—that is, skinfold thickness and body mass index—do not correlate at all well with the gold standard measurement, deuterium dilution, during at least the first 6
months of life in healthy infants. To address the question you are posing, we need to be using quite sophisticated measures of body composition, probably isotope dilution.

_Dr. Sedagathian:_ If I understood you correctly, you said that the preterm baby who is fed on breast milk has better bone mineralization in later childhood. Does this apply to babies who are partially fed on breast milk? We know that by 1 month, breast milk is insufficient for preterm infants, and we usually add supplements.

_Prof. Lucas:_ Don’t forget that these studies were done in the early 1980s, when human milk fortifiers were not in routine use. So you are looking at unfortified human milk during the period of randomization, uncontaminated by fortification.

I do want to emphasize another point. There were two hypotheses in our bone studies: one is that human milk has some factors in it that are important for the long-term programming of bone; the other is that it is suboptimal nutrition that has a long-term programming effect. We have better evidence in support of the latter. For example, we found a higher osteocalcin level in the term formula fed group than in the preterm formula fed group. One possible explanation for this is that if you feed babies on a nutrient-poor diet, perhaps a diet that is poor in calcium and phosphorus in early life, you may program them to be more retentive of calcium and phosphorus in later life, with an increase in bone formation. We have to consider programming as an adaptive, evolving event; the most useful reason one can think of, arguing teleologically, for having nutritional programming is that it allows you to adjust your metabolism to the nutritional environment in which you find yourself in early life. So if you are born into a nutrient-poor environment, you could argue that it would be useful to program yourself to be conservative with nutrients, and if you are born into a nutrient-rich environment, it would be useful to program yourself to be more wasteful with nutrients. So paradoxically, achieving good nutrition early on—in that particular respect—might not necessarily have the best long-term outcome.

**REFERENCES**