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ORIGINAL ARTICLE

The influence of parenteral protein intake on electrolyte disturbances in premature infants☆

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KEYWORDS
Hypercalcaemia;
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preterm infants

Abstract
Introduction: Aggressive parenteral nutrition with delivery of high amino acid and energy doses is used to improve growth and neurodevelopmental outcomes in very low birth weight (VLBW) preterm infants. Recent findings, however, suggest that this approach may cause electrolyte imbalances. The aim of our study was to compare the prevalence of hypercalcaemia, hypophosphataemia, and hypokalaemia in 2 groups of preterm infants that received parenteral nutrition with different amounts of amino acids and to analyse perinatal and nutritional variables associated with the development of electrolyte imbalances.
Methods: We conducted a retrospective observational study comparing 2 groups of preterm infants born before 33 weeks' gestation with birth weights of less than 1500 g managed with parenteral nutrition. One of the groups received less than 3 g/kg/day of amino acids and the other received 3 g/kg//day of amino acids or more. We analysed the prevalence of electrolyte imbalances and possible associations with aggressive parenteral nutrition, adjusting for potential confounders.
Results: We studied 114 infants: 60 given less than 3 g/kg/day of amino acids (low-intake group) and 54 given at least 3 g/kg/day (high-intake group). The prevalence of electrolyte imbalances was similar in both groups. The prevalence of hypercalcaemia was 1.67% in the low-intake group and 1.85% in the high-intake group ($P > .99$), the prevalence of severe hypophosphataemia 11.7% vs 9.3%, and the prevalence of hypokalaemia 15.0% vs 11.1% ($P > .99$). A calcium to phosphorus ratio greater than 1.05 had a protective effect against hypophosphataemia ($P = .007$).

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Conclusions: We did not find an association between hypercalcaemia, hypophosphataemia, and hypokalaemia and the amino acid dose delivered by PN in the high-intake group of preterm infants.

PALABRAS CLAVE
Hipercalcemia;
Hipofosfatemia;
Nutrición parenteral;
Síndrome de realimentación;
Recién nacidos prematuros

Influencia del aporte proteico parenteral en las alteraciones electrolíticas en recién nacidos prematuros

Resumen
Introducción: La nutrición parenteral agresiva con aportes energéticos y proteicos altos se utiliza para mejorar el crecimiento y el neurodesarrollo en recién nacidos prematuros de muy bajo peso. No obstante, hallazgos recientes sugieren que su uso puede ocasionar alteraciones electrolíticas. El objetivo del estudio era comparar la prevalencia de hipercalcemia, hipofosfatemia e hipopotasemia en dos grupos de recién nacidos prematuros que recibieron nutrición parenteral con distintos aportes de aminoácidos, y analizar variables perinatales y nutricionales asociadas a la ocurrencia de alteraciones electrolíticas.
Métodos: Estudio retrospectivo observacional, con comparación de 2 grupos de recién nacidos prematuros con peso < 1500 g y edad gestacional < 33 semanas manejados con nutrición parenteral. Uno de los grupos recibió < 3 g/kg/d de aminoácidos mientras que el otro recibió ≥3 g/kg/d. Se analizó la prevalencia de distintas alteraciones electrolíticas y su asociación con la nutrición parenteral agresiva, con ajustes para posibles factores de confusión.
Resultados: El análisis incluyó 114 recién nacidos: 60 que recibieron < 3 g/kg/d de aminoácidos (ingesta baja) y 54 que recibieron ≥ 3 g/kg/d (ingesta alta). La prevalencia de alteraciones electrolíticas fue similar en ambos grupos. La prevalencia de hipercalcemia fue de 1,67% en el grupo de ingesta baja y 1,85% en el grupo de ingesta alta ($p > 0,99$). Los respectivos valores para las otras alteraciones fueron 11,7% vs. 9,3% en el caso de la hipofosfatemia grave y 15,0% vs. 11,1% en el caso de la hipopotasemia ($p > 0,99$). Se observó que una relación calcio:fósforo superior a 1,05 ofrecía un efecto protector frente a la hipofosfatemia ($p = 0,007$).
Conclusiones: No se observó asociación entre la hipercalcemia, hipofosfatemia o la hipopotasemia y el aporte de aminoácidos mediante nutrición parenteral en la población de recién nacidos prematuros con ingestas altas de aminoácidos.

