ARTICLE

Early high amino-acid intake is associated with hypophosphatemia in preterm infants

Maria Fernanda Galletti[1]✉, Pablo Hernan Brener Dik[1], Silvia Anahi Fernandez Jonusas[1], Debora Sabatelli[2], Carla Chiesa[3], Silvana Perez[4], Mariana Brusadin[5], Larissa Genes[6], Isabel Araya[7] and Gonzalo Luis Mariani[1]

OBJECTIVE: To estimate the incidence of hypophosphatemia in preterm infants according to parenteral nutrition received and to evaluate associated risk factors.
DESIGN: A prospective multicenter cohort study included 111 patients ≤ 1250 g (7 NICUs of the NEOCOSUR Network). Two groups were compared according to the amino-acid supply in the first 48 h: aggressive parenteral group ≥ 3 g/kg/day and standard parenteral group: <2.9 g/kg/day. Hypophosphatemia was defined as serum phosphate < 4 mg/dl. A logistic regression analysis was performed to evaluate associated risk factors.
RESULTS: Fifty-eight infants received aggressive parenteral nutrition. The incidence of hypophosphatemia was significantly higher in the aggressive parenteral group (77.5% vs 53.8%, $p = 0.009$). Hypophosphatemia was independently associated with aggressive parenteral nutrition (aOR 4.16 95% CI 1.54–12.24) and negatively associated with phosphorous intake (aOR 0.92 95% CI 0.87–0.97).
CONCLUSION: Both high amino-acid intake and low phosphorus supply during the first days after birth were independently associated with hypophosphatemia.

Journal of Perinatology; https://doi.org/10.1038/s41372-022-01361-1

INTRODUCTION

Premature birth causes an abrupt disruption of nutrient supply through the placenta. Preterm (PT) infants have limited energy stores and a phase of catabolism begins after birth. PT infants who receive only supplemental glucose lose 1–2% of protein stores each day, and this may be even higher in very low birth weight infants [1–3].

Nutritional requirements after birth are higher than at any other time in life [4]. An insufficient supply of nutrients may cause a cumulative deficit leading to extrauterine growth restriction with deleterious effects on growth and neurodevelopment [1, 2, 4, 5].

Enteral feeding in the first days of life (DOL) is insufficient to meet these needs, so parenteral nutrition (PN) is currently a standard of care. Both an early start and an adequate caloric supply are critical [6]. An 'aggressive' PN with increased energy and protein supply has been studied and proposed, starting with protein intake soon after birth at a rate of ≥3 g/kg/day, resembling fetal protein accretion [2, 4, 6–8]. Several studies have shown that this strategy optimizes postnatal growth and development without increasing the risk of comorbidities or metabolic disorders [3, 5, 6, 8].

However, in the last few years many studies have reported early hypophosphatemia in PT infants [9–16]. Phosphorus (P) is the main intracellular anion. It is an essential component of membrane phospholipid, DNA, ATP and enzyme systems [9]. Furthermore, it is important for the chemotaxis, phagocytosis, and bactericidal activity of granulocytes [10, 11]. Early hypophosphatemia is a recently reported finding in PT infants with potential deleterious effects, including failure in extubating, muscle weakness, and late-onset sepsis [10–13]. Therefore, it is important to understand its associated risk factors to implement preventive strategies.

In the Neocosur Network, nutritional practice is not uniform along NICUs, with differences in the caloric and protein content of PN in the first days after birth. The present study was designed to assess the impact of the nutritional strategy on the development of hypophosphatemia. Our hypothesis was that PT infants receiving aggressive PN have a higher risk of early hypophosphatemia compared to those who received standard PN.

The primary aim was to estimate the incidence of hypophosphatemia in PT infants within the first week of life according to the PN received. Secondary aims were (1) to evaluate risk factors associated with hypophosphatemia; (2) to compare metabolic imbalances and morbidities according to the PN received; and (3) to compare morbidities according to the development (or not) of hypophosphatemia.

METHODS

Design and study population

Prospective, multicenter, cohort study conducted from October 2015 to August 2018. PT infants with BW ≤ 1250 g who were started on PN within

[1]Neonatology, Department of Pediatrics, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina. [2]Hospital Fernandez, Buenos Aires, Argentina. [3]Hospital Materno Infantil Ramón Sardá, Buenos Aires, Argentina. [4]Sanatorio Trinidad Palermo, Buenos Aires, Argentina. [5]Hospital Español de Mendoza, Mendoza, Argentina. [6]Hospital de Clínicas, Universidad Nacional de Asunción, Facultad de Medicina, San Lorenzo, Paraguay. [7]Hospital Clínico San Borja Arriarán, Santiago, Chile.
✉email: maria.galletti@hospitalitaliano.org.ar

Received: 3 August 2021 Revised: 25 January 2022 Accepted: 17 February 2022
Published online: 28 February 2022

48 h of life were included, after obtaining written informed parental consent. Infants with congenital malformations, acute kidney injury, limited probability of survival within 72 h of life and those patients with missing data regarding serum or urinary measurements were excluded.

