

NUTRITIONAL BALANCE STUDIES: EVALUATION OF A PREMATURE INFANT FORMULA

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SUMMARY

A 24 kcal/oz (81 kcal/100 ml) premature infant formula (Enfamil Premature Formula) with moderately high mineral content (117 mg Ca/100 ml and 58 mg P/100 ml) and a protein content of 3 g/100 kcal was evaluated in sixteen 3-day balance studies at 10 and 21 days of age in nine premature infants with birth weights from 1,200 to 1,400 g. Growth rates were similar to *in utero* rates, and the formula was well accepted and tolerated. Calcium retention (62.5%) was similar to *in utero* accretion, and phosphorus retention was only slightly lower. Nitrogen retention was high without the development of metabolic acidosis or abnormal serum urea nitrogen levels.

INTRODUCTION

It is generally recognized that very low birth-weight infants have unique nutritional needs because of their very rapid rates of growth and the functional limitations of their GI tracts. High calorie feedings seem to be indicated in many such infants because their immature kidneys may not be able to cope with the excess water of dilute feedings; their immature GI tracts may tolerate smaller volumes better; and susceptibility to persistent ductus arteriosus and congestive heart failure, bronchopulmonary dysplasia, and necrotizing enterocolitis may be increased or

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aggravated by excessive fluid volume (1-3). Nitrogen (protein) and mineral accretion have been shown to be higher in the 3rd trimester of pregnancy (4, 5) than can be provided to the very low birth-weight infant using mature human milk or conventional formulas. Even though preterm human milk is enriched in some of these factors (6), the levels fall to near those of term human milk within the first few weeks, at which time the growth rate of the very low birth-weight infant has reached a maximum and the need for selected nutrients is greatest. The vitamin requirements of the preterm infant are also ill-defined, although it is clear that they have an increased need for vitamin C and folic acid and can benefit from additional vitamin E and perhaps vitamin D.

Using the factorial approach based on changes in fetal composition, advisable nutrient intakes have been calculated (5). This approach provides a starting point for premature infant feedings if it is assumed that achievement of *in utero* growth rates is desirable. Another approach is to examine the nutrient content of early preterm human milk as an estimate of nutritional requirement, and to make adjustments for known mineral insufficiencies. The Nutrition Committee of the Canadian Pediatric Society has recommended that supplements to human milk of 80 mg of calcium and 50 mg of phosphorus as phosphate should be provided per kg/day to preterm infants (7), thus increasing dietary calcium to approximately 100 mg/100 kcal, and phosphorus to approximately 60 mg/100 kcal.

The purpose of this study was to evaluate a premature infant formula with mineral levels (117 mg Ca/100 kcal and 59 mg P/100 kcal) calculated to provide sufficient amounts for adequate accretion. This was accomplished by assessing GI tolerance, growth, nutrient balance, and metabolic parameters.

MATERIAL AND METHODS

Clinical Study Population

Twelve preterm, AGA (adequate for gestational age) male infants, born at the University of Miami/Jackson Memorial Medical Center, with birth weights ranging from 1,200 to 1,400 g and free of disease were enrolled in the study by five days of age. After obtaining written informed consent from the mother, they were started on enteral feedings with a diluted study formula during the first 48 hours of life (see Table 1 for composition). Concentration was increased to full strength and volume as tolerated to achieve 130 kcal/kg/day during the second week. They remained on the formula throughout the study until discharge from the hospital at 1,800 grams. All infants received a multiple vitamin supplement daily. Two babies were removed from the study; one developed a clinically significant patent ductus arteriosus, and the other, respiratory distress. The formula was discontinued in another infant because he developed NEC (necrotizing enterocolitis) at seven days of age while still receiving diluted formula. This occurred in the same period that five non-study newborns receiving other formulas also developed NEC in the Unit.

TABLE 1

NUTRIENT COMPOSITION PER 100 KCAL OF THE STUDY FORMULA

Protein, g	2.7, 3.0, 2.7 ⁺
(Whey:casein)	(60:40)
Carbohydrate, g	11.0
(Lactose:CSS*)	(50:50)
Fat, g	4.6, 4.4, 4.0 ⁺
(o/o MCT**)	(40o/o)
Na, mg	40
K, mg	111
Cl, mg	85
Ca, mg	120, 111, 117 ⁺
P, mg	54, 61, 58 ⁺
Mg, mg	12.8, 12.8, 11.9 ⁺
Zn, mg	1.0
Cu, mg	0.09
Cal. density, kcal/dl	81

⁺ Analytical values for three batches used in balance studies.