Introduction

Very low birth weight (VLBW) preterm infants (birth weight < 1500 g) are at high risk of stunted postnatal growth due to the premature interruption of placental nutrient transfer at a critical time of growth and development.[1] To address this problem, current guidelines recommend combining early aggressive parenteral nutrition (PN) to ensure high energy and protein intakes with breast milk.[2–5]

Although this strategy has proven effective in optimising postnatal nutrition and neurodevelopmental outcomes, findings from several recent studies suggest that a high amino acid intake (>3 g/kg/day) may cause electrolyte imbalances in this population, particularly involving calcium-phosphorus metabolism.[4–9]

As described in the literature, high energy and amino acid intakes in the first week of life would maintain cells in an anabolic state, leading to an increase in the cellular uptake of phosphorus and potassium, which are required for nucleic acid and adenosine triphosphate production, among other processes. Inadequate intake of phosphorus and potassium results in depleted plasma levels of these electrolytes and increased calcium levels largely resulting from bone resorption.[6–8] This process has been referred to as ''placental interrupted feeding syndrome of the preterm infant''.[10–12]

In a randomised controlled trial that compared two feeding protocols in VLBW infants, Moltu et al.[7] found a higher prevalence of hypercalcaemia, hypophosphataemia, and hypokalaemia and a higher risk of septicaemia in infants who received 3.5 g/kg/day of amino acids compared to infants in a control group receiving approximately 2 g/kg/day. On their part, Bonsante et al.[8] found a correlation between different amino acid intakes (< 1.5 g/kg/day, 1.5—2 g/kg/day, and > 2 g/kg/day) and the development of hypercalcaemia and hypophosphataemia, observing that these disturbances were more common in infants with higher intakes. More recently, Brener et al.[9] reported an association between aggressive PN and hypercalcaemia and hypophosphataemia when comparing preterm infants managed with an aggressive nutrition

regimen (2.3-3.7 g/kg/day) to controls managed with a standard regimen (2.5-3.1 g/kg/day).

Electrolyte imbalances frequently do not cause symptoms, as they are corrected early. Hypercalcaemia (defined as a total calcium level > 3.5 mmol/L or an ionized calcium level > 1.73 mg/dL), however, may cause vomiting, irritability and even ventricular arrhythmia, while severe hypophosphataemia (phosphate level < 0.9 mmol/L) is associated with muscle weakness, delayed weaning from ventilatory support, and nosocomial infections.[12,13]

The aim of our study was to evaluate the association between the amount of protein delivered through PN and the presence of electrolyte imbalances (hypercalcaemia, hypophosphataemia, and hypokalaemia) in a cohort of VLBW preterm infants.

Our secondary objectives were to analyse the timing of the onset and the duration of phosphate and calcium imbalances and to assess the appropriateness of the calcium-to-phosphorus ratio (CA:P) of the PN solution used in our hospital.

Methods

Study design

We conducted an observational, retrospective, uncontrolled, open-label study at the Maternal-Infant Unit of the Hospital Universitario Vall d'Hebron in Barcelona between January and December 2016. The study was approved by the Clinical Research Ethics Committee of the hospital (EPA(AG)58/2018(5383).

We identified candidates for the study by reviewing the PN prescription records of the Department of Pharmacy of the hospital. We included VLBW premature infants born before 33 weeks' gestation managed with PN in the neonatal intensive care unit. We excluded infants with major congenital anomalies and chromosomal abnormalities.

