2017

Prevalence, Risk Factors and Short Term Outcomes of Hypophosphatemia among Very Low Birth Weight Infants: An Exploratory Pilot Study

Al-Wassia, Haydi

UNIVERSITY OF CALGARY

Prevalence, Risk Factors and Short Term Outcomes of Hypophosphatemia among Very Low Birth Weight Infants: An Exploratory Pilot Study

by

Haydi Al-Wassia

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

GRADUATE PROGRAM IN COMMUNITY HEALTH SCIENCES

CALGARY, ALBERTA

JANUARY, 2017

Abstract

Background: Health care professionals face challenges in improving survival rates among very low birth infants who are at risk of hypophosphatemia. We aimed to identify the prevalence and predictive factors of hypophosphatemia.

Methods: Prospective, exploratory pilot study of 106 infants admitted to intensive care unit at Foothills Hospital, Calgary between October 1, 2011 and June 1, 2012.

Results: The prevalence of hypophosphatemia was 77%. Hypophosphatemic infants had significantly lower birth weight ($p< 0.001$) and gestational age ($p< 0.001$). Furthermore, respiratory distress syndrome (RDS) ($p = 0.002$), intraventricular hemorrhage (IVH) $\geq$ grade III ($p =0.020$), and hyperglycemia ($p = 0.013$) were more frequent among hypophosphatemic infants. Birth weight modified the association between RDS, IVH, hyperglycemia and hypophosphatemia.

Conclusion: Hypophosphatemia is common in premature infants. Further research should determine whether premature birth and the associated metabolic consequences as well as the severity of the accompanying comorbidities increase the risk of hypophosphatemia.

Acknowledgements

Foremost, I would like to express my deepest gratitude to my supervisor Dr. Tanis Fenton for the continuous support of my master's study and research, for her patience, motivation, care, enthusiasm and belief in me. Her guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better supervisor and mentor for my master's study.

Besides my supervisor, I would like to thank the rest of my thesis committee: Dr. Reg Sauve, Dr. Andrew Lyon and Dr. Sarah Rose for their encouragement, insightful comments, and enlightening questions.

We would like to acknowledge the support provided by Alberta children Hospital Research Institute Small Grant and the A.S.P.E.N Rhoads Research Foundation.

Last but not least, I would like to thank my loving and caring family. Thanks to my husband Shadi without whose love, encouragement, understanding and putting up with me through the toughest moments of my life, I would not have finished this thesis. Words cannot express my appreciation and love for my children Lina, Yazan, Maya, and Kindah, who are the pride and joy of my life. Despite their young age, they managed to give mommy full support, love, and understanding and tolerated mommy working on her computer days, nights, weekends and holidays. I thank my husband and kids for their patience, and I love them more than they will ever know.

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Adenosine triphosphate (ATP)

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Birth weight (BW)

Cesarean section (CS)

Generalized estimating equation (GEE)

Gestational age (GA)

Hypophosphatemia (HP)

Intraventricular hemorrhage (IVH)

Neonatal intensive care unit (NICU)

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Respiratory distress syndrome (RDS)

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Small for gestational age (SGA)

Triglycerides (TG)

Very low birth weight (VLBW)

Chapter 1: Review of the Literature

1.1 Background

Clinical and technological advances in perinatal and neonatal intensive care have improved outcomes for premature infants of gestational ages (GA) as low as 23 weeks.[1,2] Despite these improvements, reported neonatal mortality rates for very low birth weight (VLBW) infants with birth weights (BW) of 1000 to < 1500 g and those with BW < 1000 g are, respectively, about 11-fold and >100-fold that of overall neonatal mortality.[3] According to the 2010 USA National Statistic Report, although all VLBW infants constituted only about 1.4% of all live births, they accounted for approximately one-half of all infant deaths.[3] In addition to high mortality rates, the expanding limits of premature infant viability are also frequently associated with high prevalence rates of serious short-term complications and long-term morbidity.[4-6] Early clinical management challenges for the neonatal intensive care unit (NICU) health care team include the risks of electrolyte imbalances and acute metabolic disturbances, especially during the first few days of life.[7] Anticipation, early recognition, and timely intervention in these physiologically immature neonates are important aspects of continuing efforts to better identify and address the vulnerabilities of these high-risk newborns.[7-9] Among the electrolyte perturbations that are encountered, hypophosphatemia (HP) is a particularly under-recognized and under-studied disorder in the most premature of infants.[10] Early and sometimes profound HP may occur during the first week or two after birth.[11] However, reported reference ranges vary, and the data that describe HP prevalence, risk factors, and clinical consequences in VLBW infants are scant, despite recognition that HP may result in serious consequences in both children and adults.[11-15] These consequences include disturbances of musculoskeletal, cardiovascular, respiratory,

neurologic, and hematologic function.[15-21] Depletion of intracellular phosphate (PO_4) also results in widespread derangements of carbohydrate, lipid, and protein metabolism.[22,23]

Despite the potential consequences of HP, serum PO_4 (sPO_4) is neither routinely checked nor frequently monitored in VLBW infants during the early days after birth. Hence, the incidence and burden of early HP in this population are unknown.

1.2 Pathophysiology and Clinical Consequences of Hypophosphatemia

1.2.1 Phosphate Homeostasis

Phosphate is the major intracellular anion with the vast majority (85 to 90%) located in the mineral portion of the human skeleton. Most of the remaining PO_4 (10 to 15%) is predominately free unbound in soft tissues, mainly in the form of PO_4 esters and in extracellular fluid in the form of inorganic PO_4 ions.[23] In the intestine, the majority of PO_4 (80-90%) is absorbed in the jejunum. Intestinal absorption of PO_4 is dependent on the amount of PO_4 intake and the relative concentration of calcium and PO_4, since higher concentrations of calcium result in decreased absorption of PO_4 and vice versa. Renal PO_4 excretion plays a central role in PO_4 balance. In neonates, glomerular filtration is decreased and hence, renal excretion of PO_4 is normally low.[24] Conversely, preterm infants have an increased fractional excretion of PO_4, which puts them at a greater risk of HP.[25] Vitamin D is an important hormonal regulator of plasma PO_4.Vitamin D directly stimulates intestinal absorption and bone resorption and indirectly enhances PO_4 tubular reabsorption through increasing serum calcium that suppresses parathyroid hormone (PTH) secretion.[26] More recently, fibroblast growth factor 23 (FGF23), a PO_4 regulatory hormone produced by the bone was identified to play a role in PO_4 homeostasis by suppressing the activity

of vitamin D and consequently decreasing intestinal PO_4 absorption and renal PO_4 reabsorption resulting in decreasing sPO_4.[27]

Phosphate is an essential structural component in bones and teeth in the form of hydroxyapatite and it also plays several other vital roles in biological systems. As adenine triphosphate (ATP), PO_4 is involved in the principal biological form of energy storage, and therefore is used in sustaining processes such as nerve conduction, muscle contraction, and electrolyte transport.[28] As a component of phospholipids and 2,3-diphosphoglycerate (2,3-DPG), PO_4 holds central roles in cell membrane integrity and peripheral oxygen delivery.[29] A myriad of metabolic regulatory processes are mediated via protein phosphorylation and de-phosphorylation, including carbohydrate, protein, and fat metabolism.[23] Furthermore, PO_4 is a component of cyclic adenosine monophosphate and cyclic guanosine monophosphate, which are important intracellular messengers that mediate the intracellular effects of different hormones, such as PTH, antidiuretic hormone, and epinephrine.[28,30] In addition, the sPO_4 ion plays an important role in maintaining acid-base balance, through its buffering ability. It also has a role in the immune system and coagulation cascade.[28,30]

1.2.2 Requirement of Phosphate in Preterm Infants

Phosphate requirements in preterm infants are estimated based on demands for matching intrauterine bone mineral accretion rates [31] and on maintenance of normal sPO_4 concentration in breast-fed growing premature infants.[32,33] International guidelines for recommended optimal supplementation of PO_4 vary widely. The American Academy of Pediatrics recommends 4.1–4.6 mmol/kg/day enteral intake of PO_4 and 1.3–2.2 mmol/kg/day of parenteral intake,[34] whereas the European Society of Pediatric Gastroenterology, Hepatology and Nutrition recommends an enteral intake of 2–3 mmol/kg/day [35] and a wider range of parenteral intake of 0.75 to 3 mmol/kg/day. [36]

1.2.3 Definition of Hypophosphatemia

Blood samples from normal human fetuses between 20–26 weeks GA revealed higher PO_4 concentration compared to their mothers (fetuses: 2.6 ± 0.1 mmol/L, mothers 1.45 ± 0.1 mmol/L).[37] The reference range for cord blood serum concentration of PO_4 in preterm infants less than 36 weeks GA were 1.5 to 2.6 mmol/L.[11,37-39] The normal sPO_4 reference range in term neonates is between 2.1 and 2.5 mmol/L.[38,40] In preterm infants (25- 36 weeks GA) who were appropriate for gestational age, mean ± standard deviation (SD) sPO_4 levels on day 1, 3, and 5 were 1.95 ± 0.41, 1.96 ± 0.4 and 1.74 ± 0.42, respectively.[41] The proposed grading of HP in neonates regardless of GA uses sPO_4 <1.5 mmol/L, <1 mmol/L and <0.4 mmol/L to define mild, moderate and severe HP, respectively.[42]

1.2.4 Prevalence of Hypophosphatemia

Despite variation in study designs, study populations, and definitions of HP, the available literature indicates a common occurrence of HP in critically ill patients. Hypophosphatemia is one of the frequently encountered electrolyte disorders in adult intensive care units, with a reported prevalence ranging from 20 to 40%,[43] reaching 80% in sepsis cases.[44] Similarly, studies involving the pediatric age group have reported a high prevalence of HP in children admitted to the Pediatric Intensive Care Unit (PICU). de Menezes et al. identified HP in 76% of children admitted to the PICU over a one-year period.[45] In a prospective study of HP among 82 children in PICU, the prevalence of HP was 61% during the first 10 days of hospital stay.[20] More recently, Kilic et al. reported occurrence of HP in 60% of children on admission to the PICU or on the third or seventh day after admission.[19] In a group of 34 neonates with GA ranging from 30–39 weeks, the overall incidence of HP (< 2 mmol/L) was > 80% during the first three days of life.[10] In a recent retrospective cohort study of 61

infants with birth weights below 1250 g, the prevalence of HP was 91% in the first two weeks of life.[46]