Nutritional prescription

At the discretion of the neonatologists, PT infants received different amounts of amino acid (AA), glucose, lipids and minerals in the same admixture (3-in-1 solutions). We recorded the daily supply of macro and micronutrients during the first 10 DOL. According to the amount of AA received in the first 48 h two comparison groups were prospectively defined: *Aggressive PN*: AA ≥ 3 g/kg/day and *Standard PN*: AA 0.5–2.9 g/kg/day. These comparison groups were established in the study protocol regardless of NICU. Calcium gluconate and sodium glycerophosphate were standard salts used in PN formulation, but these salts could have been changed at physician discretion. Also, vitamin D was provided as a concentrated solution (Vitalipid® N Infant) containing 40 UI/ml, at a standard dose 4 ml/kg. We did not register information about the type of salt used nor the specific amount of vitamin D received. Besides, the volume and type of enteral feeding received were recorded.

Biochemical determination

We measured serum phosphate, ionized calcium ($Ca_{(i)}$) or total calcium ($Ca_{(t)}$), urea, creatinine, potassium, magnesium and glycemia within 6 h of birth (baseline determination), on DOL4 and DOL6. In addition, calciuria and phosphaturia were collected on an isolated urine sample between DOL3 and DOL5.

Primary outcome. Incidence of hypophosphatemia in the first week of life, defined as serum phosphatemia <4 mg/dl (1.29 mmol/L) on DOL4 or DOL6 [12–14, 16, 17].

Secondary outcomes. Hypercalcemia ($Ca_{(i)}$ ≥1.35 mmol/L-$Ca_{(t)}$ ≥11 mg/dl), hyperglycemia (>180 mg/dl), uremia ≥70 mg/dl, hyperkalemia (≥6.5 mEq/L), hypokalemia (≤3.5 mEq/L), hypercalciuria and hypophosphaturia (according to local references).

Other variables

Maternal and neonatal baseline characteristics were collected: gestational age (GA), birth weight (BW), antenatal steroids and prenatal $MgSO_4$ administration (either for preeclampsia or neuroprophylaxis), small for gestational age (SGA; Fenton < p10). In-hospital morbidities and mortality were also registered. Morbidities included treated patent ductus arteriosus, bronchopulmonary dysplasia (36 wk postmenstrual age, BPD), necrotizing enterocolitis (Bell ≥II), intraventricular hemorrhage (Papile Classification), late-onset sepsis (LOS) (positive blood culture or cerebrospinal fluid >72 h). We also assessed requirements of vasoactive drugs within the first 10 DOL, duration of respiratory support, presence of metabolic bone disease, retinopathy of prematurity and anthropometry data at discharge.

Ethical considerations

The study was approved by the Scientific Committee of the Neocosur Network and by the Research Ethics Committee of each participating Center.

Statistical analysis

Descriptive statistics were used to describe the study population. Continuous variables were expressed as mean and standard deviation (SD) or median and interquartile range 25–75% (IQR), according to their distribution. Comparison between the groups were performed by *T*-test or Mann–Whitney test. Categorical variables were expressed by absolute and relative frequencies. They were compared using χ^2 test or Fisher's test as appropriate. The incidence of hypophosphatemia was expressed as cumulative incidence and it was compared according to PN received. A multivariate logistic regression analysis was performed to assess variables independently associated with hypophosphatemia. Potential confounders were those variables with p value < 0.1 in the univariate analysis (Table 2) and those reported in the literature as clinically relevant. P intake, Ca:P Ratio > 0.8 (mol), aggressive PN, prenatal administration of magnesium sulfate and SGA were included in the model by forward stepwise selection. Crude (OR) and adjusted odds ratios (aOR) were reported with their 95% confidence interval (95% CI). We assessed the model´s goodness-of-fit (Hosmer–Lemeshow test).

Means of biochemical determinations at different times were compared by analyzing by repeated-measures ANOVA, which compare one or more mean scores with each other, was performed for each biochemical measure considering time as an independent variable. Baseline was set as reference.

All two-sided p values < 0.05 were considered significant. Software R 4.0.3 was used.

Sample size

Based on the lower bound of the incidence of hypophosphatemia previously reported (80%) [12], to demonstrate an absolute difference of 30% in the incidence of this disturbance, 45 neonates were needed in each group, with an α of 0.05 and 80% power. The maximum recruitment per NICU was limited to 25 PT neonates.

RESULTS

One hundred eleven patients were included. Figure 1 shows the patients flow chart and the participating Centers. Table 1 summarizes PT infants baseline characteristics and nutritional intake within 10 DOL according to nutritional strategy. Fifty-eight infants (52%) received aggressive PN, and no significant differences were found when comparing baseline characteristics between groups. PT infants in the aggressive group received a higher AA, lipids, and calories intake during the first 10 days. Conversely, the median amount of P and Ca delivered was not different.