Variables under study

At our hospital, PN for preterm infants is individualised according to the clinical condition, age (days of life), and weight of the patient as per the 4th edition of the hospital's paediatric nutrition guidelines.[14]

On the first day of life, all newborn infants that need nutritional support receive a standard PN bag if they are born after hours or an individualised PN bag if born during regular business hours.

The standard solution for day 1 (up to 2017) had a volume of 150 mL and provided 16.2 kcal, 9.5 g of carbohydrates, 3.6 g of amino acids, 0.9 g of lipids, and 1.66 mEq of calcium (osmolarity, 725 mOsm/L). As per protocol, the dose was 60 to 90 mL/kg/day for infants with weights of less than 1000 g and 65 to 80 mL/kg/day for infants with weights of 1000 to 1500 g. These volumes corresponded to a protein supply of less than 2.2 g/kg/day.

After day 1, all PN solutions are customised to the patient based on age and weight. The guideline establishes the recommended energy and nutrient intakes for days 1 to 8 of life, and doses are modified based on the clinical condition of the patient.[14] The hospital guidelines call for a starting amino acid dose of 2.5 g/kg/day and a maximum dose of 3.5 g/kg/day.

The administration of electrolytes is based on the recommendations of the guideline and imbalances are corrected by adjusting the PN solution or with electrolyte supplementation the following day. Administration of calcium (calcium gluconate 10%) starts on day 1, and administration of phosphorus (sodium glycerophosphate) and potassium (potassium chloride or potassium acetate) on day 2. Thus, the Ca:P ratio therefore ranges from 0.7 to 1.1 mmol of calcium to 1 mmol of phosphorus.[14]

Where possible, trophic enteral nutrition (EN) with maternal breast milk or with donor human milk is combined with PN on the first day of life. The starting volume of human milk is 10 to 20 mL/kg/day and is gradually increased based on tolerance. Since the necessary amount of maternal breast milk cannot always be obtained, we assume that most of the EN volume is in the form of mature donor human milk, which, based on the paediatric nutrition guideline of our hospital,[14] contains 70 mg/mL carbohydrate, 11 mg/mL protein, 45 mg/mL lipids, 150 mg/mL of sodium, 500 mg/mL of potassium, 400 mg/mL chloride, 340 mg/L calcium, 140 mg/L phosphorus, 0.5 mg/L iron, 32 mg/L magnesium, 250 μg/L copper, and 1200 μg/L zinc.

Infants received PN until they developed tolerance to total EN.

Data collection

We collected data on the following variables from the electronic health records: gestational age, sex, birth weight, history of intrauterine growth restriction, use of antenatal steroids, respiratory distress, patent ductus arteriosus, use of intravenous ibuprofen, sepsis, necrotising enterocolitis, and survival at 30 days.

We recorded the daily nutrient intakes obtained through both PN and EN for each infant, with a breakdown of the volume, energy, macronutrients (glucose, proteins, lipids) and micronutrients (sodium, potassium, chloride, calcium, and phosphorus) administered. We also recorded the details of additional electrolytes (phosphate, calcium, and potassium) given with PN and EN. The duration of follow-up in every case corresponded to the period of PN.

We retrieved the data on the plasma sodium, phosphate, calcium, potassium, urea, creatinine, and alkaline phosphatase levels that had been documented during the period of PN. We did not distinguish between plasma and serum levels. Other variables included in the analysis included the ionized calcium level, measured by arterial blood gas analysis, plasma sodium, potassium, and chloride levels measured by indirect potentiometry, the total calcium level measured with the Arsenazo III reagent test, and the phosphate level obtained by indirect calorimetry (Beckman Coulter AU5800 Chemistry Analyzer; Beckman Coulter International SA, Rue Juste-Olivier 22, PO Box 1059, CH-1260, Nyon, Switzerland).

We defined mild, moderate, and severe hypophosphataemia as plasma levels of less than 1.29 mmol/L, 1.1 mmol/L, and 0.9 mmol/L, respectively; hypokalaemia as a plasma level of less than 3.5 mmol/L; and hypercalcaemia as a total calcium level greater than 2.8 mmol/L and a plasma ionised calcium level greater than 1.3 mmol/L.

