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Egg and breast milk based nitrogen sources compared

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SUMMARY A nitrogen source based on egg protein (Vamin 9 glucose) and an alternative with an amino acid profile more similar to breast milk (Vaminolact), were compared in 14 parenterally fed infants. Subjects were randomly allocated to receive one or other amino acid solution, but were otherwise given identical diets. At the start of the study the two groups did not differ significantly in postconceptual age, postnatal age, or weight. Over a six day study period on a stable intake of intravenous nutrients there was no significant difference in growth or nitrogen retention between the two groups. Plasma amino acid profiles in those receiving Vamin 9 glucose, however, were frequently abnormal. Notably, mean concentrations of potentially neurotoxic phenylalanine and tyrosine were significantly higher (140% and 420%, respectively) in patients fed Vamin 9 compared with those given Vaminolact. An amino acid solution based on the composition of breast milk protein therefore brings plasma amino acid profiles during parenteral nutrition closer to those found in breast fed infants, and reduces in particular, the risks of hyperphenylalaninaemia and hypertyrosinaemia.

Over the past 20 years intravenous feeding has become a standard form of nutritional support for sick infants. Although the indications for its use in preterm neonates may be poorly defined, there is no doubt that parenteral nutrition has transformed the prognosis for newborns when severe congenital or acquired bowel disease precludes enteral feeding.[1] Synthetic crystalline L-amino acid solutions such as Vamin (KabiVitrum), which is based on the amino acid profile of egg protein, have commonly been used as the nitrogen source for parenterally fed children, although they were originally designed for adults. Compared with breast milk (table 1), this results in overprovision of some amino acids (such as phenylalanine) but underprovision of others (such as taurine) that are now considered essential for patients on long term parenteral nutrition.[2]

An additional consideration is that, in contrast to older patients, the most appropriate profile of amino acids administered to newborn infants may not only be different but much less flexible. The need for growth means that the young infant

Table 1 *Daily intake of individual amino acids/kg body weight for infant receiving 2·5 g/kg/day intravenous amino acids or 150 ml/kg/day breast milk*

	Vamin 9 glucose	*Breast milk*	*Vaminolact*
Alanine	107	78	250
Glycine	75	48	83
Proline	288	180	222
Valine	153	130	143
Threonine	107	87	143
Lysine	139	135	222
Serine	267	81	151
Glutamic acid	320	337	282
Leucine	189	180	278
Taurine	0	9	13
Histidine	85	46	83
Arginine	117	73	163
Tyrosine	18	57	20
Phenylalanine	196	72	108
Isoleucine	139	100	123
Cystine	50	37	40
Tryptophan	36	45	56
Methionine	68	28	52
Aspartic acid	146	165	163

requires relatively larger quantities of essential amino acids than an older child or adult. Furthermore, immaturity of enzyme systems in the neonatal liver may reduce the capability for synthesis and catabolism of certain amino acids. For example, liver cystathionase activity is low at full term, and endogenous synthesis of cystine from methionine is limited, making cystine an essential amino acid in the neonate. In addition, although histidine is not considered essential for adults, it seems to be required for normal growth in newborn infants.[3]

We recently showed that plasma amino acid profiles in babies fed with a conventional amino acid source often show increased concentrations of potentially neurotoxic phenylalanine.[4] Others have shared our concern,[5 6] and debate continues over the optimum composition of parenteral feeding solutions.[7] Attempts have been made to design amino acid preparations suited to the particular requirements of newborn infants, in some instances with the aim of producing a plasma amino acid profile during parenteral feeding that is similar to that found in breast fed babies.[8] We have therefore compared amino acid profiles, growth, and nitrogen balance during the use of our usual amino acid solution (Vamin 9 glucose), with a modified formulation containing an amino acid profile more similar to breast milk (Vaminolact, KabiVitrum), in a group of babies admitted to a regional neonatal surgical unit.

Patients and methods

The study was an open, prospective trial, with random allocation of patients to receive either Vamin 9 glucose or Vaminolact. Only patients less than 3 months of age were included. Infants below the 10th weight centile for age, those with heart failure, liver or renal impairment, or known metabolic defects, uncontrolled sepsis or receiving partial enteral feeding were excluded.

GROUP RECEIVING VAMIN 9 GLUCOSE

A group of seven patients received Vamin 9 glucose after operations for the following: small bowel volvulus (n=1), gastroschisis (n=2), small bowel atresia (n=2), and necrotising enterocolitis (n=2). At the start of total parenteral nutrition the median postconceptual age was 38 weeks (range 31–42), median weight 2470 g (range 1470–2910), and median age 16 days (range 11–29).