1.2.5 Risk Factors of Hypophosphatemia in Premature Infants

The source of PO_4 in the developing fetus is through active transport across the placenta.[47] During the last trimester of pregnancy, there is a threefold increase in fetal PO_4 uptake, which peaks at 34 weeks GA.[48] It follows that the shorter the gestation, the greater the truncation of accelerated PO_4 accretion and the resultant risk of HP.[40] The regulatory mechanism of calcium and PO_4 hemostasis in VLBW is complex and is influenced by a myriad of factors, most of which are direct results of their premature birth. Premature infants are more susceptible to develop HP due to low mineral stores, insufficient intake, and the functional immaturity of multiple organ systems.[10] Under a normal state, approximately 90% of PO_4 is absorbed in the small intestine and a similar percentage is reabsorbed in the proximal tubules of the kidney.[49] The premature birth of the neonate results in decreased both intestinal absorption and increased renal tubular losses of PO_4, a process that is amplified by the intracellular redistribution due to acid-base imbalance.[49] Metabolic acidosis, which is not an infrequent complication of preterm birth, can result in increased calcium and PO_4 renal excretion, contributing to the high prevalence of HP in this population.[50]

Reported risk factors of HP among hospitalized patients include sepsis,[51] respiratory complications,[52] malnutrition,[53,54] and drugs such as diuretics and inotropes [54,55] In a study of pediatric patients admitted to an intensive care unit, the diagnosis of respiratory disease, use of dopamine, and malnutrition were shown to be independently associated with HP.[20] Compared to healthy preterm infants (BW 2008 ± 261g), preterm infants with perinatal complications—respiratory distress syndrome (RDS), sepsis, hypoglycemia, and meningitis (BW 2160 ± 432 g)—

had lower plasma and erythrocytes PO_4 in the first month of life, despite comparable PO_4 intake for both groups.[56] More recently, Brener Dik et al. found that preterm infants < 1250 g with severe HP (< 0.64 mmol/dL) had more intrauterine growth retardation, sepsis and received more vasoactive drugs and mechanical ventilation without reaching statistical difference.[46] Although these conditions are prevalent among VLBW infants [57], their association with HP is not well understood. Additional potential risk factors specific to preterm infants, such as GA, gender, BW, and intraventricular hemorrhage (IVH) comprise an important area to explore to better understand and quantify the magnitude of HP in this vulnerable population.

1.2.6 Consequences of Hypophosphatemia

Phosphate plays a critical role in several biological processes and a multitude of metabolic functions, including energy metabolism, membrane composition, nucleotide structure, cellular signaling and bone mineralization. Therefore, evidence of its depletion in critically ill patients may manifest itself as impairment of almost all body organ systems, most importantly, the respiratory, cardiovascular, neuromuscular and hematological systems.[16,53] Adequate tissue oxygenation and energy generation for normal muscle function require sufficient amount of ATP and 2,3-DPG. Hypophosphatemia hampers the formation of 2,3-DPG and results in impaired production of ATP, disturbances that result in preventing the release of oxygen from hemoglobin and impairing muscular contraction respectively, and hence result in tissue hypoxia and neuromuscular dysfunction.[58,59] The resultant clinical consequences of severe HP may manifest as generalized muscle weakness, reduced diaphragmatic contractility, and poor ventricular function,[16] and consequently poor tissue oxygenation and a failure to wean from mechanical ventilation.[18] In 118 pediatric patients admitted to PICU, a statistically significant association

was found between HP and the duration of mechanical ventilation and length of hospital stay.[19] Further, severe HP in the adult population has been associated with myocardial dysfunction, arrhythmia and hypotension.[60-62] Other manifestations of severe HP reported in studies involving the adult population include rhadomyolysis, peripheral neuropathy, convulsions, and coma.[63,64] Erythrocytes, leukocytes and platelets require PO_4 for their normal structure and function and therefore HP may also result in hematologic disturbances that include hemolytic anemia [17,65,66] and leukocyte and platelet dysfunction.[21] Adequate amounts of ATP are required to maintain erythrocyte membrane integrity and deformability.[67] A reduction in intracellular ATP levels as a result of HP was suggested to increase erythrocyte rigidity predisposing to hemolysis in observational studies of severely HP adult and pediatric subjects.[17,65,66] Equally important, ATP is required for leukocytes phagocytosis and chemotaxis and it is not surprising that severe HP independently predicted mortality in adult patients with sepsis.[51] Moreover, Craddock et al. documented a marked decrease in chemotactic and phagocytic function in animals subjected to HP.[21]

Several studies reported high prevalence of HP in patients with sepsis.[44,68] In a case-control study of 324 adult patients treated for infectious diseases, the C-reactive protein and the number of white blood cells were higher in cases with PO_4 level below or equal to 0.6 mmol/L compared to those with higher levels.[43] It remains unclear whether PO_4 deficiency predisposed to sepsis or whether an infection state led to a drop of serum PO_4. Furthermore, several studies have examined the association between HP and mortality in diverse patient populations. In adults, HP has been associated with mortality in surgical intensive care patients and in patients with re-feeding syndrome.[69] In malnourished children, a reported death rate of 63% was observed in the presence of severe HP.[70] It is still indefinite whether HP essentially contributes to mortality, or is

purely a marker for illness severity.

1.2.7 Hypophosphatemia and Glycemic Control

Phosphate plays a central role in the intermediate metabolism of carbohydrate and its deficiency has been associated with changes in insulin sensitivity and glucose tolerance.[71,72] Impaired glucose utilization has been reported in HP animals.[73] Moreover, inorganic PO_4 has been shown to enhance erythrocyte glucose utilization when intact washed erythrocytes were mixed with excess glucose.[74] Furthermore, compared to healthy controls, adult subjects with HP tolerated less glucose and were insulin resistant.[72] Similarly, low sPO_4 was associated with high postprandial glucose and impaired insulin sensitivity in healthy subjects independent of age, gender and percent body fat.[71] Moreover, PO_4 supplementation in glucose intolerant adult patients with HP improved glucose intolerance considerably.[43]

The mechanism by which HP impairs glucose metabolism can be attributed to intracellular ATP depletion that can result in disturbances of energy production for various metabolic processes, including intracellular glucose phosphorylation and uptake by peripheral muscles and cell membranes.[44] The aforementioned disturbances can explain the glucose intolerance and abnormal insulin response seen in patients in hypophosphatemic state. In a clinical trial by Khattab et al., seven healthy male subjects randomly received oral glucose with placebo or 500 mg of PO_4. Co-consumption of PO_4 improved postprandial glucose levels and insulin sensitivity index.[44] On the other hand, there have been reports that associated HP with augmented glucose uptake and consequently resulting in hypoglycemia as a result of intracellular alkalosis and altered sodium: potassium ratio.[73,75] Hence, one can examine whether HP is associated with the occurrence of hypoglycemia, a more common problem among preterm infants, previously thought due to limited glycogen and fat stores as a result of their premature birth. Furthermore,

hypoglycemia is of concern since it has been shown to be associated with adverse neurodevelopmental outcomes.[76]

In summary, PO_4 plays a central role in numerous cellular functions and is a vital constituent of essential intracellular metabolites that are required for all body tissues. The clinical consequences of HP are principally due to the impaired cellular energy stores and tissue hypoxia as a result of ATP depletion and decreased 2,3-DPG respectively. Therefore, PO_4 deficiency, not surprisingly, can be associated with diverse and clinically important consequences affecting the function of all body systems.

Chapter 2: Methods

2.1 Purpose

The purpose of our exploratory pilot study was to provide preliminary estimates of the prevalence of HP in the first seven days after birth among VLBW infants, which would guide the sample size calculations for a future full study. We also planned to explore factors that may be predictive of HP and whether HP was associated with common neonatal outcomes.

2.2 Study Objectives

1) To determine the prevalence of HP among VLBW infants admitted to the NICU at Foothills Hospital, Calgary, Alberta between October 1, 2011 and June 1, 2012.
2) To examine possible predictive factors associated with HP in the first seven days of life among VLBW infants admitted to the NICU at Foothills Hospital, Calgary, Alberta between October 1, 2011 and June 1, 2012. In particular, we wished to examine the association between the occurrence of HP in the first seven days of life among VLBW infants and the following factors: BW, GA, sex, weight for gestational age, RDS, the administration of inotropic medications (dopamine, dobutamine, or epinephrine), sepsis, and IVH. We also planned to examine the association between aspects of nutritional intake in the first seven days of life and HP among VLBW infants, including PO_4 (mmol/kg/day), calcium (mmol/kg/day), amino acid (g/kg/day), lipids (g/kg/day), glucose (g/kg/day), and total caloric intake (Kcal/kg/day).
3) To describe the short-term outcomes, including seizures, glycemic control (hypo- and hyperglycemia), arrhythmias, need for transfusion, and mortality in infants with and without HP in the first seven days of life.

2.3 Study Design

This investigation was a prospective, observational exploratory pilot study of the prevalence and associated factors of HP during the first seven days of life among VLBW infants admitted to the NICU at the Foothills Hospital. This unit is a Level III NICU and a regional referral center located in Calgary, Alberta. The University of Calgary Conjoint Research Ethics Board reviewed and approved the study (ID: E-24134).

2.4 Operational Definition of Variables

- Hypophosphatemia was defined as one sPO_4 level less than 1.5 mmol/L in the first seven days of life.[11,77]
- Gestational age was confirmed by the best obstetric estimate using the first day of the last menstrual period and early first trimester ultrasound.[78]
- Weight for gestational age (small for gestational age) was defined as birth weight less than the 10^{th} percentile using the birth-weight charts developed by Fenton et al.[79]
- Respiratory distress syndrome was defined by the clinical signs of the three components: respiratory distress (tachypnea, grunting, and retractions), oxygen requirement, and findings on chest X-ray.[8]
- Sepsis was defined as a positive blood culture in the first seven days after birth.[80]
- Intraventricular hemorrhage was graded according to Papile's classification.[81]
- Hyperglycemia referred to blood glucose >8.3 mmol/L (> 150 mg/dL)[82,83]
- Hypoglycemia was defined as blood glucose < 2.6 mmol/L (< 47 mg/dL) [84]

2.5 Study Population and Setting

All infants with a BW of < 1500 g who were admitted to the Foothills Hospital NICU between October 1, 2011 and June 1, 2012 were eligible for this study. We included both infants of twin births in the study. Study inclusion required a sPO_4 measurement at least once during the first seven days after birth. This study automatically performed laboratory analysis on phlebotomy samples from VLBW infants that would otherwise have been discarded. A mini-lab information system of the Calgary Lab Services Chemistry Lab (IT300) was programmed to direct Roche chemistry analyzers to analyze sPO_4 whenever a serum triglyceride (TG) was measured. Serum TGs were routinely ordered almost daily for VLBW infants as intravenous lipid administration was monitored, allowing for almost daily sPO_4 measures without additional blood draws. There were no exclusion criteria.