* CSS = Corn syrup solids.

** MCT = Medium chain triglycerides.

Balance Studies

Two 3-day balance studies (at 10 and 21 days of age) were conducted in each infant while receiving 130 ± 13 (SD) kcal/kg/d. Carmine red was added to formula at the beginning of each balance period and again at 72 hours as described by Fomon (8). Three batches of the experimental formula were supplied in 100 ml, ready-to-use bottles for this study. Even though differences among the three batches were minimal, balance was calculated according to the analytical values of the one consumed. Homogeneity of each feeding was assured by vigorous agitation, immediate measurement in a 60 ml graduated cylinder, and administration by gavage bolus or nipple feeding of the total aliquot to the patient.

Stool and urine samples were frozen and subsequently analyzed for nitrogen by Kjeldahl procedure; phosphorus was determined by a modified Fiske-Subbarow procedure, and calcium by atomic absorption spectrophotometry. Fat content of stools was also determined by a modified Van de Kamer titration method (9).

Biochemical Measurements

After three to four weeks of enteral feeding, blood was drawn for biochemical assessment. Total protein, albumin, urea nitrogen, calcium, phosphorus, sodium, potassium, hemoglobin, and acid base status were all determined by the hospital clinical laboratory.

Analysis

Shaw (10, 11) has shown that fetal growth and elemental accretion is exponential, i.e. proportional to fetal size or content at the time it is determined, until the last few weeks of term gestation. Thus, for growth or a given element, the total can be expressed as $y = Ae^{kt}$ where y is the measurement of interest, t the gestational age in weeks, e the base of natural logarithms, k the fractional accumulation rate and A the base weight or element content. This equation can be made linear by taking the natural logarithm of both sides so that $\ln y = \ln A + kt$ which is of the form $x = a + bt$ allowing a and b (and hence A and k), to be determined by the the least squares method of linear regression analysis applied to growth or cumulative accretion data. The slope of the cumulative data plotted vs. time is equivalent to the rate of growth or rate of accretion (g/week or mg/week) and can be calculated as the derivative, dy/dt , of the cumulative growth or accretion data; thus, $dy/dt = kAe^{kt}$. Reference fetal growth rates were thus calculated using the equation which Shaw obtained using Lubchenko's (12) and Kloosterman's (13) growth data. Similarly, reference accretion rates were determined from equations Shaw obtained using fetal composition data from the literature (10, 11). A second set of reference fetal accretion rates was obtained from the data of Ziegler *et al.* (5). Retention was calculated as nutrient intake less stool, urine, and skin losses; the latter were estimated in mg/kg/d as 24 for N (14), 2.1 for Ca (15), and 1.4 for P (15).

All data were expressed and plotted as a function of weight in comparison to the reference fetus data. The 50th percentile weight for gestational age was used where necessary to convert gestational age (GA, weeks) to weight (wt, g), according to the following equation (11):

$$wt = 70.5e^{0.1008GA}$$

The 95/o confidence interval was also calculated using a single parameter exponential model for data from this study and plotted as the shaded area in the Figures. The two-tailed Student's "t"-test was used to compare mean retention results to calculated accretion values, assuming the pooled standard error to be equivalent to that of the mean retention. Means and standard deviations of biochemical data were calculated by common statistical procedures. These measures of central tendency and dispersion were then compared to ranges observed in premature infants at three weeks and at five weeks, respectively.

The protocol was approved by the Committee for Protection of Human Subjects of the University of Miami School of Medicine. Written informed consent was obtained for each subject.