Also, PT infants in the aggressive group received higher amounts of the macronutrients in the first 48 h of birth (Supplemental Material Table s1).

Regarding enteral feeding during 10 days, 100 (90%) patients received human milk, being exclusive in 77% of the cases. Median DOL at the onset of trophic feeding and the volume of enteral nutrition received were no different between groups (Table 1).

The overall incidence of hypophosphatemia was 66% (95% CI 56–75). Fifty-one PT infants (70%) were diagnosed on DOL4, and 22 (30%) infants on DOL6. Hypophosphatemia was significantly higher in the aggressive PN group. The incidence of this disturbance in the aggressive PN was 77.5% (95% CI 64.7–87.4) whereas in standard PN was 53.8 % (95% CI 39.4–67.7) ($p = 0.009$). There were no differences in baseline characteristics when we compared groups according to the presence of hypophosphatemia, except for prenatal MgSO4 administration. Patients who developed hypophosphatemia received significantly higher AA, calories, Ca: P Ratio (mol) and had a lower P intake within 10 day (Table 2). The amount of AA and lipids in the first 48 h of birth is shown in supplemental material Table s1. *AA* intake was higher on DOL1 and DOL2 in PT infants that developed hypophosphatemia but there was no difference in the amount of lipids prescribed. The onset of P supply was no different between PT infants with hypophosphatemia (mean 1.8 DOL; SD 0.81) and patients without this disorder (mean 1.74 DOL; SD 0.7) ($p = 0.57$).

After adjustment for potential confounders, aggressive PN and P intake were independently associated with hypophosphatemia. Figure 2 shows the results of the logistic regression model and the aOR of the variables included. Hosmer-Lemeshow test was statistically significant.

Twenty-three PT infants (40%) in the aggressive group were given rapid P IV infusion or increase in the P supply compared to 10 patients (19%) in the standard PN group ($p = 0.017$).

Biochemical determinations

No significant differences were found in any biochemical determination between PN groups, except for higher values of glycemia and potassium on DOL6 in the aggressive group. However, these values were within the normal range (Supplemental Material Table s2*)*. In SGA patients median phosphatemia at baseline (4.8 vs 5.8 mg/dl; $p = <0.0001$) and on DOL4 (3.2 vs