Statistical analysis

We have described continuous data as mean and standard deviation (SD) or median and range, and categorical data as absolute frequency and percentage distributions. We classified infants into two groups depending on whether they received less than 3 g/kg/day of amino acids (low-intake group) or 3 g/kg/day or more (high-intake group). We summarised clinical, analytical and demographic characteristics of the infants and compared them in the 2 groups. We compared continuous variables with the *t* test and categorical variables with the χ^2 test or the Fisher exact test, as applicable.

In the inferential analysis, we assessed the associations between calcium, phosphorus and potassium levels and (i) perinatal variables, (ii) nutritional intakes and (iii) Ca:P ratios by means of linear or logistic regression. The perinatal variables included in the model were gestational age, sex, birth weight, intrauterine growth restriction, use of antenatal steroids, respiratory distress, patent ductus arteriosus, use of intravenous ibuprofen, sepsis, necrotising enterocolitis and survival at 30 days. We included variables found to be significant in the univariate analysis ($P < .05$) in the multivariate analysis and then used backward elimination ($P > 0.10$). We assessed the effect of potential confounders, such as gestational age, birth weight, presence of intrauterine growth restriction, energy intake, and PN and EN dose.

The statistical analysis was performed with the software IBM SPSS Statistics, version 18.0 (SPSS Inc; Chicago, IL, USA). We considered *p*-values of less than 0.05 statistically significant.

Results

We considered 135 preterm infants managed with PN for inclusion in the study, of who we excluded 21 on account of missing data or failure to meet the inclusion criteria. This resulted in a final sample of 114 preterm infants. The analysis included a total of 306 phosphate, 503 calcium, and 464 potassium measurements.

Tables 1 and 2 present the perinatal characteristics and nutrient intakes by group. There were 60 patients in the low-intake group (amino acid intake <3 g/kg/day) and 54 in the high-intake group (≥3 g/kg/day). The groups were comparable in terms of demographic and clinical characteristics save for sepsis, which was greater in the high-intake group, although this difference was not statistically significant. When we compared PN and EN intakes in both groups, we found significant differences for all nutrients and electrolytes except the intake of magnesium through PN.

The multivariate analysis did not detect any significant associations of plasma calcium levels with perinatal variables, PN or EN intake, or Ca:P ratios. The only variables significantly associated with plasma phosphorus levels were the intake of potassium ($P = .017$) and calcium ($P = .01$) through PN. Likewise, the only significant modifiers of plasma potassium levels were the doses of calcium ($P = .002$) and phosphorus ($P = .05$) delivered through PN.

Table 3 presents the mean calcium, phosphorus, and potassium levels for each group and the absolute frequency and percentage of patients that developed hypercalcaemia, hypophosphataemia and hypokalaemia.

Fig. 1 charts the daily plasma levels of calcium and phosphate, evincing a clear trend towards hypercalcaemia and hypophosphataemia as days passed.

When we assessed the associations between the Ca:P ratio and the doses of electrolytes delivered through PN, we found that a ratio greater than 1.05:1 protected against moderate hypophosphataemia ($P = .007$), while lower ratios increased its risk ($P = .072$). This protective effect was observed for ratios up to 1.4:1.

When we analysed the associations between the Ca:P and the overall doses of electrolytes delivered through PN, EN, and additional fluids, we found that a ration greater than 1.00:1 (to up to 1.3:1) protected against moderate hypophosphataemia ($P = .043$), while lower ratios increased its risk ($P = .128$).

Discussion

In recent years, several authors have proposed that there is a link between early aggressive PN in preterm infants and serious metabolic disorders.[7–9,15–18] There is evidence that nutrition in the first days of life affects calcium, phosphorus, and potassium metabolism and may cause refeeding syndrome.[8,10–12]

We found a similar prevalence of hypercalcaemia, hypophosphataemia, and hypokalaemia in preterm infants managed with PN that received at least 3 g/kg/day of amino acids (high-intake group) and those that received doses of less than 3 g/kg/day (low-intake group).