Infants whose sPO_4 measurements were all $\geq$ 1.5 mmol/L were considered the normophosphatemic (NP) group. A trained member of the research team prospectively collected clinical and laboratory data from the written and electronic records of both the VLBW infants and their mothers. No parental consent was required as these laboratory tests, done exclusively under the research protocol, only utilized plasma samples that remained following completion of the patients' clinical laboratory studies. The University of Calgary Conjoint Research Ethics Board approved a waiver of consent for this study, to avoid selection bias by incomplete consents.

2.6 Baseline Maternal and Infant Variables

Baseline maternal characteristics in the first seven days of life in infants with HP and their normophosphatemic counterparts included maternal age, any diagnosis of diabetes or

hypertension, the use of antenatal corticosteroids, the presence or absence of chorioamnionitis, and whether delivery was achieved by Cesarean section (CS). Infants' baseline characteristics included GA, sex, BW, weight for GA and Apgar scores recorded at one and five minutes, IVH, RDS, sepsis, and administration of inotropic medications. The intake of fluid, energy, calcium, PO_4, lipid, carbohydrate, and amino acids were recorded daily and calculated. The use of PN or enteral feedings was as specified by the NICU clinical care team, and was not altered by these research results or the research team. The study dataset also included all daily sPO_4, ionized calcium, blood glucose and TG levels, as well as blood pH and creatinine test results during the first week of life.

2.7 Nutritional and Mineral Intake

In our NICU, when premature infants with a BW < 1500 g were judged to be suitably stable, our guidelines included the initiation of trophic feeds. In a physiologically stable infant, our protocol was to initiate trophic feeds within the first 24–48 hours of life at a rate of 5 mL/kg/day for a BW < 600 g, 10 mL/kg/day for a BW 600–1000 g, and 15–20 mL/kg/day for a BW > 1000 g. Expressed breast milk was preferred, although in situations where human milk was not available or was contraindicated, preterm formula or extensively hydrolyzed formula was used.

The majority of the nutritional and mineral requirements were initially provided by PN. Parenteral nutrition starter solutions began in the first few hours after birth for all infants < 1500 g. Amino acids were infused as a 3%–4% solution and the quantity per kilogram was increased stepwise to deliver 3–3.6 g/kg/day. Dextrose was increased in 1–2 mg/kg/day increments up to 6–8 mg/kg/day. Moreover, 20% lipid was started at 1 g/kg/day at 12–24 hours of age, and was increased as tolerated up to 3–4 g/kg/day. The usual PN starter solutions contained calcium

gluconate to provide 1.5–2 mmol/kg/day, which was usually delivered at 80 mL/kg/day and gradually increased as fluid orders permitted. Sodium was not generally provided during the first few days of life. Phosphate was also not typically added to PN solutions until day 2 or 3, since infusions of PO_4 preparations require delivery of sodium and potassium, which are minimized in the first days of life. Target PO_4 intakes were 1.5–2 mmol/kg/day. Once trophic feeds were tolerated, enteral feeds were increased by 10–20 mL/kg/day.

2.8 Statistical Analyses

StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP was used for the statistical analysis.

(1) Prevalence: The estimated prevalence of HP was the ratio of HP subjects to the combined total of the HP and NP groups expressed as a percentage with the estimated 95% confidence interval (CI) using the exact binomial proportions.

(2) Description of the baseline characteristics and examination of the difference between baseline variables: Continuous data are expressed as means ± SD or medians (interquartile range), depending on the data distribution, and categorical data are reported as proportions. Differences in the clinical and demographic characteristics between the HP and NP groups were examined by univariate analysis with Fisher's Exact Test for categorical data, *t*-test for continuous normally distributed data, and Wilcoxon rank-sum calculations when the assumption of normality was violated. Normality of data distribution was assessed graphically and by using the Shapiro-Wilk test. All p-values were two-sided and deemed significant if $p < 0.05$.

(3) Description of short-term outcomes: We collected data about possible adverse outcomes of HP, including seizures, arrhythmias, and the number of blood transfusions (as a surrogate for

hemolytic anemia) and mortality in the first seven days of life. Differences between the two groups were not tested statistically due to the extremely small numbers of these outcomes.

(4) The comparison of the nutritional data in the first seven days of life in infants with and without HP, using a generalized estimating equation (GEE) model: For serum PO_4, pH, and TG values and each of the nutritional intake variables measured across time (amino acid, lipid, glucose, calcium, PO_4, and total calories), we used a multiple linear regression model with GEE with autoregressive errors to account for the intra subject correlation between measurements on the same subject. For PO_4 and TG levels, there were insufficient data points to use an autoregressive error and so we used an exchangeable correlation. In each case, the variable was initially included as a quadratic variable to take into account potential non-linearity. Initially, an interaction between the two groups (HP and NP) and the variable was examined. If this interaction was significant at $p < 0.05$, it was retained in the model, and if not, the model was re-estimated with the group main effect and the quadratic variable. In this case, the significance of the group variable (HP and NP) was examined. The results of the regression models were illustrated using the marginal means with 95% CI.

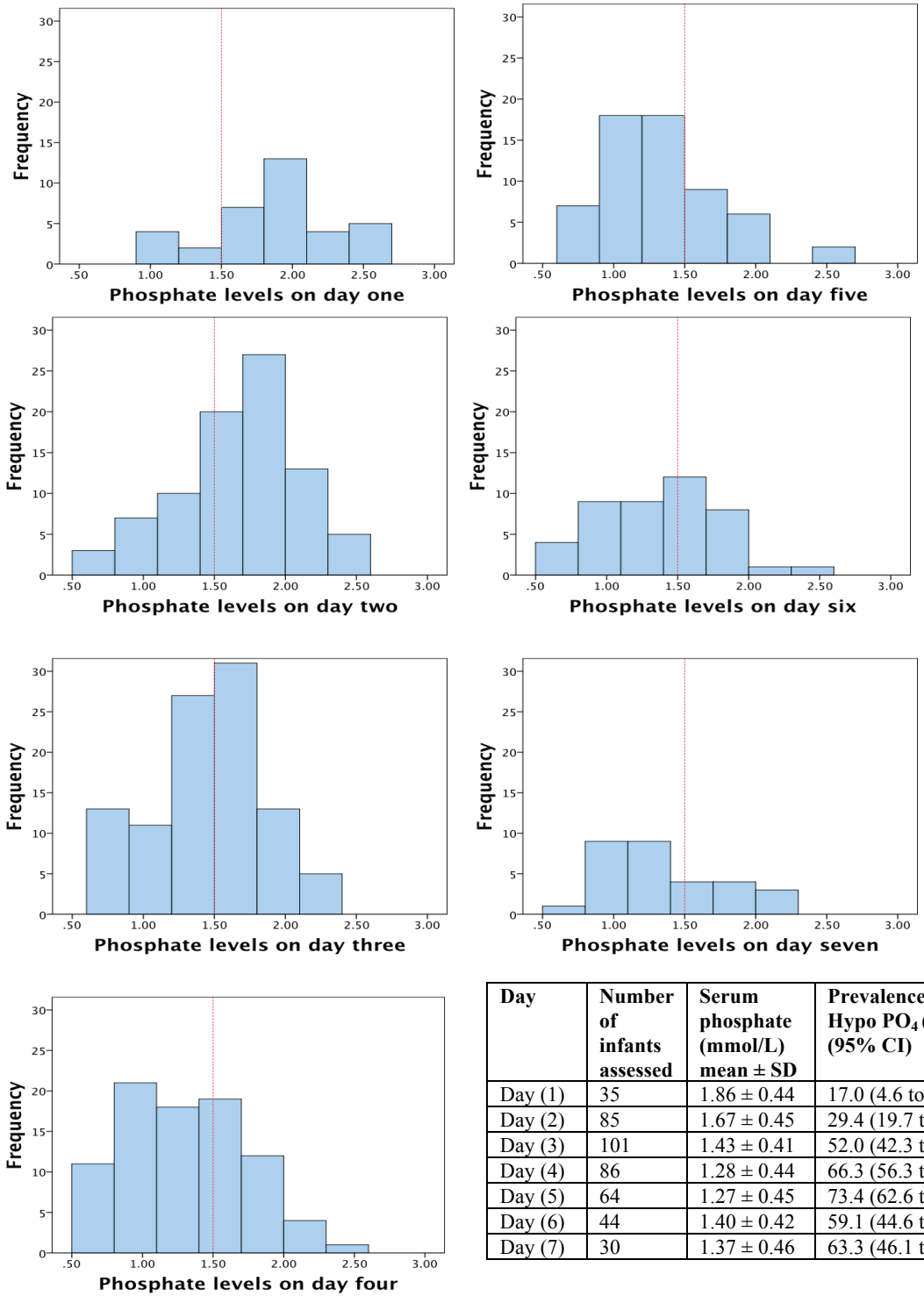
(5) Identification of risk factors or co-incidental morbidities: Any variable with a p value of less than 0.1 in the univariate analysis was considered to be a potential risk factor. Because of the small sample size and the small number of events relative to the number of predictors that we wanted to consider, logistic regression could not be used as it can result in overfitted risk model and produce unreliable results and inaccurate prediction. Moreover, logistic regression was not attempted as odds ratios could not be calculated when no events were observed in any cell. To overcome these statistical challenges, we used stratified analysis to be able to examine the associations between HP and potential risk factors and calculated the risk difference as the effect

measure of choice in our study. The risk difference was calculated by subtracting the risk of HP in infants without the risk factor of interest from the risk of HP in infants with the risk factor of interest. The risk difference was reported along with its 95% CI. The 95% CI of the risk difference was calculated according the methods described by Rothman. [85] The p-value provided is for the chi-square test for the two by two table. As there was a significant correlation between BW and GA ($r = 0.7$, $p < 0.001$), we used the median BW (since nutritional stores are proportional to BW) to stratify our sample to examine for possible interaction or confounding. If the magnitude of risk difference across strata of BW was determined to be different clinically, we kept the results stratified. If the stratum-specific measures of association were similar, but there was a difference between the crude and adjusted estimates by 10% or more, the Mantel-Haenszel method was used to calculate a pooled estimate and p-value.

