

ORIGINAL REPORT

TITLE: Severe hypercalcaemia and hypophosphataemia with an optimised preterm parenteral nutrition formulation in two epochs of differing phosphate supplementation

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ABSTRACT

Objective: To compare in two epochs of differing parenteral nutrition phosphate provision serum calcium, phosphate, potassium, and sodium concentrations and the frequency of abnormality of these electrolytes and of sepsis in preterm infants who received an optimised higher amino acid-content parenteral nutrition formulation.

Design and setting: Retrospective cohort study at a single tertiary-level neonatal unit.

Patients: Preterm infants given parenteral nutrition in the first postnatal week during two discrete 6-month epochs in 2013-4.

Interventions: In epoch 1 the $\text{Ca}^{2+}:\text{PO}_4$ molar ratio of the parenteral nutrition formulation was ~1.3–1.5:1 (1.7 mmol Ca^{2+} and 1.1 mmol PO_4 per 100 mL aqueous phase) and in epoch 2 was 1.0:1 via extra phosphate supplementation (1.7 mmol Ca^{2+} and 1.7 mmol PO_4 per 100 mL).

Main outcome measures: Peak calcium and nadir phosphate and potassium concentrations, and proportions with severe hypercalcaemia ($\text{Ca} > 3.0$ mmol/L), hypophosphataemia ($\text{PO}_4 < 1.5$ mmol/L), and hypokalaemia ($\text{K}^+ < 3.5$ mmol/L) within the first postnatal week.

Results: In epoch 2, peak calcium concentrations were lower than in epoch 1 (geometric means: 2.83 vs. 3.09 mmol/L, P-value <0.0001), fewer babies were severely hypercalcaemic (10/49, 20%, vs. 31/51, 61%; P-value <0.0001); nadir plasma phosphate concentrations were higher (means: 1.54 vs. 1.32 mmol/L, P-value =0.006), and there were fewer cases of hypophosphataemia (17/49, 35% vs. 31/51, 61%, P-value=0.009) and hypokalaemia (12/49, 25% vs 23/51, 45%, P-value=0.03).

Conclusions: Reverting from a parenteral nutrition $\text{Ca}^{2+}:\text{PO}_4$ molar ratio of 1.3-1.5:1 to a ratio of 1.0:1 was associated with a lower incidence and severity of hypophosphataemia and hypercalcaemia. For preterm infants given higher concentrations of amino acids ($\geq 2.5\text{g/kg/day}$) from postnatal day 1, an equimolar $\text{Ca}^{2+}:\text{PO}_4$ ratio may be preferable during the first postnatal week.

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INTRODUCTION

Parenteral nutrition (PN) is essential to meet the nutritional requirements of many preterm babies until enteral feeds are established. Higher amino acid intakes in the first postnatal week have been recommended to meet their accelerated ex-utero metabolic demands,[1] and may improve somatic and brain growth.[2] Yet uncertainty remains about ideal early amino acid intakes for optimal growth and whether higher early protein intakes are safe and improve neurodevelopmental outcomes.[3]

Current European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines have, since 2005, recommended PN provision for growing preterm infants of amino acids up to 4 g/kg/day, Ca^{2+} 1.3–3.0 mmol/kg/day, and PO_4 1.0–2.3 mmol/kg/day, with a $\text{Ca}^{2+}:\text{PO}_4$ ratio in the range 1.3–1.7:1.[1] Between 2006-2012 our neonatal intensive care unit (NICU) aimed to provide this amino acid intake via a preterm PN formulation with a higher amino acid concentration of 3.2 g per 100 mL of aqueous phase PN. It had equivalent Ca^{2+} and PO_4 concentrations, each of 1.0 mmol/100 mL, and thus an equimolar (1.0:1) $\text{Ca}^{2+}:\text{PO}_4$ ratio. Electrolyte disturbances appeared uncommon in this era.

In January 2013, a new PN formulation was introduced into all neonatal units in the East of England region to better accord with the ESPGHAN guidelines for higher calcium and amino acid intakes for growing preterm infants.[1] In the ensuing months, our neonatal unit and several others reported frequent cases of severe hypercalcaemia and hypophosphataemia occurring during the first postnatal week. Our hypothesis was that these biochemical disturbances were secondary to inadequate phosphate provision in the PN leading to a 'refeeding-type syndrome'. [4] Because a recent report had associated severe hypophosphataemia with sepsis in

preterm infants[5], the NICU of the Norfolk and Norwich University Hospital introduced an urgent temporising workaround measure that aimed to alleviate the incidence of these biochemical abnormalities. The change, made in December 2013 with the approval of our hospital's Paediatric Nutrition Support Team, involved the daily addition of extra phosphate to each baby's PN on an individualised basis to match the Ca^{2+} concentration, so effectively reverting to our NICU's former equimolar (1:1) $\text{Ca}^{2+}:\text{PO}_4$ ratio.