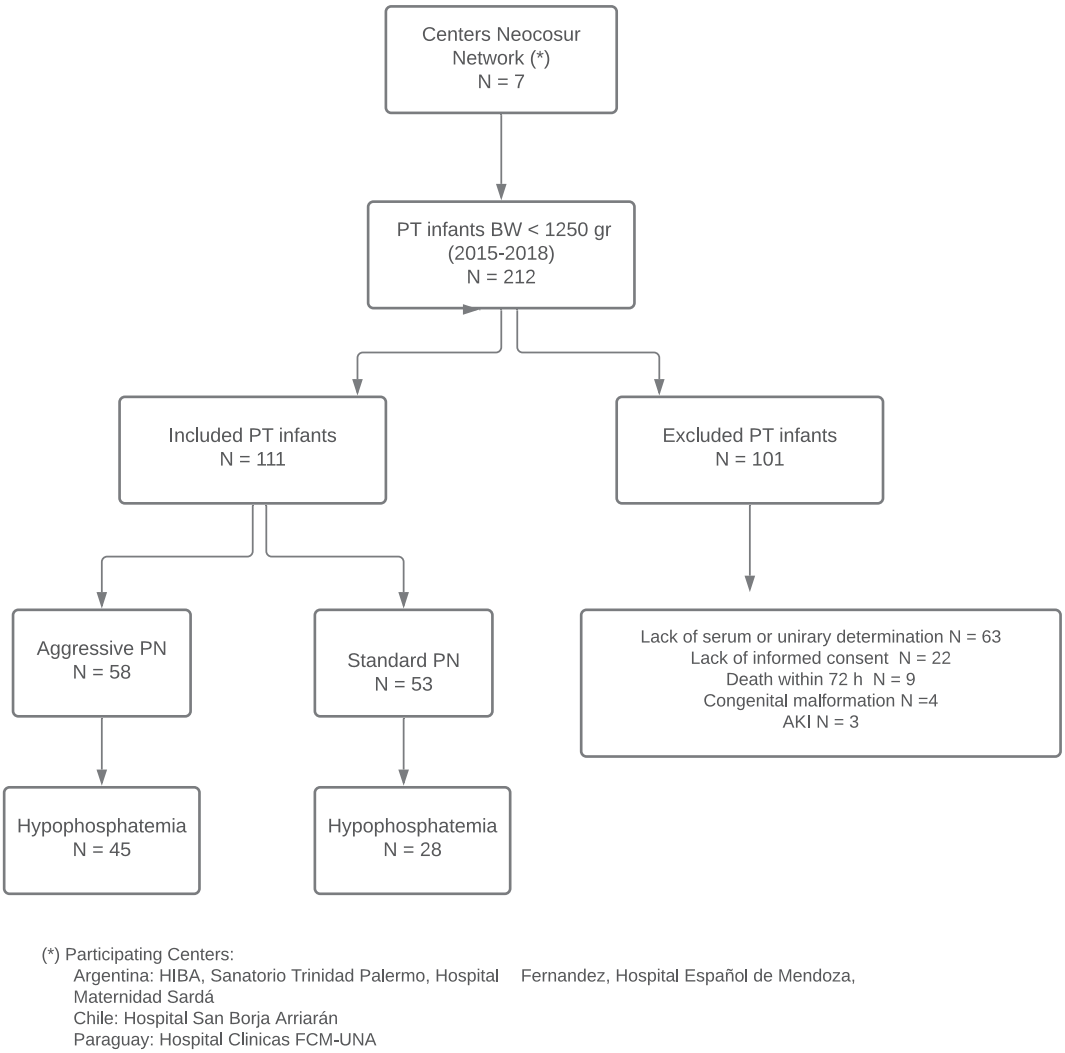

Fig. 1 Patient flowchart. Patients included and excluded, distribution according to parenteral nutrition received. *Participating centers belonging to Neocosur Network.

4.4 mg/dl; $p = 0.0018$) was lower compared to appropriate for GA infants.

Changes in total and ionized calcium, serum phosphate and potassium comparing baseline determination with DOL4 and DOL6 in both nutritional strategies are shown in Supplemental Material Fig. s1. On DOL4 and DOL6 phosphatemia was significantly lower than baseline in both nutritional strategies. Conversely, total calcium was significantly higher on DOL4 and DOL6 when compared with baseline.

Hypercalcemia was observed in 58 patients, and it was more frequent in patients with hypophosphatemia (66% vs 31%, $p = 0.001$). Only three patients presented hyperkaliemia, whereas 44% ($N = 32$) of patients with hypophosphatemia developed hypokalemia compared to 16% ($N = 6$) of patients with normal phosphatemia ($p = 0.004$).

Urinary samples were analyzed in 104 (94%) patients. We found no differences in the frequency of hypercalciuria or hypophosphaturia comparing nutritional strategies. The incidences of hypophosphaturia and hypercalciuria were different when PT infants who developed hypophosphatemia were compared to those who did not. Forty-six patients (67%) with hypophosphatemia developed hypophosphaturia compared to 50% of PT infants with normal phosphate values ($p = 0.045$). Hypercalciuria was observed in 28% of patients with this disturbance in comparison to 5.5% of PT infants without hypophosphatemia ($p = 0.001$)

Clinical outcomes and anthropometry

Three patients were transferred to other Institutions, so data were not available. Overall, in-hospital mortality was 13% ($N = 14$). No differences were observed in the frequency of neonatal morbidities or mortality during hospitalization when comparing both the nutritional strategy received and the presence (or not) of hypophosphatemia (Table 3). PT infants with hypophosphatemia required more days of mechanical ventilation (MV). The median of MV was 8 days (IQR 3–21.5) in PT with hypophosphatemia in contrast to 2 days (IQR 1–13) in patients with normal values ($p = 0.04$). Conversely, no differences were found in the incidence of moderate-severe BPD comparing both groups (31% vs 26%, $p = 0.65$).

We found no differences in anthropometric measures at discharge from NICU comparing nutritional strategies.

DISCUSSION

Our data demonstrated a high incidence of early hypophosphatemia in PT infants BW ≤ 1250 g and that high AA intake and low P supply were associated risk factors. The global incidence of hypophosphatemia in this study was 66%, while the frequency reported by other authors has a wide range [7, 17, 18]. Differences in the evaluated nutritional strategy, in the PT infants included and differences in the definition of hypophosphatemia could explain this variability.

Table 1. Maternal and neonatal baseline characteristics and median nutritional intake within 10 DOL ($N = 111$).