RESULTS

Growth rates in premature infants receiving the study formula are shown in Figure 1 not to differ from fetal growth rates. While nitrogen retention (Figure 2) is higher than in the reference fetus, the premature infants' capacity to handle it apparently was not exceeded, as shown by

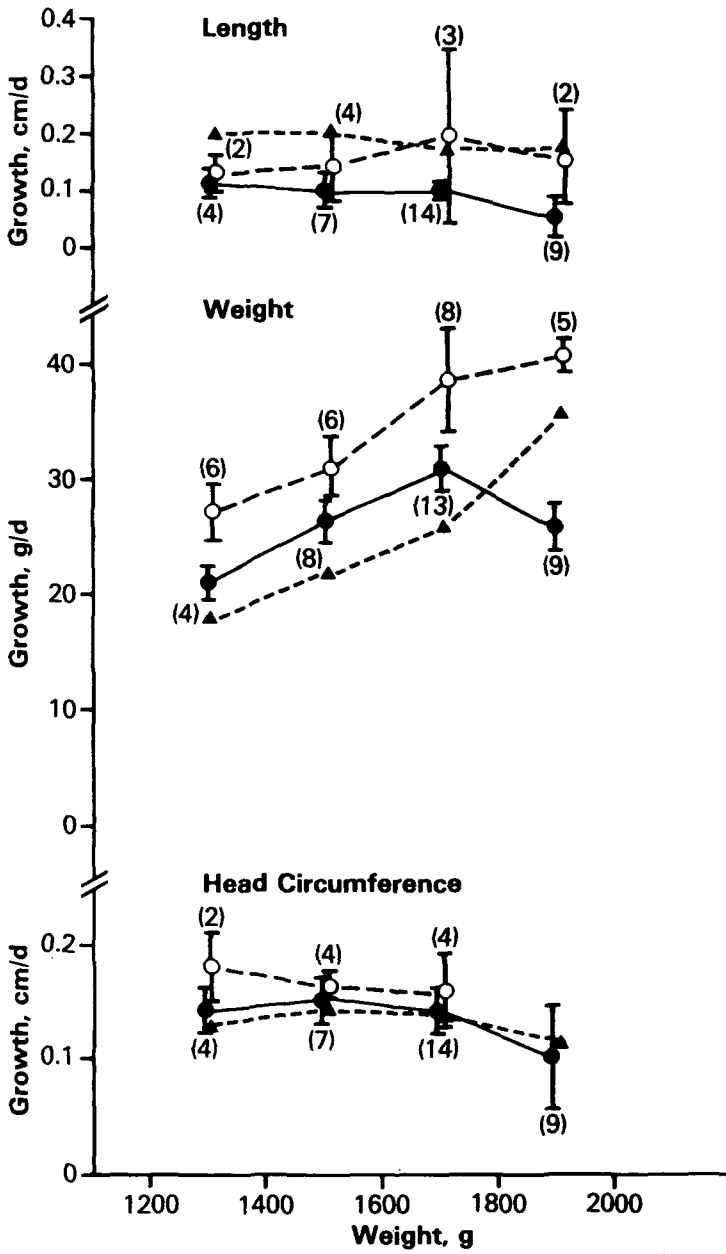


FIGURE 1

Growth rates ± 1 SEM (n) of premature infants receiving Enfamil Premature Formula (O—O) or preterm human milk (21) (●—●) compared to intrauterine growth rates (▲—▲)

