Proceedings of the Nutrition Society (2005), **64**, 297–303 DOI:10.1079/PNS2005435

Biochemical homeostasis and body growth are reliable end points in clinical nutrition trials

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Studies of biochemical homeostasis and/or body growth have been included as outcome variables in most nutrition trials in paediatric patients. Moreover, these outcome variables have provided important insights into the nutrient requirements of infants and children, and continue to do so. Examples of the value of such studies in improving parenteral nutrition, in defining essential fatty acid metabolism and requirements of infants and in defining the protein and energy needs of low-birth-weight infants are discussed. Data from such studies have helped to define the mechanism of metabolic acidosis and hyperammonaemia associated with the use of early crystalline amino acid mixture and, hence, how to prevent these disorders. Such studies have allowed the development of parenteral amino acid mixtures that circumvent grossly abnormal plasma concentrations of most amino acids and appear to be utilized more efficiently. These studies have also helped define micronutrient requirements, including requirements for several such nutrients that had not been previously recognized as essential (e.g. Cr, Se, Mo, α-linolenic acid). Studies of body growth have been particularly valuable in defining the nutritional requirements of low-birth-weight infants. Finally, studies of metabolic homeostasis coupled with more sophisticated metabolic studies have provided considerable insight into the metabolism of the essential fatty acids, linoleic acid (18:2*n*-6) and α-linolenic acid (18:3*n*-3). Although such studies have not defined the amount of the longer-chain PUFA synthesized from each of these essential fatty acids, i.e. arachidonic acid (20:4*n*-6) and DHA (22:6*n*-3), they have shown that the rates of conversion are extremely variable from infant to infant, suggesting a possible explanation of why some studies show developmental advantages from intake of these fatty acids while others do not.

Biochemical homeostasis: Growth: Total parenteral nutrition: Essential fatty acid metabolism: Preterm and low-birth-weight infants

For decades, biochemical homeostasis and body growth have been among the most commonly included outcome variables of clinical nutrition trials, particularly clinical trials in infants and children. Moreover, data from such studies have contributed immeasurably to the current understanding of a number of nutritional issues. Equally, or perhaps more important, the information obtained from these studies has led to improvements in nutritional management, which in turn have helped decrease both morbidity and mortality. Some of the many contributions of studies of biochemical homeostasis and body growth in clinical nutritional trials are discussed in the present paper. Although not necessarily the most important such contributions, the examples discussed are based largely on the author's studies of biochemical homeostasis and body growth in clinical nutrition trials in paediatric patients. Three examples have been chosen for discussion: (1) the contributions of studies of biochemical homeostasis and body growth to refining and improving parenteral nutrition; (2) the contribution of such studies to the current understanding of essential fatty acid requirements during infancy and childhood; (3) the contribution of these studies to defining nutrient requirements of preterm and low birth weight (LBW) infants.

Contributions of biochemical homeostasis and growth studies to improving parenteral nutrition

The idea of providing nutrients intravenously to patients with severely compromised gastrointestinal function first

Abbreviation: LBW, low birth weight.
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surfaced just after description of the circulatory system several centuries ago. However, a successful method of delivering nutrients intravenously eluded practitioners until approximately 40 years ago, when Wilmore & Dudrick (1968) documented normal growth and development of an infant with virtually no remaining small intestine who received only parenterally-administered nutrients for the first several months of life. The breakthrough that made this possible was infusing a concentrated nutrient mixture into a large vein with rapid flow rather than infusing a large volume of a more dilute mixture into a peripheral vein. The components of the nutrient mixture included a protein hydrolysate, glucose, vitamins, some minerals and electrolytes. Although subsequently proven inadequate, periodic infusions of plasma were given as a source of trace minerals and essential fatty acids.