GROUP RECEIVING VAMINOLACT

A group of seven patients received Vaminolact after operations for gastroschisis (n=3) and necrotising enterocolitis (n=4). At the start of total parenteral nutrition, the median postconceptual age was 39·5 weeks (range 31–43), median weight 2320 g (range 1070–3200) and median age 36 days (range 11–84).

NUTRITIONAL INTAKE

Total parenteral nutrition was prescribed for all patients in accordance with a standard protocol and administered by central venous catheter.[9] Intakes/kg body weight were progressively increased over the first six days of feeding and then maintained at constant amounts as follows: carbohydrate, 8 g, 10 g, 10 g, 12 g, 12 g, 14 g; amino acids, 0·5 g, 1·0 g, 1·5 g, 2·0 g, 2·5 g; fat, 1 g, 2 g, 2 g, 3 g, 3 g, 3·5 g. The maximum daily energy intake was therefore 0·41 MJ/kg. Detailed records of intravenous infusions including additional peripheral infusions were kept so that precise calculation of intakes of fluid, electrolytes, and nutritional components could be made.

ANTHROPOMETRY

Patients were weighed daily to the nearest 10 g using electronic scales (Seca). Additional anthropometric measurements were made on day 0 and day 6 by a single experienced observer; head circumference was measured to the nearest 0·1 cm using a paper tape measure, and triceps and subscapular skinfold thickness to the nearest 0·2 mm with skin calipers (Holtain Ltd).

HAEMATOLOGICAL AND BIOCHEMICAL MONITORING

Infusion of Intralipid was suspended at 5 am each morning until venous blood from a peripheral vein was sampled, between 9 and 10 am. Vamin was constantly infused through the central venous feeding catheter and was not interrupted before the peripheral blood was sampled. Blood was taken on the day after the maximum intake of nutrients was reached (day 0) and then 72 and 144 hours later. In addition to plasma amino acid profile, estimation of sodium, potassium, creatinine, albumin, total bilirubin, calcium, phosphate, magnesium, glucose, and haemoglobin concentrations, full blood count, and alanine aminotransferase and γ glutamyltranspeptidase activities were also carried out. Plasma for amino acid analysis was deproteinised by addition of an equal volume of 3% sulphosalicylic acid immediately after collection. The supernatant was then stored at −20°C until analysis. Plasma concentrations were measured with a Kontron Chromakon 500 amino acid analyser using a lithium buffer system and ninhydrin detection.

NITROGEN BALANCE

All urine, ileostomy, and nasogastric losses were

collected during the final 72 hours of the study for subsequent analysis of nitrogen content. During this period patients were nursed on a metabolic bed placed inside an incubator.[10] Urine, nasogastric aspirate, and ileostomy specimens were stored at 4°C until the 72 hour collection was completed. As subjects received no enteral feed during the study, stool losses from the rectum were minimal and were ignored. Specimens were homogenised, total volume measured, and then 20 ml aliquots stored at −20°C until analysis. Nitrogen content was determined using the micro Kjeldahl method[11] and phenol-hypochlorite (Bertholet) reaction.

STATISTICAL ANALYSIS

Comparisons between groups of clinical characteristics, plasma amino acid concentrations, and nitrogen retention were made by the Mann-Whitney U test. Other biochemical observations were compared by Student's *t* test. The mean concentration of each amino acid was calculated after $\log_{10}$ transformation of all the measurements made in one group throughout the study period. Within group changes in variables from day 0 to day 6 were compared using a paired *t* test. A probability of $<0{\cdot}05$ was accepted as significant.

Approval for the study was obtained from the research ethical committee of the Central Birmingham Health Authority. Informed parental consent was obtained before starting total parenteral nutrition.

Results

There were no significant differences between patients given Vamin 9 glucose and those given Vaminolact in age in days, postconceptual age, weight at the start of total parenteral nutrition, or intakes of fluid, nitrogen, carbohydrate, or energy. No adverse reactions to feeding solutions were seen in either group and no patients were septic during the period of study.

ANTHROPOMETRY

Each group showed a significant increase in weight, head circumference, and triceps skinfold thickness over the six day study period ($p<0{\cdot}05$) (table 2). Increases in subscapular skinfold thickness were not significant. Median weight gain was 37 g/day (range 23–60 g) in the Vamin 9 glucose group, and 32 g (10–38 g) in the Vaminolact patients ($p=0{\cdot}26$). No significant differences were found in changes in other anthropometric variables between groups.