In the randomised controlled trial that compared two groups of VLBW infants, Moltu et al.[7] found that infants who received a mean 3.7 g protein/kg/day (SD, 0.11) exhibited better postnatal growth compared to those who received a mean of 2.5 g/kg/day (SD, 0.18), but the incidence of electrolyte imbalances (hypercalcaemia, hypophosphataemia and hypokalaemia) and septicaemia was also higher in the high-protein group. Bonsante et al.[8] also observed a higher incidence of hypophosphataemia and hypercalcaemia in preterm infants that received high amino acid doses (2.3 ± 0.8 g/kg/day) compared to infants receiving intermediate doses (1.49 ± 0.34 g/kg/day) or low doses (1.2 ± 0.8 g/kg/day). Brener et al.[9] reported similar findings of a retrospective study that compared infants given 3.3 to 3.7 g of amino acids/kg/day versus 2.5 to 3.1 g/kg/day.

In our cohort, the mean amino acid intakes were 2.67 ± 0.3 g/kg/day in the low-intake group and 3.21 ± 0.24 g/kg/day in the high-intake group. The main reason the difference was this small that formulation of PN was dictated by the hospital guidelines.[14] Besides being similar, these values were quite high, so that differences in amino acid intake may not have been large enough to have an impact on outcomes, a fact that set our sample apart from the samples analysed in the studies discussed above. This may also explain why we found no correlation between amino acid intake and electrolyte imbalances. In other words, most of the infants in our study received less than 2.6 g of protein/kg/day so that their risk of developing electrolyte imbalances was similar.[7–9,15–18] On the other hand, although we found differences between

Table 1 Perinatal characteristics of patients with high and low amino intakes.

Characteristics		Subgroups		
		Low-intake group <3 g/kg/d N = 60	High-Intake Group ≥3 g/kg/d N = 54	P
Perinatal variables				
Female sex	N (%)	34 (52.31)	31 (57.41)	> 0.99
Gestational age (weeks)	Mean ± SD	28.26 ± 2.43	28.79 ± 2.71	> 0.99
Birth weight (g)	Mean ± SD	1075 ± 281	1062 ± 276	> 0.99
Intrauterine growth restriction	N (%)	25 (41.67)	27 (50.00)	> 0.99
Antenatal steroids	N (%)	58 (96.67)	50 (92.59)	> 0.99
Respiratory distress	N (%)	49 (81.67)	37 (68.52)	> 0.99
Patent ductus arteriosus	N (%)	17 (28.33)	17 (31.48)	> 0.99
Intravenous ibuprofen	N (%)	11 (18.33)	12 (22.22)	> 0.99
Sepsis	N (%)	10 (16.67)	17 (31.49)	> 0.99
Necrotising enterocolitis	N (%)	1 (1.67)	1 (1.85)	> 0.99
Duration of parenteral nutrition (days)	Mean ± SD	8.88 ± 8.45	8.74 ± 5.81	> 0.99
Mortality at 30 days	%	3.3	7.4	> 0.99
Standard solution given on day 1	N (%)	15 (25)	27 (50)	< 0.001

Table 2 Characteristics of nutrition delivered to patients in the high- and low-protein intake groups.