	Aggressive PN ($N = 58$)	**Standard PN ($N = 53$)**	***p* value**
Baseline characteristics			
GA, wk, mean (SD)	28 (2.2)	28 (2)	0.96[a]
BW, g, mean (SD)	956 (210)	960 (170)	0.92[a]
Female gender, *n* (%)	28 (48)	20 (38)	0.26[b]
SGA, *n* (%)	16 (28)	12 (23)	0.54[b]
Antenatal steroids, *n* (%)	51 (88)	49 (92)	0.42[b]
Prenatal MgSO4, *n* (%)	41 (71)	31 (59)	0.17[b]
Preeclampsia, *n* (%)	18 (31)	16 (30)	0.92[b]
Parenteral intake, median			
Fluid, ml/kg/day, median (IQR)	94 (86–103)	95 (83–106)	0.92**
AA, g/kg/day, median (IQR)	3.4 (3.2–3.6)	3.1 (2.6–3.3)	**<0.0001**[c]
Lipid, g/kg/day, median (IQR)	2.4 (2.1–2.6)	2.1 (1.6–2.4)	**0.001**[c]
Carbohydrate, g/kg/day, median (IQR)	10.3 (9.3–11.5)	9.5 (8.6–10.6)	0.06[c]
Ca, mg/kg/day, median (IQR)	26 (22–30)	27 (22–30)	0.67[c]
P, mg/kg/day, median (IQR)	22 (16–26)	19 (13–28)	0.52[c]
Ca:P Ratio (mol), median (IQR)	0.92 (0.8–1.2)	0.98 (0.78–1.5)	0.38**
Energy, Kcal/kg/day, median (IQR)	72 (65–76)	67 (59–71)	**0.0001**[c]
Enteral intake, median (IQR 25–75)			
Fluid, ml/kg/day	32 (18–44)	22 (12–51)	0.43[c]
Exclusive Human milk, *n* (%)	40 (68%)	37 (71%)	0.80[b]
Onset Trophic feeding, days, median (IQR)	2 (2–3)	3 (2–4)	0.44[c]

Bold values indicate statistical significance $p < 0.05$.
DOL day of life, *GA* gestational age, *BW* birth weight, *SGA* small for gestational age (Fenton < p10), *prenatal* $MgSO_4$ prenatal magnesium sulfate administration, *AA* amino-acid, *Ca* calcium intake, *P* phosphorous intake.
[a]*T*-test.
[b]χ^2.
[c]Mann–Whitney.

Table 2. Maternal and neonatal baseline characteristics and median parenteral intake within 10 DOL according to the development of hypophosphatemia.

	Hypophosphatemia ($N = 73$)	**Normal phosphatemia ($N = 37$)**	***p* value**
GA, wk, mean (SD)	28 (2.2)	28.1 (2)	0.78[a]
BW, g, mean (SD)	940 (193)	986 (184)	0.24[a]
SGA, *n* (%)	20 (27)	8 (22)	0.58[b]
Female gender, *n* (%)	28 (38)	20 (54)	0.07[b]
Prenatal MgSO4, *n* (%)	53 (72)	18 (47)	**0.013**[b]
Preeclampsia, *n* (%)	27 (37)	7 (19)	0.07[b]
AA, g/kg/day, median (IQR)	3.3 (3.1–3.6)	3.1 (2.8–3.3)	**0.0002**[c]
Lipid, g/kg/day, median (IQR	2.3 (2–2.5)	2.1 (1.8–2.5)	0.13[c]
Carbohydrate, g/kg/day, mean (SD)	10.2 (1.7)	9.5 (2)	0.06[a]
Ca, mg/kg/day median (IQR)	26 (22–30)	27 (24–31)	0.33[c]
P, mg/kg/day, median (IQR)	19 (13–26)	24 (16–41)	**0.006**[c]
Ca:P Ratio (mol), median (IQR)	1.04 (0.83–1.5)	0.83 (0.56–1.45)	**0.02**[c]
Energy, Kcal/kg/day, median (IQR)	71 (65–75)	67 (59–73)	**0.04**[c]

Bold values indicate statistical significance $p < 0.05$.
DOL day of life, *GA* gestational age, *BW* birth weight, *SGA* small for gestational age (Fenton < p10), *prenatal* $MgSO_4$ prenatal magnesium sulfate administration, *AA* amino-acid; Ca: calcium intake, *P* phosphorous intake.
[a]*T*-test.
[b]χ^2.
[c]Mann–Whitney.

In PT infants, normal phosphatemia is around 6.5 mg/dl (2 mmol/L). The kidney plays a crucial role in the homeostasis of P and Ca. The minimum phosphatemia in the PT infant below which tubular reabsorption is almost complete and phosphaturia is close to zero is 5 mg/dl. Some authors suggest this threshold should be used to define hypophosphatemia [19].

One of the factors involved in the development of hypophosphatemia is protein supply. After birth, the PT infant begins a catabolic phase and the early onset of PN has been described as a "nutritional emergency" [20]. However, this can trigger hypophosphatemia due to the release of hormones, mainly insulin, similar to a re-feeding syndrome [9, 10, 12, 13, 21]. In a state of anabolism, the consumption of P and K increases and approximately 0.3 mmol (9.3 mg) of P is needed for 1 g of protein retention [9, 10, 17]. Bonsante et al. suggested that, after "placenta feeding disruption", hypophosphatemia could be related to a suboptimal provision of these minerals during the anabolic state generated by the continuous AA supply [9]. This study was designed to evaluate the incidence of hypophosphatemia according to the AA supply. The incidence of this disturbance was higher in the aggressive PN group. However, half of the control group also presented this disturbance. We have to recognize that our control group also received a "high supply" of AA compared to routine PN used years ago [5, 6, 9]. In the present study, patients who developed early hypophosphatemia received significantly more AA in the first 48 h of birth and within the 10 DOL. Also, aggressive PN was independently associated with the development of hypophosphatemia after adjusting for confounders. Several authors have reported similar results [10, 12]. Moltu et al. reported a higher incidence of hypophosphatemia in the group that received high AA supply. This disturbance was correlated with the protein intake of the first week of life, but not with glucose or lipids supplementation [10]. Bonsante et al. reported hypophosphatemia in the group that received AA > 2 g/kg/day and that AA supply in the first DOL was the main determinant of phosphatemia by linear regression model [9].