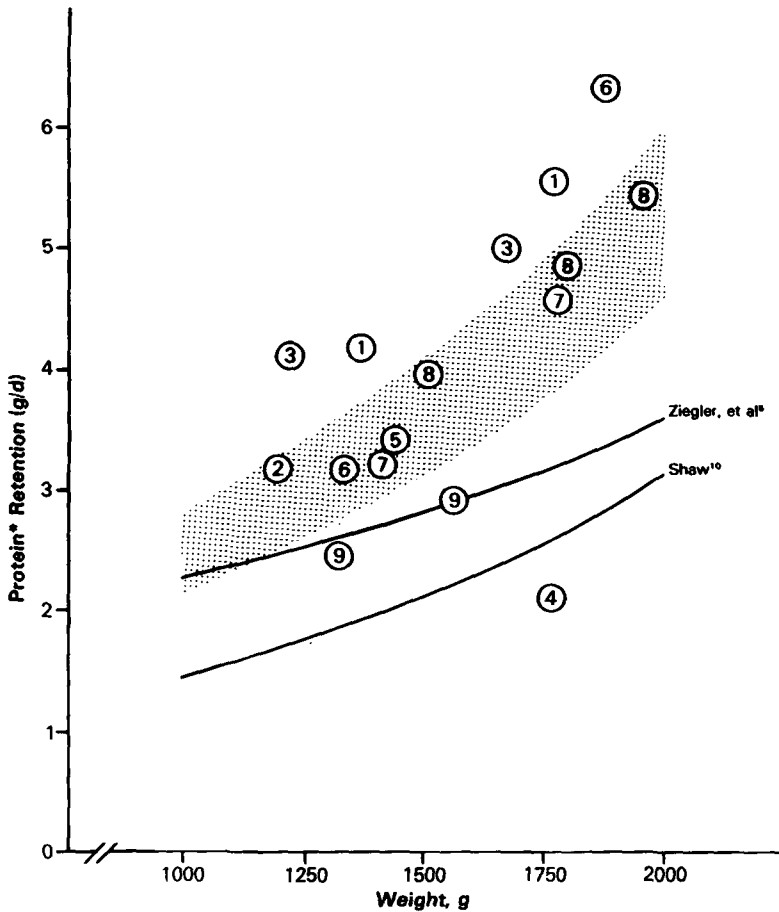


FIGURE 2

Protein retention rates ($6.25 \times$ nitrogen retention) of infants receiving Enfamil Premature Formula compared to intrauterine accretion rates of Ziegler *et al.* (15) and Shaw (10). The shaded area represents the 95% confidence interval about the exponential regression line fit to the data

serum urea nitrogen levels, 4.4 ± 1.1 (SD) mg/dl, (see Table 2) in the lower part of the normal range. Fat absorption was very high at $93.0 \pm 0.80\%$. Calcium retention averaged 62.5% and is shown in Figure 3 to encompass mean reference fetus accretion rates and not differ significantly; furthermore, serum calcium remained normal throughout the study period. Serum phosphorus also remained at normal levels (Table 2); however, retention appears to be slightly low (Figure 4), although it does not differ significantly from Shaw's reference fetal values.

The serum sodium and potassium concentrations as well as blood pH values also were in the normal range (Table 2).

TABLE 2
SERUM BIOCHEMISTRY

	Study group*	Values in premature infants (19)	
		3 weeks	5 weeks
Urea nitrogen, mg/dl	4.4 ± 1.1	2.1–31.4	2.0–26.5
Calcium, mg/dl	9.9 ± 0.6	8.1–11.0	8.6–10.5
Phosphorus, mg/dl	6.3 ± 0.9	6.2–8.7	5.6–7.9
Sodium, mEq/l	139 ± 2.0	129–142	133–148
Potassium, mEq/l	5.3 ± 0.9	4.5–7.1	4.5–6.6
pH	7.4 ± 0.04	7.38	

* Mean ± SD after three weeks of formula usage.

DISCUSSION

Assessment of the utility of specific nutrients has long presented a challenge to the scientist. Growth rates provide a gross, but useful index in spite of relative non-specificity and difficulties with precision over short terms. Biochemical determinations provide a snapshot of dynamic physiologic processes and do not always reflect body stores. Even specific nutrient balance determinations, which would theoretically be ideal, have been criticized (16). They are fraught with potential errors, virtually all of which bias the results toward falsely high retention rates. The difficulty and expense associated with conducting well-done metabolic balance studies also limits the amount of such data that is feasible to obtain.

The challenge of interpreting balance studies in premature infants is further compounded by uncertainties regarding appropriate reference curves and values. Ziegler, *et al.* (5) and Shaw (10, 11), among others, have gone to great lengths to provide reference values using fetal body composition studies as a function of body weight and/or gestation; nutrient accretion can be estimated from such data as the first derivative (slope) at a given body weight or gestational age. Even with all these efforts, questions persist as to the appropriateness of comparing totally different methods, i.e. retention rates determined from balance studies to accretion rates determined from body composition data. Nevertheless, keeping these limitations in mind, and for lack of better methods for the assessment of specific nutrient utilization, this study combines determinations of growth, biochemical levels, and metabolic balance compared to *in utero* growth, neonatal blood chemistry, and fetal accretion.