Chapter 3: Results

The study cohort comprised 106 premature infants with a BW < 1,500 g. During the first week of life, 24 (22%, 95% CI 16%, 32%) infants had only normal sPO_4 levels and 82 (77%, 95% CI 68%, 84%) infants met the study criteria for HP by having at least one measurement < 1.5 mmol/L.[11] The distributions of PO_4 levels in the first seven days of life are shown in Figure 3.1. Greater than half of the infants had HP on days 3 through 7. The highest prevalence of HP was on day four and five of age. The cumulative occurrence of HP is depicted in Figure 3.2 and Table 3.1.

There were no statistically significant differences in maternal characteristics between the NP and HP groups (Table 3.2). The HP infants had significantly lower BW (HP: 950g ± 248, NP: 1217g ± 250; $p< 0.001$) and GA (HP: 27.4 weeks ± 2.00, NP: 29.0 weeks ± 2.40; $p< 0.001$) compared to those in the NP group (Table 3.2). Among the clinical parameters examined, RDS (HP: 83%, NP: 50 %; $p = 0.002$), IVH ≥ grade III (HP: 18%, NP: 0%; $p = 0.020$) and hyperglycemia (HP: 41%, NP: 13%; $p = 0.013$) occurred more frequently in HP infants compared to NP infants, reaching statistical significance (Table 3.2).



Day	Number of infants assessed	Serum phosphate (mmol/L) mean ± SD	Prevalence of Hypo PO_4 (%) (95% CI)
Day (1)	35	1.86 ± 0.44	17.0 (4.6 to 29.4)
Day (2)	85	1.67 ± 0.45	29.4 (19.7 to 39.0)
Day (3)	101	1.43 ± 0.41	52.0 (42.3 to 61.7)
Day (4)	86	1.28 ± 0.44	66.3 (56.3 to 76.3)
Day (5)	64	1.27 ± 0.45	73.4 (62.6 to 84.2)
Day (6)	44	1.40 ± 0.42	59.1 (44.6 to 73.7)
Day (7)	30	1.37 ± 0.46	63.3 (46.1 to 80.6)

Figure 3.1: Distribution of serum phosphate in the first seven days of life in very low birth weight infants.

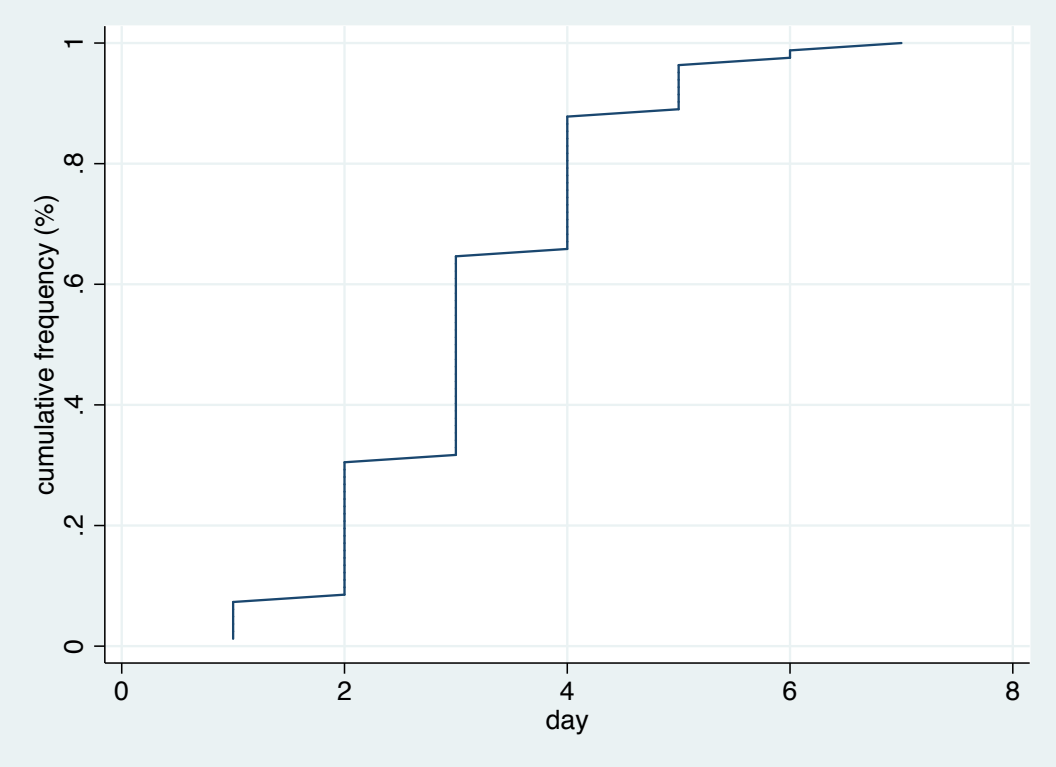


Figure 3.2: Cumulative occurrence of hypophosphatemia in the first seven days of life.

Table 3.1: Cumulative occurrence of hypophosphatemia in the first seven days of life.

Day	**Number of infants with Hypo PO_4**	**Cumulative occurrence of Hypo PO_4 (%)**
Day (1)	6	7.32
Day (2)	19	30.49
Day (3)	28	64.63
Day (4)	19	87.80
Day (5)	7	96.34
Day (6)	2	98.78
Day (7)	1	100

Abbreviation: N/A, not applicable.

Table 3.2: Maternal and infant characteristics of the very low birth-weight infants with and without hypophosphatemia in the first week of life

	Hypo PO_4 N = 82	**Normal PO_4 N = 24**	**P-value**
Maternal characteristics			
Maternal age (years) (mean ± SD) Median (IQR)	30.4 ± 5.92 31 (26, 35)	31.04 ± 5.48 31 (27, 36)	0.657 0.784
Diabetes (%)	6 (7)	0 (0)	0.333
Hypertension (%)	22 (27)	8 (33)	0.608
Antenatal steroid (%)	73 (89)	23 (96)	0.450
Cesarean section (%)	65 (79)	16 (67)	0.273
Chorioamnionitis (%)	14 (17)	6 (25)	0.385
Infant characteristics			
Gestational age (weeks) (mean ± SD) (Median, [IQR])	27.4 ± 2.00 28 (26, 29)	29.0 ± 2.40 29 (28, 30)	<0.001 0.003
Birth weight (grams) (mean ± SD) (Median, [IQR])	950 ± 248 915 (750, 1179)	1217 ± 250 1307 (1093, 1405)	< 0.001 0.001
Small for gestational age (SGA) (%)	21(26)	8 (33)	0.448
Male (%)	55 (67)	15 (63)	0.807
Apgar at 1m (Median, [IQR])	5 (3, 6)	6 (4, 7)	0.458
Apgar at 5m (Median, [IQR])	7 (6, 8)	8 (7, 9)	0.353
Respiratory distress syndrome (%)	68 (83)	12 (50)	0.002
Pneumothorax (%)	3 (4)	0 (0)	1.000
Intraventricular hemorrhage (%) All grades ≥ Grade III	 24 (29) 15 (18)	 3 (13) 0 (0)	 0.116 0.020
Inotropic support (%)	21 (26)	3 (13)	0.268
Sepsis (%)	6 (7)	0 (0)	0.333
Hyperglycemia (%)	33 (41)	3 (13)	0.013
Hypoglycemia (%)	13 (16)	2 (8)	0.511

Abbreviations: IQR, interquartile range; SD, standard deviation

No infants died during the seven-day study period. Only one infant in the HP group developed a seizure and no infants developed arrhythmias in either group in the first week of life. Seventeen infants (21%) required blood transfusions in the HP group and 3 (13%) infants in the NP group (Table 3.3).

Table 3.3: Associations of hypophosphatemia with adverse outcomes in very low birth weight infants with and without hypophosphatemia in the first week of life

	Hypo PO_4 N = 82	**Normal PO_4 N = 24**
Mortality, N (%)	0 (0)	0 (0)
Seizures, N (%)	1 (1.2)	0 (0)
Arrhythmias, N (%)	0 (0)	0 (0)
Need for transfusion, N (%)	17 (21)	3 (13)

Abbreviation: N/A, not applicable.

In the analysis of the changes in variables over the first week of life, sPO_4 ($p = 0.003$), PO_4 intake ($p = 0.003$) and calcium intake ($p = 0.01$) all had significant interactions with the quadratic time function (Figure 3.3 –3.5). Serum PO_4 of the HP group decreased to a nadir around day 4-5, while the NP group maintained their PO_4 levels at an almost constant level (Figure 3.3). There was an increase in the intake of PO_4 and calcium over time; the significant interaction indicated that the shape of the rate of increase differed between NP and HP infants (Figures 3.4–3.5). Calcium and PO_4 intakes increased more rapidly in the NP than the HP group.