Characteristics		Subgroups		
		Low-intake group <3 g/kg/d N = 60	High-Intake Group ≥3 g/kg/d N = 54	P
Perinatal variables				
Patient mean daily composition of parenteral nutrition				
Volume (mL/kg/day)	Mean ± SD	85.58 ± 12.63	103.48 ± 17.91	< 0.001
Energy (kcal/kg/day)	Mean ± SD	54.51 ± 6.29	63.48 ± 7.47	< 0.001
Protein (amino acids) (g/kg/day)	Mean ± SD	2.67 ± 0.30	3.21 ± 0.24	< 0.001
Carbohydrates (glucose) (g/kg/day)	Mean ± SD	6.83 ± 0.81	8.10 ± 1.35	< 0.001
Lipids (g/kg/day)	Mean ± SD	1.68 ± 0.28	1.92 ± 0.33	< 0.001
Sodium (mEq/kg/day)	Mean ± SD	2.20 ± 0.69	2.60 ± 0.86	< 0.05
Potassium (mEq/kg/day)	Mean ± SD	1.32 ± 0.34	1.51 ± 0.28	< 0.05
Phosphorus (mmol/kg/day)	Mean ± SD	0.65 ± 0.14	0.70 ± 0.14	< 0.001
Calcium (mEq/kg/day)	Mean ± SD	1.35 ± 0.24	1.59 ± 0.15	< 0.001
Ca:P ratio (mmol:mmol)	Mean ± SD	1.04:1 ± 0.21	1.17:1 ± 0.20	< 0.001
Magnesium (mEq/kg/day)	Mean ± SD	0.12 ± 0.09	0.12 ± 0.11	> 0.99
Mean daily composition of enteral nutrition in patients (donor human milk)				
Volume (mL/kg/day)	Mean ± SD	58.73 ± 23.22	40.52 ± 23.87	< 0.001
Additional electrolyte doses				
Sodium (mEq)	Mean ± SD	0.15 ± 0.37	0.11 ± 0.25	> 0.99
Potassium (mEq)	Mean ± SD	0.01 ± 0.04	0.02 ± 0 0.05	> 0.99
Phosphorus (mmol)	Mean ± SD	0.02 ± 0.07	0.00 ± 0.01	> 0.99
Calcium (mEq)	Mean ± SD	0.02 ± 0.12	0.01 ± 0.03	> 0.99

Ca:P = calcium to phosphorus ratio.

groups the amount of electrolytes delivered through PN, once the contribution through EN was taken into account, there were no overall differences in the total amounts of electrolytes received by each group. In addition, as PN solutions in our hospital are customised to meet individual requirements every day, the early correction of plasma electrolyte imbalances may explain the low prevalence rates we observed. Another factor to take into account is that although the use of supplemental electrolytes was similar between groups, it could have contributed to such

Table 3 Prevalence of hypercalcaemia, hypophosphataemia and hypokalaemia in preterm infants based on amino acid dose delivered by PN.

Characteristics		Subgroups		
		Low-intake group <3 g/kg/d N = 60	High-Intake Group ≥3 g/kg/d N = 54	P
Perinatal variables				
Total calcium (mmol/L)	Mean ± SD	2.22 ± 0.32	2.27 ± 0.18	> 0.99
Ionized calcium (mg/dL)	Mean ± SD	1.13 ± 0.39	1.12 ± 0.42	> 0.99
Phosphate (mmol/L)	Mean ± SD	1.5 ± 0.41	1.53 ± 0.36	> 0.99
Potassium (mmol/L)	Mean ± SD	4.45 ± 0.76	4.42 ± 0.47	> 0.99
Hypercalcaemia (plasma	Infants, *n* (%)	1 (1.67)	1 (1.85)	> 0.99
calcium >2.8 mmol/L)	Abnormal measurements, *n* (%)	1/261 (0.38)	1/242 (0.41)	> 0.99
Hypercalcaemia (ionized	Infants, *n* (%)	42 (70)	43 (79.6)	> 0.99
calcium > 1.3 mmol/L)	Abnormal measurements, *n* (%)	164/303 (54.13)	196/338 (58 0.99)	> 0.99
Mild hypophosphataemia	Infants, *n* (%)	22 (36.67)	21 (38.89)	> 0.99
(P < 1.29 mmol/L)	Abnormal measurements, *n* (%)	62/148 (41.89)	50/158 (31.65)	0.063
Moderate	Infants, *n* (%)	13 (21.67)	15 (27.78)	> 0.99
hypophosphataemia (P < 1				
.1 mmol/L)	Abnormal measurements, *n* (%)	33/148 (22.30)	25/158 (15.82)	0.150
Severe hypophosphataemia	Infants, *n* (%)	7 (11.67)	5 (9.26)	> 0.99
(P < 0.9 mmol/L)	Abnormal measurements, *n* (%)	16/148 (10.81)	10/158 (6.33)	0.161
Hypokalaemia	Infants, *n* (%)	9 (15.00)	6 (11.11)	> 0.99
(K < 3.5 mmol/L)	Abnormal measurements, *n* (%)	21/237 (8.86)	10/227 (4.41)	0.055