While effective, the therapy was labour-intensive. In addition, as use of the technique spread to other groups of patients, some of the raw materials (e.g. protein hydrolysates) became scarce. Thus, when an inexpensive method for manufacturing crystalline amino acids became available, crystalline amino acid mixtures replaced the protein hydrolysates that had previously been used. Shortly thereafter, metabolic acidosis and hyperammonaemia were noted, particularly in infants and children. In addition, although the crystalline amino acid mixtures provided approximately the same pattern of indispensable amino acids as egg protein, most mixtures contained large amounts of only a few dispensable amino acids (e.g. glycine). Thus, marked derangements of the plasma amino acid pattern of both paediatric and adult patients requiring parenterally-delivered nutrients were common. Finally, despite periodic infusion of a reasonable volume of plasma from normal volunteers, deficiencies of many trace minerals and essential fatty acids were common.

Resolution of metabolic acidosis associated with use of early crystalline amino acid mixtures

Since metabolic acidosis had not been encountered before the use of crystalline amino acid mixtures, the occurrence of low blood pH, low plasma bicarbonate concentrations and low blood base excess concentrations in the first few subjects receiving parenteral nutrition regimens containing crystalline amino acid mixtures was somewhat surprising (Heird *et al*. 1972*a*).

The problem was approached by evaluating the known causes of metabolic acidosis, i.e. excessive gastrointestinal or renal loss of base or intake of actual or potential H^+. This exercise documented that neither gastrointestinal nor renal loss of base was greater than expected. For example, stool loss of base, defined as undetermined anion, i.e. the difference between the concentrations of common cations and anions, usually the concentration of $Na^+ + K^+$ – the concentration of Cl^-, was found to be only approximately 0·5 mmol/kg per d; in comparison, infants with diarrhoea lose four to five times this amount daily (Torres-Pinedo *et al*. 1966). Renal net acid excretion was actually found to be higher than expected for the extent of metabolic acidosis, indicating that renal base loss also was not excessive. Further, the preformed acid load of the crystalline amino acid mixtures was found to be only approximately 25 % of the preformed acid load, or titratable acidity, of the hydrolysates (10–12 mmol/l *v*. 30–35 mmol/l).

Comparison of the undetermined anion content of the crystalline amino acids mixtures and the hydrolysates showed that the crystalline amino acid mixtures actually had a 'cation gap' rather than an 'anion gap', i.e. the concentration of Cl^- and other anions exceeded the sum of the concentrations of Na^+ and K^+. The protein hydrolysates, on the other hand, had the expected 'anion gap', i.e. Na^+ and K^+ concentrations exceeded the concentration of Cl^- and other anions. It was further shown that the concentrations of the cationic amino acids (i.e. histidine, arginine and lysine) in the crystalline amino acid mixtures equalled the cation gap. These amino acids, when metabolized in the absence of anionic amino acids, result in release of H^+, suggesting, as subsequently shown, that metabolic acidosis could be prevented by addition of anionic amino acids or other organic anions that consume H^+ when metabolized. Decreasing the cationic amino acid content of the mixture should also be effective, but is not practical because most of these amino acids are indispensable amino acids. A common practice that has been employed since description of metabolic acidosis and its cause is to supply the cationic amino acids as acetate rather than chloride salts so that metabolic acidosis is no longer a problem.

Understanding and preventing hyperammonaemia associated with parenteral nutrition

The protein hydrolysates that were utilized initially as a N source for parenteral nutrition regimens contained free NH_3 and, hence, resulted in slightly elevated plasma NH_3 concentrations (Johnson *et al*. 1972). However, the NH_3 content of these mixtures was less than the amount of NH_4Cl that was commonly used at that time to treat metabolic acidosis without evidence of hyperammonaemia or other problems. Thus, it was somewhat surprising to encounter a patient who rapidly and unexpectedly became lethargic and developed grand mal seizures after having been maintained uneventfully on a parenteral nutrition regimen containing a crystalline amino acid mixture for approximately 5 weeks (Heird *et al*. 1972*b*). The electroencephalogram suggested a 'metabolic disorder' and subsequent evaluation revealed a blood NH_3 concentration >8000 μg/l. The amino acid intake was stopped and the blood NH_3 concentration was found to drop precipitously to a near-normal value. However, the blood NH_3 concentration rose precipitously when the amino acid intake was re-introduced. Administration of supplemental arginine with the crystalline amino acid mixture maintained a normal blood NH_3 concentration provided the total arginine intake from both the crystalline amino acid mixture and supplemental arginine was ≥1·0 mmol/kg per d.