NITROGEN BALANCE

The median nitrogen intake for Vamin 9 glucose

Table 2 *Growth during study period*

	Vamin 9 glucose	*Vaminolact*
Median daily increase in weight (range)	37 g (23–60)	32 g (10–38)
Median increase in triceps skinfold thickness during six day study (range)	0·6 mm (0–1·6)	0·4 mm (0–0·6)
Median increase in subscapular skinfold thickness during six day study (range)	0·4 mm (0·2–3·0)	0·4 mm (0–0·4)
Median increase in head circumference during six day study (range)	0·6 cm (0–1·2)	0·4 cm (0–0·9)

Table 3 *72 Hour nitrogen balance*

Weight (g)	*Nitrogen intake (mg)*	*Urinary nitrogen loss (mg)*	*Nasogastric nitrogen loss (mg)*	*Ileostomy nitrogen loss (mg)*	*Nitrogen retention (%)*
		Group receiving Vamin 9 glucose			
2550	2340	228	118	0	85
1470	1215	225	7	17	80
2470	2175	330	128	0	79
2910	2262	490	66	0	75
2000	1800	525	23	0	70
1930	1653	460	0	0	72
2630	1872	904	0	0	52
		Group receiving Vaminolact			
2550	2340	343	0	0	85
3200	2304	453	0	0	80
2740	2187	440	14	0	79
2320	1863	347	0	139	74
1070	957	278	4	0	70
1440	1210	404	0	171	52
2180	1584	875	17	0	44

Table 4 *Mean (range) plasma amino acid concentrations (μmol/l) in patients receiving Vamin 9 glucose and Vaminolact*

Amino acid	*Target range*[12]	*Vamin 9 glucose*	*Vaminolact*	*p Value*
Alanine	125–647	251 (87–872)	305 (125–509)	0·66
Glycine	77–376	460 (232–937)	448 (220–701)	0·68
Proline	83–319	312 (85–458)	153 (171–356)	0·03
Valine	88–222	222 (69–331)	152 (106–237)	0·0002
Threonine	70–197	248 (70–440)	367 (145–699)	0·003
Lysine	80–232	82 (43–195)	206 (71–388)	0·0001
Serine	0–326	268 (86–689)	218 (110–579)	0·15
Glutamic acid	24–243	78 (20–764)	98 (40–550)	0·8
Leucine	53–169	94 (49–144)	108 (47–226)	0·03
Taurine	1–167	48 (24–186)	88 (52–245)	0·01
Histidine	34–119	92 (50–153)	99 (60–145)	0·5
Arginine	42–148	32 (16–78)	51 (21–148)	0·0005
Tyrosine	38–119	146 (43–572)	28 (5–69)	0·0004
Phenylalanine	22–70	192 (55–800)	80 (47–167)	0·0028
Isoleucine	27–90	55 (11–124)	50 (22–100)	0·029
Cystine/cysteine	99–208	52 (25–108)	38 (10–70)	0·047
Tryptophan	19–100	30 (20–42)	49 (26–69)	0·0001
Methionine	22–50	39 (12–69)	25 (13–40)	0·0001
Aspartic acid	5–51	19 (7–73)	23 (12–72)	0·6

patients was 857 mg/kg/72 hours (range 712–918) and for Vaminolact patients 803 mg/kg/72 hours (range 720–916). The median nitrogen retention calculated from the three day balance study was 75% (range 52–85) for Vamin 9 glucose and 74% (range 44–85) for Vaminolact (table 3). The nitrogen intakes and retentions were not significantly different between the groups.

HAEMATOLOGICAL AND BIOCHEMICAL MEASUREMENTS

No haematological or biochemical measurements changed significantly in either group over the six day study period. In particular, no patients showed biochemical evidence of liver dysfunction. Other than plasma amino acid concentrations, no haematological or biochemical measurements in the two groups were significantly different.