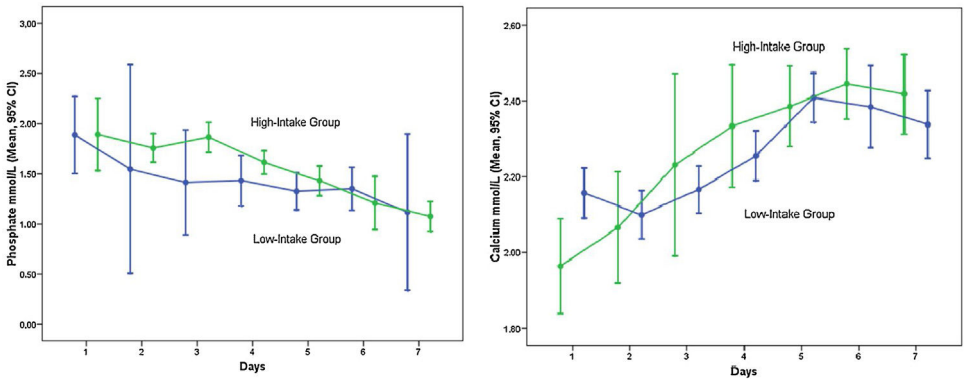

Figure 1 Plasma levels of calcium and phosphorus in 60 infants that received 3 g/kg/day of amino acids (low-intake group) and 54 infants that received 3 or more g/kg/day (high-intake group). Mean and 95% confidence interval.

early correction, but we are unable to establish causality with the data available to us.

Electrolyte imbalances were considerably less frequent in our cohort compared to other studies except for ionized calcium. We classified hypophosphataemia into 3 categories based on plasma phosphorus levels. We found mild hypophosphataemia (<1.29 mmol/L) in 36.6% (22/60) of patients in the low-intake group and 38.9% (21/54) of patients in the high-intake group. Applying the same cut-off point to define hypophosphataemia as us, Moltu et al.[7] and Brener et al.[9] reported frequencies of 77% (36/40 patients) and 90% (17/22 patients), respectively, in the groups of infants with a high amino acid intake. The frequency of moderate hypophosphataemia (<1.1 mmol/L) in our sample was 21.7% (13/60 patients) in the low-intake group and 27.8% (15/54 patients) in the high-intake group. In yet another study that applied the same cut-off point, Brener et al.[20] found that 58.3% (35/60) of patients that received 3 to 3.5 g/kg/day of amino acids developed moderate hypophosphataemia. Last of all, we found severe hypophosphataemia (<0.9 mmol/L), which can be clinically relevant, was 11.7% (7/60) of patients in the low-intake group and in 9.3% (5/54) of patients in the high-intake group.[12,13] Applying a similar cut-off point (< 1 mmol/L), Bonsante et al.[8] found that 18.9% (11/53) of infants in the high-intake group developed severe hypophosphataemia.

We analysed total and ionized calcium plasma levels, as the former vary in relation to albumin levels, which are gen-

erally low in preterm infants. Based on the total calcium level, only 1 patient in each group had hypercalcaemia. This low prevalence contrasts sharply with the rates of 6.2% (3/48 patients) and 30.2% (16/53 patients) reported by Bonsante et al.[8] for infants receiving low and high amino acid doses, respectively. The prevalence of hypercalcaemia based on plasma levels of ionized calcium, however, was much higher: 70% (42/60) in the low-intake group and 79.6% (43/54) in the high-intake group. Brener et al.[9] reported a similar proportion of 87% (35/40) in patients given higher doses of amino acids.