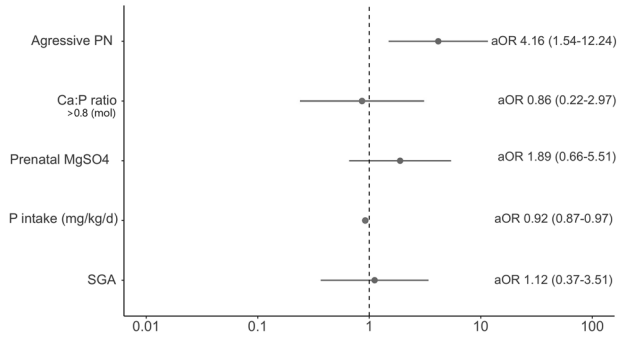

Fig. 2 Multivariate logistic regression model. The figure shows the adjusted odds ratio (aOR) with their 95% confidence interval. The point is the odds ratio (OR) and the line the 95% confidence interval. The vertical dotted line indicates the limit of no effect (OR = 1), that is, the OR of the factors that overlap this line have no effect. To the right of it, are the factors that increase the OR, and to the left, the factors that reduce the OR. Test Hosmer–Lemeshow $p = 0.19$. Ca:P ratio (>0.8 mol), prenatal magnesium sulfate administration, Phosphorus (P) intake, SGA: small for gestational age (Fenton < p10).

Other risk factors associated with hypophosphatemia are the amount and onset of P supply and the Ca:P ratio [9, 15, 19, 22]. Yang et al. reported that PT infants with severe hypophosphatemia began P supplementation significantly later and that the amount of P supply was significantly lower from DOL4 [15]. Conversely, in the present study P supply was initiated early in both groups and the onset of P supplementation was not different when comparing patients with and without hypophosphatemia. In contrast, the amount received within the first week was significantly lower in those who developed this disturbance and in the multivariate regression model P supply was independently associated with hypophosphatemia. Bonsante et al. also demonstrated that P intake is an independent factor of both phosphatemia ($p < 0.001$) and calcemia ($p = 0.03$) [9]. Moe et al. evaluated the impact of an unintended reduction in the P intake and concluded that patients with the lowest phosphatemia received less P and more AA, similar to our results [23]. In contrast, Bustos Lozano et al. found no significant differences in the parenteral P intake in patients with and without hypophosphatemia [14].

Current nutritional guidelines recommend that mineral fetal accretion may be used as a reference of Ca and P provision. They also suggest a lower Ca:P ratio (0.8–1 mol) in PT infants in early PN to reduce the incidence of hypophosphatemia, in contrast to growing stable infants [24, 25]. This is consistent with the results reported by Mulla et al. when comparing two different periods according to the Ca:P ratio. Patients who received a Ca:P Ratio 1.3–1.5:1 developed most frequently hypophosphatemia [26]. In the present study, P supply in both groups was lower than the

Table 3. Morbidities and in-hospital mortality according to the development of hypophosphatemia ($n = 108$).

	Hypophosphatemia ($N = 73$)	**Normal phosphatemia ($N = 37$)**	***p* value**
LOS, *n* (%)	17 (23)	6 (16)	0.38[a]
Vasopressor drugs, *n* (%)	15 (20)	7 (19)	0.84[a]
NEC, *n* (%)	12 (17)	7 (19)	0.76[a]
BPD, *n* (%)	20/64 (31)	8/30 (26)	0.65[a]
PDA, *n* (%)	35 (48)	14 (40)	0.43[a]
Metabolic bone disease, *n* (%)	9 (13)	5 (14)	1[a]
IVH, *n* (%)	14 (19)	7 (19)	0.97[a]
Retinopathy of prematurity, *n* (%)	17 (23)	6 (17)	0.40[a]
Mortality ($n = 109$), *n* (%)	8 (11)	6 (16)	0.45[a]
MV, days ($n = 68$), median (IQR)	8 (3–21,5)	2 (1–13)	**0.04**[b]

Bold values indicate statistical significance $p < 0.05$.
LOS late onset sepsis, *NEC* necrotizing enterocolitis, *BPD* bronchopulmonar dysplasia, *PDA* patent ductus arteriosus, *IVH* intraventricular hemorrhage, *MV* mechanical ventilation.
[a]χ^2 (or Fisher).
[b]Mann–Whitney.

recommended intake, and it was significantly lower in patients with hypophosphatemia. In addition, in these patients Ca:P Ratio was higher than recommended for PT infants in early PN. Concerns of hyperkalemia or hypernatremia due to use of phosphate salts may also contribute to this disturbance. In our study, hyperkalemia was rarely observed. In contrast, one-third of the patients developed hypokalemia, mainly in the hypophosphatemic group. A positive correlation between both has been described [7, 10, 14, 15] In the present study we did not record the type of salt used nor the natremia. However, a recently published study did not report cases of hypernatremia when receiving sodium phosphate from the first DOL [17].