Other crystalline amino acid mixtures available at the time contained more arginine and, hence, did not result in metabolic acidosis. As a result of similar findings in a total of three patients, the manufacturer of the culprit crystalline amino acid mixture increased its arginine content and

https://doi.org/10.1079/PNS2005435 Published online by Cambridge University Press

symptomatic hyperammonaemia related to the composition of the amino acid infusate has not been observed since.

Improving the composition of parenteral amino acid mixtures

While the resolution of metabolic acidosis and hyperammonaemia associated with use of crystalline amino acid mixtures resulted in major improvements in the composition of these mixtures, the plasma concentration of many amino acids remained abnormal (Winters *et al*. 1977). The concentrations of some dispensable amino acids (e.g. glycine) were quite high, as were the concentrations of some indispensable amino acids (e.g. phenylalanine and methionine). These high concentrations were of particular concern among paediatricians because of their experience with inborn errors of metabolism (e.g. phenylketonuria). In these severe conditions the activity of enzymes involved in metabolism of specific amino acids is low, resulting in a high plasma concentration of the specific amino acids. Moreover, these conditions are usually accompanied by severe developmental delays which, although perhaps a coincidence, were assumed to be related directly to the high plasma amino acid concentration. Of equal or greater concern was the fact that the concentrations of other indispensable amino acids (e.g. the branched-chain amino acids, tyrosine and cyst(e)ine) were low. This situation is most probably a reflection of the known relationship between the quality of protein intake and the plasma amino acid pattern. For example, soyabean protein, which has a low methionine content and fails to support normal growth, results in a low plasma concentration of methionine. Both growth and plasma methionine concentration are restored to normal by methionine supplementation of the native protein (Fomon *et al*. 1979).

The concept of amino acid imbalance, although described only in animals (Harper *et al*. 1970), cannot be ignored. In this condition the addition of a single amino acid to an otherwise well-balanced-protein diet results in poor growth that is secondary, most probably, to decreased intake.

A mixture of amino acids has been formulated that results in 'normal' plasma concentration of each amino acid (Heird *et al*. 1987). Tyrosine and cyst(e)ine, both of which are thought to be indispensable amino acids for young infants, are insoluble and/or unstable in aqueous solution; thus, it is impossible to include these amino acids in crystalline amino acid mixtures. For these amino acids the inclusion of their precursors, phenylalanine and methionine, does not result in higher plasma tyrosine and cyst(e)ine concentrations. The mixture does contain some N-acetyl-L-tyrosine but the bioavailability of this soluble tyrosine salt is less than optimal (Heird *et al*. 1988; Van Goudoever *et al*. 1994). Moreover, cyst(e)ine can be provided as the HCl salt, remembering that it is likely to cause metabolic acidosis (Laine *et al*. 1991). It also can be provided as the poorly-bioavailable N-acetyl-L-cyst(e)ine (Van Goudoever *et al*. 1994). Dipeptides containing the amino acids are now available and, in theory, should be the ideal source of tyrosine and cyst(e)ine. Preliminary data (Roberts *et al*. 2001) are promising.

Limited evidence (Helms *et al*. 1987) suggests that the mixture of crystalline amino acids that results in normal plasma concentrations of most amino acids may be used somewhat more efficiently for anabolism than mixtures that result in a less-normal plasma amino acid pattern (e.g. retention of approximately 78% N intake *v*. approximately 63% N intake).