Lastly, we found hypokalaemia in 15% (9/60) of patients in the low-intake group and 11.11% (6/54) of patients in the high-intake group. Once again, these proportions contrast sharply with the corresponding proportions of 46% (11/24 patients) and 88% (21/24 patients) reported by Moltu et al.[7]

Both hypophosphataemia and hypercalcaemia tended to occur towards the end of the PN period in our series, which was consistent with the findings of Moltu et al.[7] and Bonsante et al.,[8] although their groups reported lower phosphorus and higher calcium levels. This could be due to refeeding syndrome, which typically develops after 2 or 3 days, or to increments in enteral feeding volumes during the PN period, which reduced the opportunity to correct electrolyte imbalances.

Phosphorus is an essential mineral for cell metabolism, as it is involved in adenosine triphosphate production and correct immune cell function. Phosphorus depletion has been linked to neuromuscular weakness, insulin resistance, extubation failure, and sepsis.[7,12,13,19–21] Sepsis was more common in preterm infants fed higher amounts of amino acids in the randomized controlled trials of Moltu et al.[7] and Brener et al.[7,20] In our study, sepsis was also more frequent in the high-intake group, although not significantly so.

According to both Senterre et al.[21] and the ESPGHAN/ESPEN/ESPR/CSPEN guidelines on paediatric PN, a Ca:P ratio of 0.8 to 1.1:1 should be maintained in the first week of life in VLBW preterm infants. This ratio achieves a reduction in the incidence of hypophosphataemia and therefore hypercalcaemia in patients with optimised protein and energy intakes who do not receive nutrition high in calcium and phosphorus (0.8-2 and 1-2 mmol/kg/day, respectively). A higher ratio is recommended after the first week (1-1.3:1), as calcium and phosphorus requirements also increase during this period (to 1.6–3.5 and 1.6–3.5 mmol/kg/day, respectively).[13,21]

In our series, a Ca:P ratio greater than 1.05 had a protective effect against moderate hypophosphataemia ($P = .007$) while lower ratios increased the risk ($P = .072$). Thus, use of higher-than-recommended ratios (up to 1.4:1 possibly) in the first week of life might reduce the incidence of hypophosphataemia in VLWB preterm infants.

Perinatal disorders and congenital anomalies cause 76.2% of deaths in infants aged less than 1 year in our setting, and mortality in newborns under 28 days of age in 2016 was 16.5%.[22] In our series, the mortality at 30 days was 3.3% in the low-intake group, and 7.4% in the high-intake group. Considering that we excluded infants with congenital anomalies, this figure is consistent with data from our setting. None of the study variables was associated with higher mortality.

There are some limitations to our study intrinsic to its retrospective and observational design. Reviews of health records are typically limited by missing information and potential bias. Daily blood test results were not available for all infants, as these tests are ordered as deemed necessary. Another important limitation was that both groups end up having high protein intakes because PN prescription is dictated by hospital guidelines. Besides, there was a difference in the protein intake on day 1 depending on whether patients were given standard PN the study protocol was applied. Another limitation is that we assumed that most of the EN administered to the infants was in the form of mature donor human milk, but we were unable to differentiate between different compositions based on the maturity of the milk. Nevertheless, data were available for a considerable number of the included patients for the period under study.

In conclusion, our analysis of a sample of VLBW preterm infants given high amino acid doses did not find an association between hypercalcaemia, hypophosphataemia, and hypokalaemia and the amino acid dose delivered through PN. However, we did find that optimising the Ca:P ratio in PN could have a protective effect against hypophosphataemia in this population, and therefore believe that this is an option worth considering.

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