Also, SGA has been associated with hypophosphatemia, mainly in very low birth weight infants [13, 18, 21, 27]. In the present study, SGA patients had lower phosphatemia at baseline determination and on DOL4 when compared with adequate for GA patients, in concordance with Mena et al. [28]. Conversely, we found no difference in the proportion of SGA comparing patients with and without hypophosphatemia and the logistic regression model failed to demonstrate a significant association between SGA and hypophosphatemia. Even so SGA patients had 12% higher odds of this disturbance when compared with patients adequate for GA. Probably the lack of statistical significance is related to the small number of patients with this condition.

P is widely distributed and is critical for many biological processes. Therefore, hypophosphatemia may result in muscle weakness, failure in extubation, more days of VM and development of BPD, increased requirement of vasopressor drugs, impaired leukocyte function with increased risk of LOS and abnormal bone metabolization [10, 12, 13, 15, 18, 21]. Conversely, in the present study, the incidences of BPD and LOS were not different between groups. The lack of differences observed could be related to an early diagnosis and treatment of hypophosphatemia and to the multicausal pathogenesis of both morbidities.

This study has limitations. The design of the study does not allow to establish causality between associated factors and hypophosphatemia. However, four of the Bradford Hill criteria are met: strength of association, consistency, temporality, and biological plausibility. The overlap in AA supply between both groups could be considered a limitation of the study, but the amount of AA received was different on DOL1 and DOL2. Also, the exclusion of many patients due to lack of serum or urinary determinations could be considered a source of selection bias. Excluded patients had lower BW (data not shown) so we consider that the results of the present study would not have been modified. P measurements only in three intervals, not data regarding maternal or neonatal vitamin D status and lower calcium and phosphorus intake could also be considered limitations of this study.

The strengths of this study include an exhaustive recruitment of the daily supply of both, enteral and PN. Also, the participating Centers were from different locations and have diverse nutritional protocols, so the results of this study may be generalizable.

We conclude that early hypophosphatemia is a common disturbance in infants BW ≤ 1250 g. A high initial intake of AA along with an insufficient intake of P interacts in a susceptible host triggering this disturbance. It is important to agree on the definition of hypophosphatemia, determine the appropriate time to assess it and discuss its treatment to avoid potential adverse effects.