PLASMA AMINO ACID PROFILES

For each group, the mean and range of plasma concentrations for individual amino acids are shown in table 4; the quoted target ranges represent the 95% confidence interval derived from amino acid profiles in breast fed infants.[12] Few amino acids were consistently found at concentrations within the target range. Phenylalanine concentrations in subjects fed Vaminolact were significantly lower than in the Vamin 9 group (p=0·0028) as were concentrations of tyrosine (p=0·0004). In the group given Vaminolact, 80% of the tyrosine values fell below, and 20% within, the target range, while in the Vamin 9 group 58% were above the upper target range and none below. Total cystine/cysteine concentrations always fell below the lower target range in the Vaminolact group, and only one value in the Vamin 9 group was above this. In the subjects fed Vaminolact, the mean concentrations of all amino acids except threonine, lysine, histidine, and cystine were closer to the reference means than in Vamin 9 glucose fed infants.[12]

Discussion

The results of this study indicate that the use of a neonatally adapted amino acid source leads to a reduction in the derangement of plasma amino acid profiles, while growth and nitrogen retention are unchanged. Previous studies have shown that plasma phenylalanine is higher in parenterally fed babies than in those receiving enteral feeds,[13 14] and particular attention has been drawn to the observation that high phenylalanine intakes in neonates receiving Vamin 9 glucose may be associated with potentially neurotoxic plasma phenylalanine concentrations.[4–6] Two of the seven babies given Vamin 9 glucose during this study had plasma phenylalanine concentrations above 600 μmol/l, which would be considered unacceptably high in a child with treated phenylketonuria.[15] Phenylalanine concentrations in children receiving Vaminolact were much lower (p=0·0028), although a high proportion (68%) were still above the upper limit of the target range.

There are several reasons why plasma amino acid profiles in patients given Vaminolact still differ from

those in breast fed infants. An intravenous amino acid intake of 2·5 g/kg/day is 25% higher than with breast milk of average composition. Furthermore, plasma amino acid profiles in breast fed infants reflect modification of relative amino acid concentrations during small intestinal absorption and subsequent passage of portal blood through the liver. That such modification occurs is one reason why attempts to reproduce those plasma amino acid profiles found in breast fed infants exactly in parenterally fed infants are probably misguided.

The mean tyrosine concentration in infants fed Vamin 9 glucose was also high compared with that reported in breast fed infants,[12] and tyrosine concentrations were significantly higher than those found in the Vaminolact group ($p=0·0004$). This too is a cause for concern, as it has been suggested that raised tyrosine concentrations may be related to long term developmental impairment.[16] The raised tyrosine concentrations probably reflect phenylalanine catabolism, as the intake of tyrosine with both Vamin 9 glucose and Vaminolact is similar. The low mean concentration of tyrosine found in the Vaminolact group suggests possible underprovision of this amino acid, which is said to be 'semi-essential' in the very low birthweight infant. This might pose problems in long term parenteral nutrition, although the consequences of a low tyrosine intake are unknown.

The increased threonine concentrations with Vaminolact reflect the fact that intake of threonine is about 50% higher than in the Vamin 9 glucose or breast fed infants, but does not exceed the recommended maximum for preterm infants.[17] Although concentrations of cystine/cysteine were low, clinical studies have shown that cystine deficient diets have no apparent adverse effects on growth or nitrogen balance in preterm infants and the precise requirements for cystine remain unknown.[18 19]

The implications of the hyperphenylalaninaemia observed in the Vamin 9 glucose group are difficult to assess. Recent evidence suggests that appreciable intellectual impairment may occur in patients with phenylketonuria even when plasma phenylalanine concentrations have been kept as low as 600 μmol/l.[20] Although courses of parenteral nutrition will in most cases be measured in weeks rather than months, the neonatal brain is undergoing a period of rapid growth and development and might therefore be particularly sensitive to injury. Studies in animals have shown that even transient rises of plasma phenylalanine may be associated with enduring behavioural changes[21] and poor brain growth.[22] High concentrations of phenylalanine during parenteral nutrition must therefore be regarded as potentially harmful. The widespread experience with Vamin 9 glucose suggests, however, that the risk of clinically apparent neurological injury in the short term is extremely small.

Amino acid solutions such as Vaminolact that are specifically designed to meet the needs of infants are likely to lessen the risk of neurotoxicity resulting from amino acid imbalance, and we now use a neonatally adapted formula (Vamin Infant*) in patients less than 6 months of age. We reject the view that measurement of plasma amino acid concentrations during parenteral nutrition is unnecessary,[23] and recommend weekly monitoring —at least of phenylalanine—in patients up to 6 months of age who are receiving an unmodified, high phenylalanine, amino acid solution.

*Vamin Infant is available in the United Kingdom and is identical to Vaminolact except that it does not contain taurine. Vaminolact does not yet have a product licence in the United Kingdom.

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