Compared with historical data, the incidence of total parenteral nutrition cholestasis appears to be lower in the subjects in whom the crystalline amino acid mixture is initially evaluated (Heird *et al*. 1987). Other studies, however, have shown no real difference in the incidence of total parenteral nutrition cholestasis as a function of the pattern of amino acids infused (Forchiella *et al*. 1995).

Defining parenteral micronutrient requirements

As mentioned earlier, the initial assumption that adequate amounts of trace minerals and essential fatty acids could be provided by periodic infusions of normal plasma to subjects requiring parenteral nutrition has not proved to be true. Thus, a number of previously-undescribed micronutrient deficiencies have been described in patients receiving parenteral nutrients exclusively. This information, in turn, has improved the understanding of the role of these nutrients in human nutrition irrespective of the route of nutrient administration. Trace minerals that had not been recognized previously as essential but have been recognized in patients receiving only parenteral nutrients include Cr (Jeejeebhoy *et al*. 1977), Se (Kien & Ganther, 1983), and Mo (Abumrad *et al*. 1981). The development of neurological abnormalities in a child who had received long-term parenteral nutrition without a source of α-linolenic acid (18:3*n*-3) has established this fatty acid as a second essential fatty acid (Holman *et al*. 1982). Before the availability of acceptable lipid emulsions for use in parenteral nutrition, classical essential fatty acid deficiency with poor growth and scaly skin lesions were common (Friedman *et al*. 1976). Currently, the issues concerning parenteral lipid requirements no longer concern the need for linoleic acid (18:2*n*-6) and 18:3*n*-3 but, rather, the potential need for their longer-chain more-unsaturated metabolites arachidonic acid (20:4*n*-6) and DHA (22:6*n*-3). This issue, too, is pertinent to nutrition generally, independent of the route of nutrient delivery, and will be discussed.

Understanding the essential fatty acid needs of infants

As mentioned earlier, 18:2*n*-6 and 18:3*n*-3 are recognized as essential, or indispensable, fatty acids and the requirements for these fatty acids are reasonably well defined. Each of these fatty acids is converted to longer-chain more-unsaturated fatty acids, the most important of which are 20:4*n*-6, which is synthesized from 18:2*n*-6, and 22:6*n*-3, which is synthesized from 18:3*n*-3. These two long-chain PUFA are the major *n*-6 and *n*-3 fatty acids respectively in the developing brain and 22:6*n*-3 is highly concentrated in retinal photoreceptor membranes (Martinez,1992). Together with other *n*-6 and *n*-3 long-chain

https://doi.org/10.1079/PNS2005435 Published online by Cambridge University Press

PUFA, 20:4*n*-6 is also a precursor of various series of eicosanoids (Innis, 1991).

The plasma lipid content of 22:6*n*-3, but not necessarily that of 20:4*n*-6, is lower in formula-fed *v*. breast-fed infants (Carlson *et al*. 1986). Since breast milk contains 22:6*n*-3 and other long-chain PUFA, whereas conventional formulas available at that time did not (Jensen, 1999), it has been assumed by many researchers that 20:4*n*-6 and 22:6*n*-3 as well as 18:2*n*-6 and 18:3*n*-3 are essential nutrients for infants. On the basis of these assumptions as well as some studies showing that breast-fed infants and infants supplemented with 20:4*n*-6 and 22:6*n*-3, particularly the latter, have more optimal visual and neurodevelopmental outcomes (Carlson *et al*. 1996; Birch *et al*. 2000; Lauritzen *et al*. 2001), infant formulas have recently been supplemented with these fatty acids.