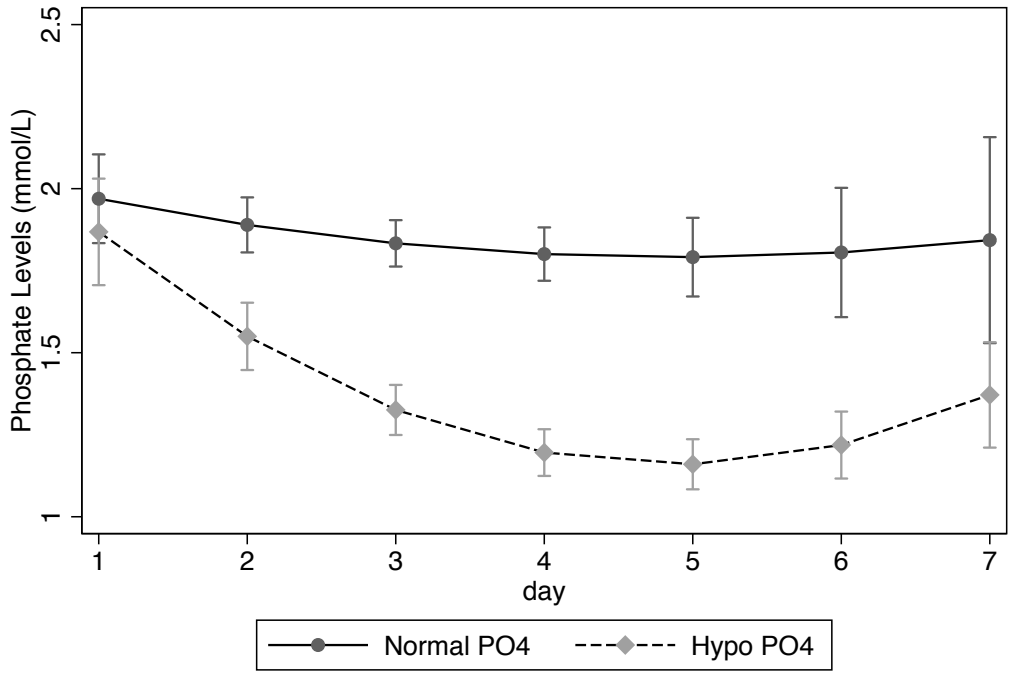


Figure 3.3: Phosphate levels in the first seven days of life.

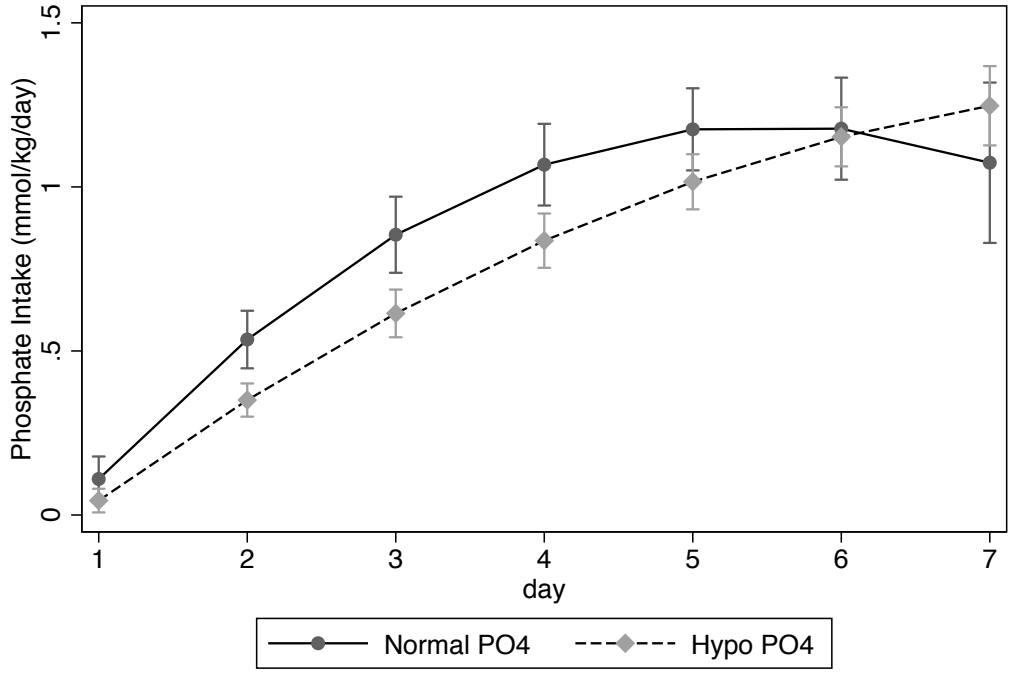


Figure 3.4: Phosphate intake in the first seven days of life.

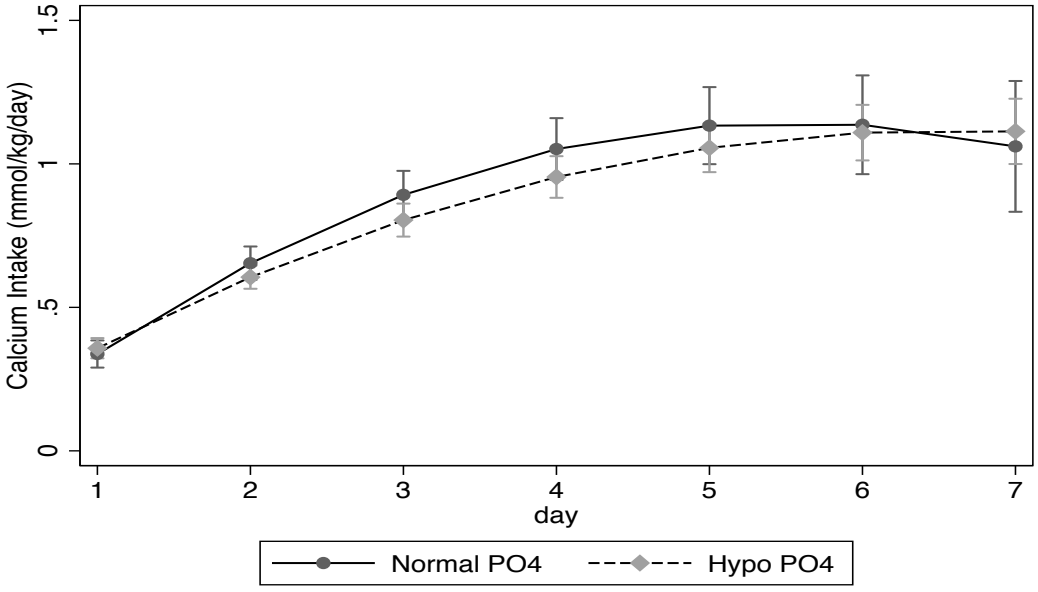


Figure 3.5: Calcium intake in the first seven days of life.

For the remainder of the variables—glucose intake, amino acid intake, lipid intake, total caloric intake and TG levels—there was no evidence of a significant interaction at $p < 0.05$, and so no evidence of a significant difference in the curves between the NP and HP infants (Figures 3.6–3.10). The intake of amino acids, glucose, lipids and total calories increased over time for both HP and NP groups similarly, as the two curves were parallel on the graphs. Moreover, there was no significant difference between the two groups (interaction p-value, group p-value):
amino acid ($p = 0.12$, $p = 0.326$), glucose ($p = 0.670$, $p = 0.468$), lipid ($p = 0.052$, $p = 0.154$), total calories ($p = 0.112$, $p = 0.726$) and TG ($p = 0.099$, $p = 0.295$) and in the graph the lines for the two groups are rather close together (Figures 3.6 –3.10).

In the analysis of the changes in pH values over the first week of life, although there was a statistically significant effect of a quadratic relationship over time ($p = 0.041$), this was not considered a clinically relevant effect since the largest difference for both groups between the maximum predicted value and the minimum predicted value was 0.01units. In addition there was a statistically significant difference between the two groups ($p = 0.043$), which again was equivalent to 0.01units (95% CI 0.0003units, 0.02units) (Figure 3.11). The statistically significant effects were due to the very small variation in the pH values over time. For creatinine levels, there were many missing values and insufficient data to do a longitudinal analysis, so a single mean value for each patient with at least one non-missing value was calculated, then the t-test applied to the single mean. There was no significant difference between the groups ($p = 0.773$).

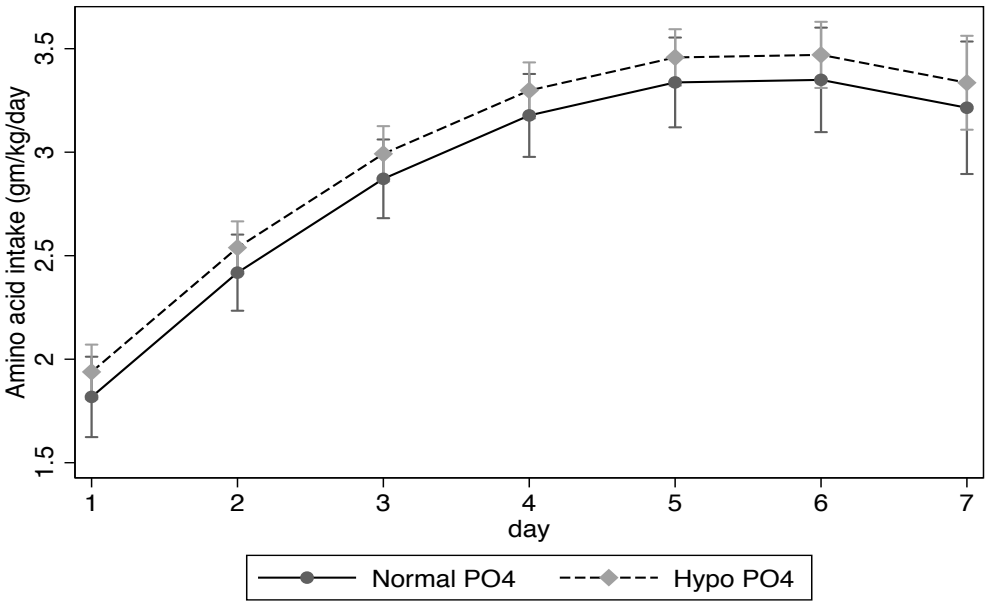


Figure 3.6: Amino acid intake in the first seven days of life.