A sense of the utility of the study formula can thus be obtained, particularly where all the methods used are consistent with the same conclusion. Growth and biochemical data in our case are limited to the study infants, admittedly a small number (nine babies) for such data; but the consistency in the conclusions to which the various parameters lead justifies their inclusion here. Furthermore, these data are supported by similar data from infants in another study of the same formula (17).

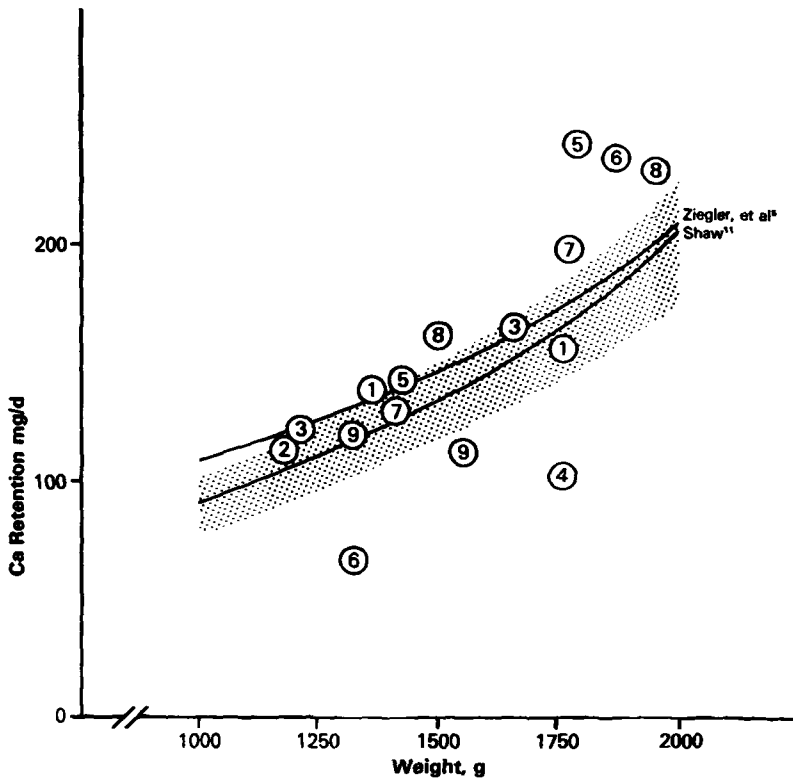


FIGURE 3

Calcium retention rates of infants receiving Enfamil Premature Formula compared to intrauterine accretion rates of Ziegler *et al.* (5) and Shaw (11). The shaded area represents the 95% confidence interval about the exponential regression line fit to the data

Moderately high-protein, high-calorie infant formulas have been available and used successfully for the past several years in rapidly growing very low birth-weight infants. The formula used in this study resulted in rates of weight gain comparable to that experienced by the fetus in the third trimester. The fact that rates of growth in length and head circumference were similar to weight relative to fetal growth, suggests that all aspects of growth were appropriately proportional, not merely the accumulation of excess fat and/or water. The high nitrogen retention observed was consistent with the anabolism measured anthropometrically, although it could partly be explained by implicit overestimation in nitrogen balance methodology, or to an underestimation of the requirements of the reference fetus (17). The low serum urea nitrogen values and the lack of acidosis in the face of abundant nitrogen retention is evidence of