Whether infants are capable of endogenous synthesis of 20:4*n*-6 and 22:6*n*-3 if given adequate 18:2*n*-6 and 18:3*n*-3 remained unknown until the early 1990s, when it was shown by several investigators that 18:2*n*-6 and 18:3*n*-3 labelled with stable isotopes of either C or H are converted to the respective long-chain PUFA (Demmelmair *et al*. 1995; Carnielli *et al*. 1996; Salem *et al*. 1996; Sauerwald *et al*. 1996, 1997). This finding suggests that it might be possible to supply the requirements of these fatty acids by increasing the content of the precursors in formula, particularly that of 18:3*n*-3, the precursor of 22:6*n*-3.

However, plasma lipid concentrations of 22:6*n*-3 remain lower than those of breast-fed infants, even in infants receiving a reasonably high intake of 18:3*n*-3 (Ponder *et al*. 1992; Jensen *et al*. 1997). Thus, it appears that endogenous synthesis of 22:6*n*-3 is not sufficient to maintain the same plasma lipid content of this fatty acid as that observed in subjects who receive a preformed source of 22:6*n*-3. Nonetheless, it is important to note that not all studies have shown that supplementation of formulas with these fatty acids confers developmental benefits (Lucas & Morley, 1999; Makrides *et al*. 2000; Auestad *et al*. 2001).

Despite the widespread availability of supplemented formulas throughout the world, the role of 22:6*n*-3 in infant nutrition remains an active area of investigation. Current understanding, based primarily on studies of biochemical homeostasis plus studies of metabolism of labelled precursor fatty acids as well as assessment of visual and neurodevelopmental function, suggests that the rate of conversion of 18:2*n*-6 and 18:3*n*-3 to 20:4*n*-6 and 22:6*n*-3 respectively varies widely from infant to infant (Carnielli *et al.* 1996; Salem *et al*. 1996; Sauerwald *et al*. 1996). This variation may help to explain some of the apparent discrepancies among studies of long-chain PUFA supplementation on development, some of which show advantages of supplementation and some of which do not.

Contributions of studies of growth in defining protein needs of preterm and low-birth-weight infants

Growth has long been a major outcome variable of clinical nutrition trials in infants; for example, most studies of treatment of malnourished infants include growth as the major outcome variable. Currently, demonstration of normal growth is a common requirement for regulatory approval of newly-designed infant formulas. More recently, studies of body composition have been added to studies of growth, providing additional insights into the nutrient needs of infants.

Table 1. Clinical characteristics of low-birth-weight (LBW) infants participating in studies of protein requirements of LBW infants

Birth weight 750–1750 g (%*)	
750–1250	29
1251–1500	39
1501–1750	32
Gestational age 26–34 weeks (%*)	
< 30 weeks	16
30–32 weeks	59
> 32 weeks	25
Age at first feeding (d)	1–10
Age at full intake (d)	28
Age when birth weight regained (d)	13–19
Respiratory distress (%*)	75
Antibiotic therapy (%*)	74
Parenteral nutrition (%*)	62
Duration of total parenteral nutrition (d)	7–14

*Percentage of the infants participating in the studies.

One of the more pressing needs in paediatric nutrition concerns the nutrient requirements of LBW infants. Unlike the term infant, for whom the nutrient requirements can be defined as the amount of each in a reasonable volume of human milk, LBW infants fed human milk do not grow at the same rate as they would had they not been born prematurely (Kashyap *et al*.1990). Even if all protein, Ca and Na in a reasonable volume of human milk are absorbed, the intake of these nutrients will remain considerably less than the amount of each that accumulates during the last trimester of gestation (Forbes, 1982).

The fact that LBW infants fed formulas with a higher content of protein and other nutrients grow at a more rapid rate than infants fed human milk was recognized as long as a half century ago (Gordon *et al*. 1947). Unfortunately, the formula used in this initial study provided a protein intake of 6 g/kg per d, or approximately three times the amount provided by the same volume of human milk. Hence, although the infants were found to exhibit higher rates of growth, they had high plasma concentrations of some amino acids, high blood urea-N concentrations and many developed metabolic acidosis. Eventually, it was shown that preterm infants who receive a protein intake of 4 g/kg per d grow as well as those who receive 6 g/kg per d and also appear to tolerate this intake quite well (Davidson *et al*. 1967). Nonetheless, what is now considered an irrational fear of a moderate protein intake persisted until approximately the early 1990s. In part, this situation reflected the desire to provide the non-nutritional benefits of human milk (e.g. protection against infection and neurodevelopmental advantages) and the belief that the higher protein content of preterm milk (Atkinson *et al*. 1980) might support both better growth and non-nutritional benefits.