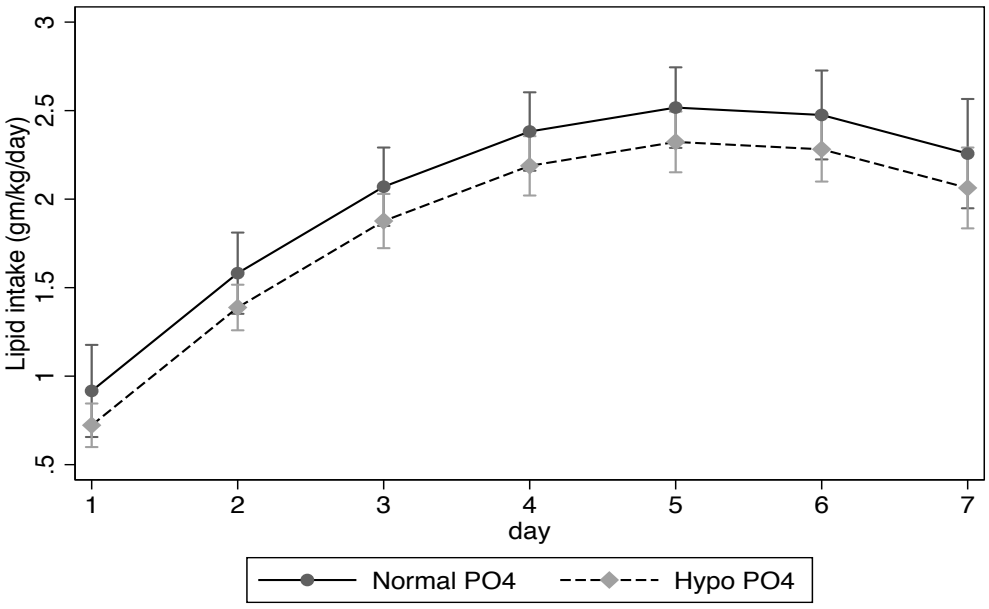


Figure 3.7: Lipid intake in the first seven days of life.

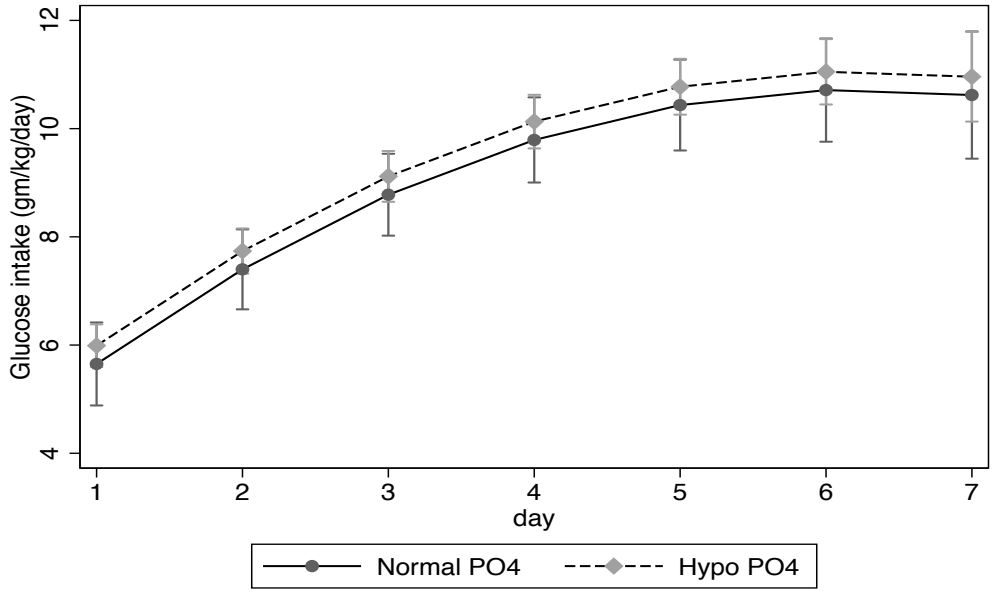


Figure 3.8: Glucose intake in the first seven days of life.

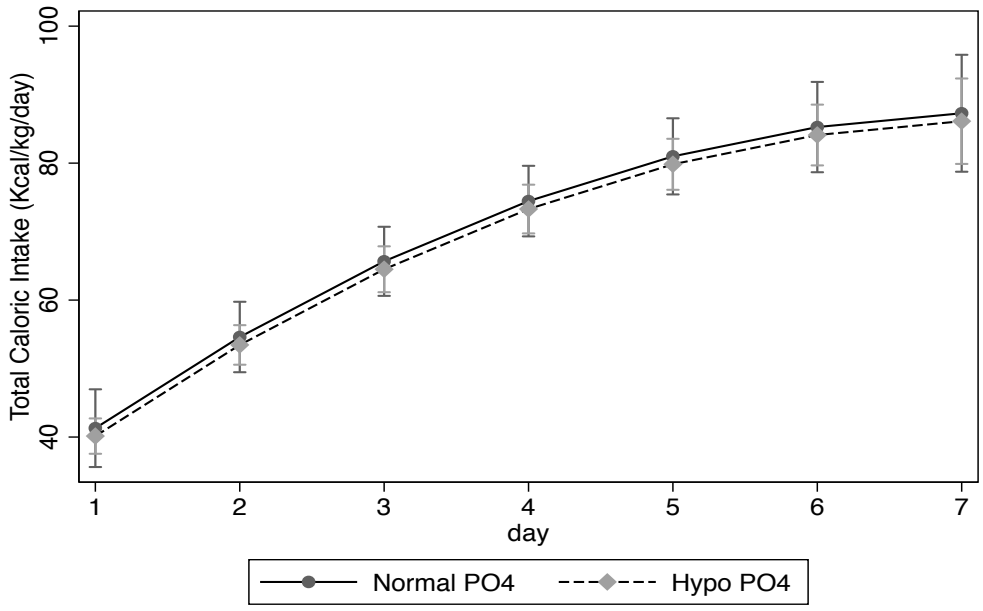


Figure 3.9: Total caloric intake in the first seven days of life.

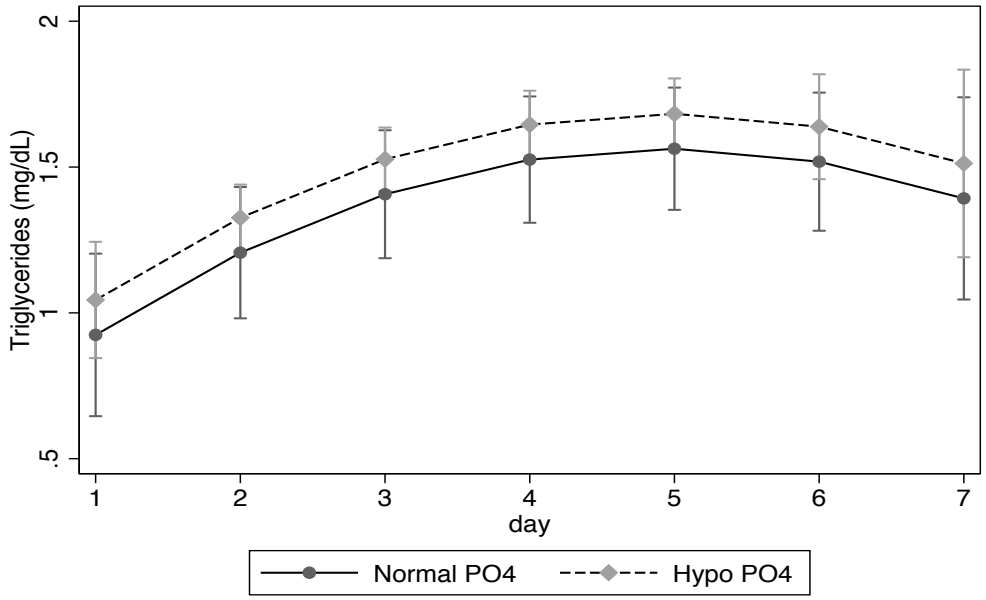


Figure 3.10: Triglyceride levels in the first seven days of life.

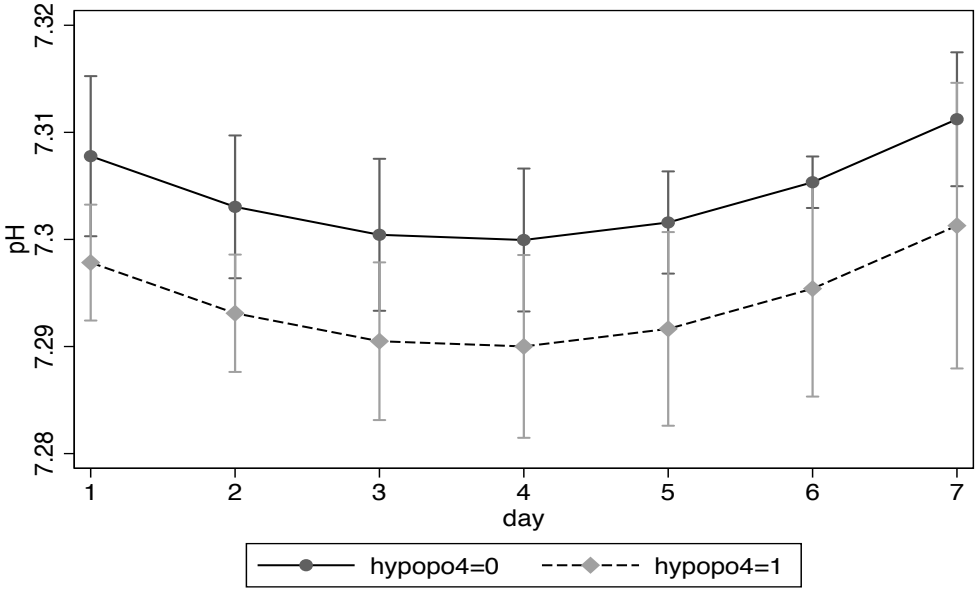


Figure 3.11: pH values in the first seven days of life.

To adjust for the association between HP and potential predictor variables including RDS, IVH of all grades, severe IVH (≥ grade III) and hyperglycemia, we used approximately the median BW, 1 kilogram, the cut-point for extremely low BW infants, to stratify our sample and calculated the stratum specific risk differences along with their 95% CIs. The risk of HP in infants < 1000 g (94%) was higher than that observed in infants ≥ 1000 g (61%) for a risk difference of 33% (95% CI 19%, 48%; $p < 0.001$). The unadjusted univariate and stratified analyses of HP risk in the presence of co-morbidities are depicted in Table 3.4. In the univariate analysis, there were statistically significant excess cases of HP in VLBW infants in the presence of RDS, ≥ grade III IVH, and hyperglycemia (31, 26, and 22 excess cases per 100 infants, respectively) compared to VLBW infants without those comorbidities in the first week of life (Table 3.4). For infants who weighed less than 1000 g there was little difference in risk of HP in infants with RDS (93%) compared to those without RDS (100%) during the first 7 days of life (95% CI 14%, 1%). However, for larger infants (1000-1500 g), the risk of HP was lower for both infants with RDS (74%) and those without (37%) for a statistically significant risk difference of 37% (95% CI 11%, 64%; $p = 0.007$) during the study period (Table 3.4).