REFERENCES

1. Van Den Akker CH, Vlaardingerbroek H, Van Goudoever JB. Nutritional support for extremely low-birth weight infants: abandoning catabolism in the neonatal intensive care unit. Curr Opin Clin Nutr Metab Care. 2010;13:327–35.
2. Thureen PJ. Early aggressive nutrition in the neonate. Pediatr Rev. 2007;20: 45e–55.
3. Senterre T, Rigo J. Reduction in postnatal cumulative nutritional deficit and improvement of growth in extremely preterm infants. Acta Paediatr. 2012;101: 64–70.
4. Dinerstein A, Nieto RM, Solana CL, Perez GP, Otheguy LE, Larguia AM. Early and aggressive nutritional strategy (parenteral and enteral) decreases postnatal growth failure in very low birth weight infants. J Perinatol. 2006;26:436–42.
5. Ehrenkranz RA. Early, aggressive nutritional management for very low birth weight infants: what is the evidence? Semin Perinatol. 2007;31:48–55.
6. Hay WW Jr. Aggressive nutrition of the preterm infant. Curr Pediatr Rep. 2013;1:1–17.
7. Senterre T, Abu Zahirah I, Pieltain C, de Halleux V, Rigo J. Electrolyte and mineral homeostasis after optimizing early macronutrient intakes in VLBW infants on parenteral nutrition. J Pediatr Gastroenterol Nutr. 2015;61:491–8.
8. Ehrenkranz RA. Early nutritional support and outcomes in ELBW infants. Early Hum Dev. 2010;86:21–5.
9. Bonsante F, Iacobelli S, Latorre G, Rigo J, de Felice C, Robillard PY, et al. Initial amino acid intake influences phosphorus and calcium homeostasis in preterm infants - it is time to change the composition of the early parenteral nutrition. PLoS ONE. 2013;8:1–9.
10. Moltu SJ, Strømmen K, Blakstad EW, Almaas AN, Westerberg AC, Brække K, et al. Enhanced feeding in very-low-birth-weight infants may cause electrolyte disturbances and septicemia–a randomized, controlled trial. Clin Nutr. 2013;32: 207–12.
11. Craddock PR, Yawata Y, VanSanten L, Gilberstadt S, Silvis S, Jacob HS. Acquired phagocyte dysfunction. A complication of the hypophosphatemia of parenteral hyperalimentation. N Engl J Med. 1974;290:1403–7.
12. Brener Dik PH, Galletti MF, Fernández Jonusas SA, Alonso G, Mariani GL, Fustiñana CA. Early hypophosphatemia in preterm infants receiving aggressive parenteral nutrition. J Perinatol. 2015;35:712–5.
13. Ross JR, Finch C, Ebeling M, Taylor SN. Refeeding syndrome in very-low-birth-weight intrauterine growth-restricted neonates. J Perinatol. 2013;33:717–20.
14. Bustos Lozano G, Hidalgo Romero Á, Melgar Bonis A, Ureta Velasco N, Orbea Gallardo C, Pallás, et al. Early hypophosphataemia in at risk newborns. Frequency Magnit Pediatr. 2018;88:216–22.
15. Yang SM, Noh OK, Lee JH, Park MS. Late and insufficient phosphorus supplementation is associated with early severe hypophosphatemia in extremely low birth weight infants with early amino acid administration. Perinatology. 2018;29:13.
16. Trivedi A, Sinn John KH. Early versus late administration of amino acids in preterm infants receiving parenteral nutrition. Cochrane Database Syst Rev. 2013. http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD008771.pub2/abstract.
17. Bustos Lozano G, Soriano-Ramos M, Pinilla Martín MT, Chumillas Calzada S, García Soria CE, Pallás-Alonso CR. Early hypophosphatemia in high-risk preterm infants: efficacy and safety of sodium glycerophosphate from first day on parenteral nutrition. J Parenter Enter Nutr. 2019;43:419–25.
18. Pająk A, Królak-Olejnik B, Szafrańska A. Early hypophosphatemia in very low birth weight preterm infants. Adv Clin Exp Med. 2018;27:841–7.
19. Senterre J, Salle B. Renal aspects of calcium and phosphorus metabolism in preterm infants. Biol Neonate. 1988;53:220–9.
20. Torrazza RM, Neu J. Evidence-based guidelines for optimization of nutrition for the very low birthweight infant. Neoreviews. 2013;14:e340–9.
21. Ichikawa G, Watabe Y, Suzumura H, Sairenchi T, Muto T, Arisaka O. Hypophosphatemia in small for gestational age extremely low birth weight infants receiving parenteral nutrition in the first week after birth. J Pediatr Endocrinol Metab. 2012;25:317–21.
22. Vileisis RA. Effect of phosphorous intake in total parenteral nutrition infusates in premature neonates. J Pediatr. 1987;110:586–90.
23. Moe K, Beck-Nielsen SS, Lando A, Greisen G, Zachariassen G. Administering different levels of parenteral phosphate and amino acids did not influence growth in extremely preterm infants. Acta Paediatr. 2015;104:894–9.
24. Mihatsch W, Fewtrell M, Goulet O, Molgaard C, Picaud JC, Senterre T, et al. ESPGHAN/ESPEN/ESPR/CSPEN guidelines on pediatric parenteral nutrition: calcium, phosphorus and magnesium. Clin Nutr. 2018;37:2360–5.
25. Johnson PJ. Review of micronutrients in parenteral nutrition for the NICU population. Neonatal Netw. 2014;33:155–61.
26. Mulla S, Stirling S, Cowey S, Close R, Pullan S, Howe R, et al. Severe hypercalcaemia and hypophosphataemia with an optimised preterm parenteral nutrition formulation in two epochs of differing phosphate supplementation. Arch Dis Child Fetal Neonatal Ed. 2017;102:F451–5.
27. Boubred F, Herlenius E, Bartocci M, Jonsson B, Vanpée M. Extremely preterm infants who are small for gestational age have a high risk of early hypophosphatemia and hypokalemia. Acta Paediatr. 2015;104:1077–83.

28. Mena Nannig P, Cubillos Celis MP, Toro Jara C, Zuñiga Vergara C. Prematuro Extremo - Perfil Bioquímico en Sangre Cordón y Crecimiento Fetal. Rev Chil Pediatr. 2016;87:250–4.

AUTHOR CONTRIBUTIONS

MFG was responsible for designing and writing the protocol, conducting the search, extracting, analyzing data, interpreting results, drafted the initial manuscript, and approved the final manuscript as submitted. PHBD contributed to the statistical analysis of data, revised the manuscript, and approved the final manuscript as submitted. SFJA aided in the design of the study, reviewed, and revised the manuscript, and approved the final manuscript as submitted. DS, CC, SP, MB, GL, and IA coordinated the study in their Institutions and contributed to the acquisition of patient's data. They also revised the manuscript and approved the final manuscript as submitted. GLM mentored MFG in study conception, critically reviewed the manuscript, and approved the final manuscript as submitted.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41372-022-01361-1.

Correspondence and requests for materials should be addressed to Maria Fernanda Galletti.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.