During the 1980s and early 1990s a number of protein intakes were studied, ranging from 2·25 g/kg per d to approximately 4·5 g/kg per d, all with a concomitant

https://doi.org/10.1079/PNS2005435 Published online by Cambridge University Press

energy intake of approximately 504 kJ (120 kcal)/kg per d (Kashyap *et al*. 1986, 1988, 1990, 1994). The clinical characteristics of the >200 infants enrolled in these studies are summarized in Table 1. Across this range of protein intakes a direct relationship was found between protein intake and weight gain as well as N balance, plasma concentrations of albumin and transthyretin, and blood urea-N concentration (Kashyap & Heird, 1994). It was also found that the intrauterine rate of weight gain could be achieved with a protein intake of 2·8–3·0 g/kg per d. An intake in this range was also shown to support the intrauterine rate of N retention, while a higher intake was required to maintain normal plasma albumen and transthyretin concentrations. Even at the highest protein intake studied blood urea-N concentrations did not exceed 100 mg/l. Finally, the plasma concentration of most amino acids were found to remain within 2 SD of the concentration observed 2 h postprandially in normally-growing 30-d-old breast-fed term infants (Wu *et al*. 1986).

Formulas designed specifically for preterm and LBW infants have been available for approximately the last 25 years. Studies by other researchers have shown that infants who receive these formulas *v*. a standard term formula during initial hospitalization perform better on standard developmental tests at 18 months as well as at 7–8 years of age (Lucas, 1999). Although the formulas provide more of several nutrients other than protein, this advantage is usually attributed to the protein content.

Despite the availability of these special formulas for preterm and LBW infants and their obvious advantages over standard term formula, most preterm and LBW infants continue to grow at a slower rate than the fetus *in utero*. Recent data from the National Institute of Child Health Neonatal Network Centers document that >90% of such infants weigh <10th percentile of intrauterine standards at discharge (Ehrenkrantz *et al*. 1999). Moreover, although there are no firm data showing that this growth pattern is associated with neurodevelopmental delays, there is a growing consensus that such delays occur. Certainly, preterm and LBW infants have a high incidence of both developmental and growth delays (Saigal *et al*. 2001). However, the neurodevelopmental delays may not be a consequence of their poor early growth secondary to inadequate nutrient intake but, rather, a consequence of a myriad of other factors to which they are subjected during their frequently long initial hospital stays. Of these possibilities, it is far easier to improve nutrient intake than control the many non-nutritional factors. Thus, doing so seems wise.

Irrespective of the contribution of poor nutrition and growth to the long-term neurodevelopmental deficits of preterm and LBW infants, it is clear that growth will continue to be an important outcome variable of studies for defining optimal growth and optimal nutrient intakes of these vulnerable infants.

A major problem appears to be the fact that currently-available formulas, although supporting normal growth and some catch-up growth once full intakes are achieved, do not support sufficient growth to allow the infant to overcome the deficits often encountered during the first several days to few weeks of life when nutrient intake is inadequate (Heird, 1999, 2001). Interestingly, the recently introduced 'post-discharge formulas', which have a higher protein and a slightly higher energy content, support growth at rates in excess of that supported by term formulas, and infants fed these formulas are both somewhat heavier and somewhat longer at 18 months of age. However, these formulas do not appear to support greater rates of growth than term infant formulas beyond approximately 2–4 months post term (Cooke *et al*. 1998, 2001; Carver *et al*. 2001; Lucas *et al*. 2001).

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