For infants who weighed less than 1000 g there was very little difference in risk of HP between infants with IVH of all grade (86%) and those without IVH of all grade (100%). In the stratum of BW ≥ 1000 g, there were 56 cases per 100 infants of HP in those without IVH of all grades, while all infants with IVH of all grades developed HP (100%) ($p = 0.038$; Table 3.4). When only severe IVH (≥ grade III) was considered, for infants of BW < 1000 g, there was no statistically significant difference in the risk of HP in infants with severe IVH (100%) and those without severe IVH (93%) during the first seven days of life ($p = 0.328$). In infants ≥ 1000 g, all infants

with severe IVH had HP (100%) and there were 59 cases of HP per 100 infants with no severe IVH (59%) with a risk difference of 41% (95% CI 28%, 55%).

When hyperglycemia was studied as a risk for HP in different BW categories, we found that in infants with BW < 1000 g, there was no statistically significant difference in the risk of HP in infants with hyperglycemia (90%) and those without hyperglycemia (100%) ($p = 0.127$). In infants with BW > 1000 g, all infants with hyperglycemia developed HP while 55% of those without hyperglycemia developed HP, for a risk difference of 45% (95% CI 30%, 59%; $p = 0.035$). As RDS, IVH (all grades and severe IVH) and hyperglycemia were associated with an increased the risk of HP to a different degree in both BW categories, there may be an effect modification by weight on the association between RDS, IVH and hyperglycemia with HP. The association between HP and comorbidities including RDS, IVH and hyperglycemia was modified by BW and for that confounding by BW was not examined.

Table 3.4: Hypophosphatemia with co-incidental morbidities stratified by birth weight in very low birth weight infants in the first week of life

	Univariate risk difference (95% CI, p - value) N=106	**Birth weight < 1000 g**				**Birth weight ≥ 1000 g**			
		Hypo PO_4	Normo PO_4	Total	**Risk of Hypo PO_4**	Hypo PO_4	Normo PO_4	Total	**Risk of Hypo PO_4**
Respiratory distress syndrome	0.31 (0.10, 0.52) p = 0.001								
Yes		42	3	45	**0.93**	26	9	35	**0.74**
No		7	0	7	**1**	7	12	19	**0.37**
Total		49	3	52		33	21	54	
Risk difference (95%CI, P-value)		**-0.07 (-0.14, 0.01), p = 0.482**				**0.37 (0.11, 0.64), p = 0.007**			
Intraventricular hemorrhage (all grades)	0.15 (0.00, 0.31) p = 0.097								
Yes		18	3	21	**0.86**	**6**	**0**	6	**1**
No		31	0	31	**1**	27	21	48	**0.56**
Total		49	3	52		33	21	54	
Risk difference (95%CI, P-value)		**-0.14 (-0.29, 0.01), p = 0.030**				**0.44 (0.28, 0.56, p = 0.038)**			
Severe intraventricular hemorrhage (≥ grade III)	0.26 (0.17, 0.35) p = 0.024								
Yes		12	0	12	**1**	3	0	3	**1**
No		37	3	40	**0.93**	30	21	51	**0.59**
Total		49	3	52		33	21	54	
Risk difference (95%CI, P-value)		**0.08 (-0.01, 0.16), p = 0.328**				**0.41 (0.28, 0.55), p = 0.155**			
Hyperglycemia	0.22 (0.08, 0.36) p = 0.011								
Yes		27	3	30	**0.90**	6	0	6	**1**
No		22	0	22	**1**	26	21	47	**0.55**
Total		49	3	52		32	21	53	
Risk difference (95%CI, P-value)		**-0.10 (-0.21, 0.01), p = 0.127**				**0.45 (0.30, 0.59), p = 0.035**			

Chapter 4: Discussion

In this exploratory study, we prospectively evaluated sPO_4 levels in a convenience sample of 106 consecutive VLBW infants who were admitted to a tertiary care level NICU and had their PO_4 levels measured whenever their TG were measured over the first week of life. Hypophosphatemia occurred in 77% of our VLBW study group, a prevalence rate that is consistent with previous reports in the critically ill populations in the neonatal,[32,46] pediatric,[19,77] and adult intensive care units.[86,87] Despite its high prevalence, there are limited published data about the factors that contribute to HP and its clinical impact in VLBW infants. Numerous studies have described HP-associated morbidity and mortality in critically ill children and adults.[19,20,45,62,88] However, it is not known whether clinically relevant HP thresholds, and the risks of short- and long-term consequences, can be extrapolated to VLBW infants, perhaps at least in part since infants have higher reference intervals for PO_4 relative to other groups.[49] Recent studies have provided greater insights into PO_4 metabolism, but with the exception of metabolic bone disease of prematurity,[15,24,89,90] little is known about the clinical spectrum and outcomes of HP in these particularly vulnerable immature neonates.

The VLBW infants with HP in our study had significantly lower GA (27.4 weeks) compared to those who had normal sPO_4 levels (29.0 weeks; $p < 0.001$). Likewise, the BW of HP infants (950 g) were lower than that of their NP counterparts (1217 g; $p < 0.001$). This observation that smaller and lower GA preterm infants were at higher risk of HP is supported by several other studies.[13,46,91] In a retrospective cohort study of 61 preterm infants less than 1250 g, those with severe HP ($PO_4 < 2$ mg/dL) had significantly lower BW (803 g) compared to those with moderate HP (964 g).[46] El Hassan et al. studied 52 preterm infants less than 1500 g in an attempt to establish PO_4 norms in the first four weeks of life.[13] Phosphate levels were significantly lower

in infants with BW between 500 to 1000 g compared to those with BW between 1001 and 1500 g.[13] In contrast to recently published studies that showed a higher rate of HP in SGA (40%) compared to the appropriate for gestational age (AGA) (9%) infants,[77,92] in our sample there was no statistically significant difference in the incidence of SGA infants between the HP and NP groups. It is worth mentioning here that although the aforementioned studies[77,92] included VLBW, the authors used more stringent definition of HP (0.9mmol/L and 1.3mmol/L) and that could at least in part explain their lower risk of HP in their samples.

In our sample, there was a trend for a daily decrease in sPO_4 levels in both groups during the first week of life with a nadir of mean sPO_4 levels at days 4-5 of life (Figure 3.3). However, the HP group had a significantly more rapid decrease and lower sPO_4 concentrations (Figure 3.3, $p = 0.003$). These findings of a daily decrease in sPO_4 levels in the first 4-6 days of life are in agreement with previous studies that observed a fall in sPO_4 in the first week of life in VLBW infants.[13,46]

The disturbed placental transfer of PO_4 because of premature birth is one of the reasons that could contribute to the high prevalence of HP in this age group, as the PO_4 transfer across the placenta peaks (60-70 mg/kg/day) during the third trimester of gestation.[49] One of the other postulated reasons for the increased incidence of HP in the VLBW infants is the latest nutritional recommendation for preterm infants that entitles early introduction of PN with rather high protein and caloric intake to support post-natal growth and good neurodevelopmental outcomes.[93,94] The early initiation of aggressive PN has been recently recognized to be associated with electrolyte imbalances, including HP.[25,95,96] Adequate amino acid and energy supply support tissue anabolism and enhance nitrogen retention and somatic growth, processes that require

nucleic acids, ATP and membrane phospholipids, in all of which PO_4 is an essential component. Phosphate utilization is increased in the rapidly growing preterm infants and for each gram of protein retained, 0.3 mmol of PO_4 is required for tissue growth.[49] In a prospective observational study of 154 infants less than 33 weeks, infants with higher mean protein intake (> 2g/kg/day) in the first week of life had significantly lower sPO_4 concentrations compared to those with medium (1.5-2 g/kg/day) and low (< 1.5 g/kg/day) mean protein intake (sPO_4 mmol/L: 1.38 ± 0.33, 1.49 ± 0.34, 1.67 ± 0.32 respectively, $p < 0.001$). [95] In a randomized clinical trial of 50 VLBW infants, infants who were allocated to the enhanced PN protocol (starting protein at 3.5 g/kg/day and lipids at 2 g/kg/day on the first day of life) had significantly more cases of HP (PO_4 < 1.4 mmol/L, prevalence = 77%) compared to infants who received a standard protocol (starting protein at 2g/kg/day and lipids at 0.5g/kg/day, prevalence =26%) in the first week of life ($p = 0.001$).[97] In our study, and similar to the study by Brener Dik et al.[46] of a cohort of infants weighing < 1250 g, there was no significant difference in protein and caloric intakes between the two groups during the study period. However, in our sample, the NP group had a significantly more rapid increase of PO_4 intake compared to the HP group. This observation is supported by other studies. In a small, randomized clinical trial conducted in Duke University Medical Centre in 1987, a total of 27 infants less than 1500 g were randomly assigned to three different levels of PO_4 intake in the first two weeks of life. Infants in the low PO_4 intake group (1.00 ± 0.04 mmol/kg/day) had a significantly more rapid fall of serum PO_4 levels in the first week and remained low at two weeks of life compared to those in the moderate PO_4 (1.34 ± 0.03 mmol/kg/day) and high PO_4 (1.67 ± 0.05 mmol/kg/day) intake groups in which sPO_4 levels were maintained within the normal reference range (1.8-2.2 mmol/L).[98,99] In a quality control prospective cohort study of infants less than 34 weeks designed to achieve mineral homeostasis

in the first five weeks of life following the revision of the institution standard nutritional protocol according to the ESPGHAN 2005 recommendation, the infants achieved the goal parenteral intake of PO_4 of 1.9 mmol/kg/day on day 3 of life.[32] Despite average PO_4 intakes of 2.3 mmol/kg/day in the first week of life in this cohort of infants, 34% had sPO_4 concentration below 1.8 mmol/L and the mean levels were below 2 mmol/L from birth till day 9 of life. These authors suggested the need to increase PO_4 dosing for preterm infants in the early postnatal period for preterm infants.[32]