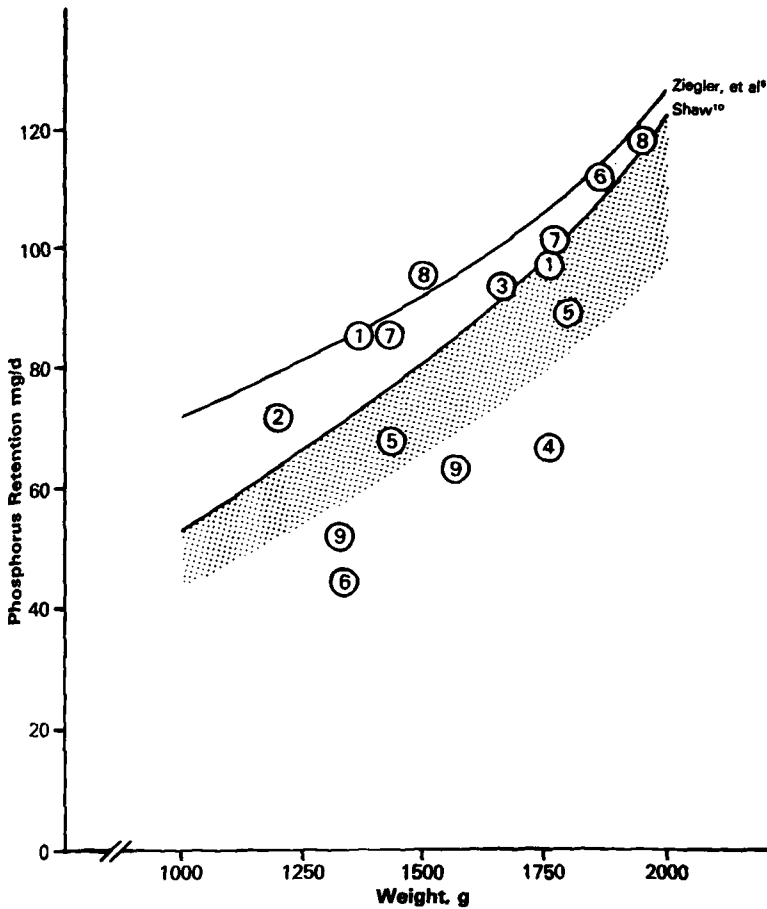


FIGURE 4

Phosphorus retention rates of infants receiving Enfamil Premature Formula compared to intrauterine accretion rates of Ziegler *et al.* (5) and Shaw (10). The shaded area represents the 95% confidence interval about the exponential regression line fit to the data.

the adequate protein utilization and that quantitatively, the protein intake was also appropriate.

The mineral requirements of premature infants have not been clearly established. One balance study using a premature infant formula containing 198 mg Ca/100 kcal showed calcium retention in excess of mean fetal accretion rates in 10 of 10 determinations (18). A moderate amount of calcium (117 mg Ca/100 kcal) was present in the formula used in the current study and resulted in retention rates in very low birth-weight infants similar to, and distributed about mean fetal accretion. Additional

calcium would not appear to be necessary in general, although the individual infant who goes without adequate calcium for several weeks before starting enteral feeding may need a supplement for a short period of time to make up for any accumulated deficit. Furthermore, increasing mineral concentration (particularly Ca) in the formula may increase the sediment because of solubility problems. This may result in an unpredictable intake of Ca by the infant, since delivery will depend not only on the total calcium content, but also on the method used to administer the formula (18).

Serum phosphorus levels were normal using this formula and phosphorus retention rates were not significantly different from Shaw's reference fetus accretion rates. It is concluded that phosphorus levels in the formula are adequate with a Ca:P ratio of 2:1, although calculated phosphorus retention appeared to be slightly low. None of the study infants developed rickets or other findings of inadequate mineral or vitamin D intake. In another study with this formula conducted by Gross (21) in a similar group of prematures, normal serum alkaline phosphatase values were observed during the eight weeks of feeding.

In conclusion, the study of the premature infant formula meets the protein, caloric, and mineral requirements of the rapidly growing low birth-weight infant without inducing unusual metabolic stresses. It is well tolerated and they achieve fetal growth (weight, length, and head circumference) and nutrient accretion rates.

RESUMEN

ESTUDIOS DE BALANCE NUTRICIONAL: EVALUACION DE UNA FORMULA INFANTIL PARA PREMATUROS

Se evaluó una fórmula infantil para prematuros (Enfamil Premature Formula) de 24 Kcal/oz (81 Kcal/100 ml) con un contenido mineral moderadamente alto (117 mg Ca/dl y 58 mg P/100 ml) y un contenido proteínico de 3 g/100 Kcal, en 16 períodos de balance de tres días de duración cada uno, al 10º y 21º día de edad en nueve recién nacidos prematuros cuyo peso al nacer fluctuaba entre 1,200 y 1,400 g. La velocidad de crecimiento observada fue similar a la velocidad intrauterina, y la fórmula fue bien aceptada y tolerada. Las retenciones de calcio (62.5%) fueron similares al aumento intrauterino, y la retención de fósforo fue ligeramente menor. Se notó una retención nitrogenada elevada sin desarrollo de acidosis metabólica ni anomalía en los valores séricos de urea.

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