The increased amino acid and energy supply to replace the disrupted placental feeding has been hypothesized to trigger PO_4 depletion because of the enhanced protein synthesis that uses PO_4 for ATP production resulting in a reduction of PO_4 concentrations.[97] Further, the use of early high protein intake is often coupled with suboptimal provision of PO_4 contributing to the occurrence of HP. Several factors contribute to the limited and wide variation of PO_4 supplementation in PN solutions, including fluid restriction based on the severity of the clinical condition of the infant and also the need to start other infusions (inotropes), which further limit the amount of PO_4 and other electrolytes that can be supplied.[100,101] Moreover, PO_4 is provided as sodium and potassium salts (in this center as a mixture of monobasic [NaH_2PO_4 and KH_2PO_4] and dibasic [Na_2HPO_4 and K_2HPO_4]), which are often restricted until the urine output is established, delaying PO_4 intake in the first few days.[102,103] Moreover, if the infant is acidotic, the acetate salt of sodium and potassium is used, limiting the addition of PO_4 in the PN.[104] In addition, the solubility of calcium and PO_4 in PN solutions and the risk of precipitation when they are needed in large amounts in small volumes is another limitation to increasing the supply to match the protein load ordered.[30,105,106]

The severity of illness and extreme prematurity may play a role in defining the amount of PO_4

needed to match the metabolic demand and cellular requirements of the compromised organ systems normally responsible for handling PO_4. In our univariate analysis, infants with HP were more likely to have RDS, hyperglycemia and severe IVH (≥ grade III). When we adjusted for BW in the stratified analysis, infants < 1000 g had a high risk of HP for both those with and without RDS (93% vs. 100 %), IVH of all grades (86% vs. 100%), severe IVH ≥ grade III (100% vs. 93%) and hyperglycemia (90% vs. 100%), which may reflect the extreme prematurity. The co-incidence with acuity of illness in these infants likely signifies their very low BW, low body stores of PO_4, immaturity of organ systems required to handle PO_4, [15,107] and their concurrent risk of these morbidities of prematurity.

While severe IVH was associated with a higher risk of HP to different degrees in both BW categories, there may be an effect modification by weight on the association between RDS and hyperglycemia with HP. While we have no biological explanation for the possible relationship between IVH and HP, it is possible that the HP infants were sicker and at greater risk of IVH.

Respiratory distress syndrome is the commonest respiratory disorder in infants born before 32 weeks of gestation and carries a high rate of morbidity and mortality.[4] The incidence of RDS is inversely related to GA. The associated hypoxemia, hypercarbia and acidosis trigger further biochemical disturbances of cellular metabolism resulting in impairment of oxygen delivery.[108] Delivoria-Papadopoulos et al. studied 12 premature infants with RDS (BW 1490 ± 240 g) and compared them to premature infants without RDS (BW 1500 ± 400 g) and term infants (BW 3100 ± 520 g). There was a significantly lower red cell 2,3-DPG in the preterm infants with RDS.[109] Phosphate is an important component of 2, 3-DPG, which regulates the release of oxygen from hemoglobin, especially in conditions with high metabolic activity, as is the case in RDS.[29] Hypophosphatemia hampers the formation of 2,3-DPG and that may signify the role of

PO_4 in infants with RDS.[18,59] Tsirka et al. studied 21 infants with mean GA 32 ± 2 weeks and mean BW 1990 ± 400 g with respiratory distress at birth in the first week of life and found a significantly lower plasma and red blood cell PO_4 as well as red blood cell ATP and 2,3 DPG compared to a control group matched for GA and BW.[110] Attaining satisfactory 2,3 DPG levels in the sick preterm infants by increasing PO_4 intake could theoretically improve oxygen delivery to the tissues and alleviate illness severity.

In a quasi-experimental trial, preterm infants less than 34 weeks were allocated alternatively to either receiving PN with PO_4 intake of 1mmol/kg/day (30mg/kg/day) (n = 10) or PN with PO_4 intake of 2 mmol/kg/day (60mg/kg/day) (n = 15). An additional observational control group consisted of 16 infants matched for BW and GA, who were well enough to start oral milk intake in the first two days of life. Infants in the lower PN PO_4 intake group had significantly lower plasma and red blood cell PO_4 on the fourth day of life and remained significantly lower by the end of the first month in comparison with the other two groups.[111] In the same study, the 2,3-DPG levels were lower in infants assigned to the low PO_4 intake in comparison with the control group throughout the study period, and on the 30th day they were lower than in infants assigned to the high PO_4 intake group infants.

On the other hand, PO_4 depletion could just be a marker of illness severity. As shown in the study by Cholevas et al., who studied preterm infants with and without perinatal problems, although PO_4 intake was comparable between the two groups, plasma and red blood cell PO_4 levels as well as 2,3 DPG and ATP were lower in the former group.[56] We do not know at this point whether HP in the VLBW is a marker of the severity of the concurrent comorbidities or it is a consequence of the deficient stores because of their premature birth and the limited provision of PO_4 in the first few days of their lives.

Critically ill VLBW infants frequently experience poor glycemic control during the first week of life as they adjust to extra-uterine life.[112,113] In preterm infants, early hyperglycemia is not uncommon, with a prevalence ranging between 20% and 86% depending on the definition used, BW, GA, intravenous glucose infusions and severity of illness.[82,114,115] Early hyperglycemia in preterm infants has been identified as a risk factor for death,[116] white matter abnormalities,[116] IVH,[82] and prolonged hospital stay.[117] In terms of risk factors, hyperglycemia in the first week of life in VLBW infants is associated with low GA, small size, use of inotropes, lipid infusions, and sepsis.[115] Some of these risk factors have also been associated with HP.[10,20,55,118] In our study, HP was common in the smaller infants (< 1000 g) for both those with (90%) and without hyperglycemia (100%). However, in the larger infants (1001–1500 g), there was a statistically significant greater risk of HP in those with hyperglycemia (100% and 55% respectively). Hypophosphatemia could potentially contribute to hyperglycemia in preterm critically ill infants, which, as far as we are aware, has not been previously studied in this population. Phosphate is an essential component of ATP that is needed for glucose metabolism, including oxidative phosphorylation, glycogen synthesis, and glycolysis. Hypophosphatemia can result in reduction of ATP compounds resulting in intracellular disturbance of glucose metabolism and consequently glucose intolerance. [44,119] Further, PO_4 is an important constituent of cell-membrane protein and its deficiency could alter transmembraneous phospholipids resulting in abnormality in insulin binding and glucose transport.[120] It is possible that HP is a mediating variable in some or all of the cases of hyperglycemia in VLBW infants. However, prospective randomized studies controlling for PO_4 supplementation and monitoring for glucose hemostasis are needed to establish whether HP is a cause for hyperglycemia in VLBW infants or whether it is a consequence of the stress-induced hyperglycemia in the very sick ones with the

resultant metabolic disturbances and cellular shifts.

No infants died during the first week of life, and the rather short study period may have limited the number of morbid events seen. Among episodes of seizures, arrhythmias and the need for blood transfusion, there were no statistically significant differences between the two groups.

In summary, HP occurred in the majority of VLBW neonates in this observational study. These newborns did not receive the expected amount of transplacental PO_4 and were at a greater risk of illness severity as a consequence of the degree of prematurity. Moreover, in this study, lower PO_4 delivery was an important and potentially avoidable factor that may have contributed to HP risk. However, in addition to more aggressive and earlier provision of PO_4, our findings suggest that other potential factors may impede sPO_4 normalization or increase requirements, including the effects of comorbidities and illness severity. Additional studies will be necessary to clarify the foregoing, as well as the relationship of HP to RDS and other morbidities characteristic of this population. We suggest that a longer duration of observational assessment may uncover morbidity and mortality risks that had not yet manifested during the first week of life.

4.1 Strengths and limitations

Finally, we wish to point out several strengths and limitations in our study. We feel that the strengths include the use of a prospective design and the inclusion of consecutive VLBW infants admitted to the NICU, which lessened the chance of selection bias. By employing a consecutive sampling of patients that met the predefined inclusion and exclusion criteria, factors that could influence subjects' participation and affect the study's outcome were avoided. This consequently minimized the chance of selection bias. Further, repeated sPO_4 measurements were obtained independently of patients' condition and treatments received, giving a degree of

strength to internal validity. The careful calculation of all nutritional data, including PO_4 intake and other variables that could affect sPO_4 levels in studying risk factors of HP, gave strength to credibility of our study. An additional strength is that we used a criteria for HP based on the usual definition of reference intervals, from a study of 480 preterm infants.[11] Another strength of our study is the longitudinal nature of the data over the first week of life and the accounting for repeated measures and correlated responses using GEE analysis.

We would like to acknowledge some of the limitations of our study. The level of PO_4 in plasma or serum is subject to interference by hemolysis and by heparin (routinely used to maintain patent central lines). Both hemolysis and blood samples contaminated with heparinized solution can cause an artefactual increase in sPO_4 measurements.[121,122] Another limitation was that sPO_4 levels were obtained with TG measures, so we did not have measures for each infant each day; this created unbalanced data as some infants had missing observations. Further, we did not obtain maternal or infant vitamin D and PTH levels, both of which influence the transcellular distribution and serum level of PO_4.[123-125] In addition, our study was not powered to detect differences of small magnitude or to support a more in depth multivariable analysis. However, it was a pilot study intended to guide the planning of a larger-scale prospective study and facilitates power calculations for study proposals.

As we only included one center in our study and we used a convenience sample, the generalizability of our findings may be limited. Nevertheless, our NICU may have typical characteristics of a tertiary level NICU in a developed country, and the prevalence of HP in our center was comparable to that reported in the literature giving strength to external validity.

4.2 Conclusion

Insufficient intake of PO_4 in VLBW infants during the first week of life was associated with prevalent HP, yet this condition remains under-recognized. Our study identified other metabolic and clinically important co-incidental events—IVH, RDS and hyperglycemia—whose exact roles in VLBW HP need to be better defined. Whether it is the premature birth and the associated metabolic consequences, or whether it is the severity of the accompanying comorbidities that increase the risk of HP in this population, is yet to be investigated. To confirm or refute our findings, prospective studies are needed to identify risk factors, assess the benefits and safety of more aggressive and earlier PO_4 delivery and to assess short and long-term clinical consequences of HP. Further, improved HP surveillance of critically ill premature infants and reevaluating the nutritional guidelines regarding PO_4 delivery in units caring for these infants is advised.

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