This retrospective study reports the incidence of electrolyte disturbances of Ca^{2+} , PO_4 , K^+ , and Na^+ and the incidence of sepsis in preterm infants who received PN in periods before and after our bespoke additional PO_4 supplementation of the new regional PN formulation.

METHODS

We reviewed PN provision of amino acids and electrolytes and the associated incidence and severity of abnormalities of Ca^{2+} , PO_4 , K^+ , and Na^+ in babies born within two discrete 6-month epochs. Epoch 1 was 1st June to 29th November 2013 during which the newly-introduced regional PN aqueous phase formulation contained 1.7 mmol Ca^{2+} and 1.1 mmol PO_4 per 100 mL; including the PO_4 provision from lipid, this delivered an overall $\text{Ca}^{2+}:\text{PO}_4$ molar ratio of ~1.5:1 from day 1 of PN and ~1.3:1 on days 2-7 (Table 1). Epoch 2 was 5th December 2013 to 31st May 2014, the period of bespoke daily additional PO_4 supplementation to match the higher calcium provision thereby achieving an overall $\text{Ca}^{2+}:\text{PO}_4$ ratio of 1.0:1 (1.7 mmol Ca^{2+} plus 1.7 mmol PO_4 per 100 mL less the PO_4 contribution from lipid). In epoch 2, the additional PO_4 was added to the PN aqueous phase by our hospital pharmacy's sterile production unit as sodium glycerophosphate (Glycophos® Sterile Concentrate, Fresenius Kabi, Runcorn, UK) containing phosphate 20 mmol and Na^+ 40 mmol per 20 mL vial); this thereby provided additional supplementation of 2 mmol Na^+ for every mmol of added PO_4 . Amino acid provision remained unchanged (3.3 g per 100 mL aqueous phase) and, except for the supplementary sodium glycerophosphate, the PN formulation regime was identical to that in epoch 1 (Table 1).

We included in our analyses all admitted preterm infants (<37 weeks' gestational age) that received any PN during their first postnatal week, excluding those who died aged <1 week. In both epochs our NICU's practice for delivering PN was to commence amino acids and lipid in infants eligible for PN on the first postnatal day, ideally within 6 hours of birth. The duration of 'day 1' was variable, commencing from the time of birth and ending at the first 24:00h. Using a standardised protocol (**supplementary file S1**), amino acid intake commenced on day 1 at 2.5 g/kg/day,

and increased daily to reach a maximum of 4.3 g/kg/day by day 5 (Table 1). Supplementary sodium 3.0 mmol and potassium 1.9 mmol per 100 mL aqueous phase was provided from day 1. Enteral feeding with human milk was commenced from day 1 and increased gradually over subsequent days according to our regional standardised feeding protocol (**supplementary file S2**).

We reviewed case notes, PN charts, and daily biochemistry and microbiology results during the first postnatal week. We recorded actual aqueous phase volumes delivered in each infant on each day during the first week, from which we derived the daily amino acids and electrolytes intakes received. The main outcome measures were incidences in the first week of severe hypercalcaemia (plasma Ca^{2+} >3.0 mmol/L), hypophosphataemia (plasma PO_4 <1.5 mmol/L), severe hypophosphataemia (PO_4 <1.0 mmol/L), and hypokalaemia (plasma K^+ <3.5 mmol/L), and the peak plasma Ca^{2+} and nadir PO_4 and K^+ concentrations. Secondary outcomes were peak plasma Na^+ concentration, incidence of hypernatraemia (plasma Na^+ >150 mmol/L), and incidence of sepsis as defined by a positive blood or cerebro-spinal fluid culture with accompanying antibiotic treatment for ≥ 5 days between birth and discharge. We used t-tests, Mann-Whitney U test and the Chi-squared test to compare data between the two cohorts. Multivariable logistic and linear regression models were used to adjust for potential confounding factors. This study was registered as a clinical audit and service evaluation and did not require formal ethics approval.

RESULTS

101 preterm infants received PN over the 12-month study period. One 23-week gestation infant who died aged 12 h postnatal was excluded from analysis. Of 100 included infants, 26 were transfers in from other hospitals and commenced or continued PN upon arrival which was usually within 24-48 hours of birth. Baseline characteristics (Table 2) and PN nutrient and electrolyte intakes within the first week (**Table A, supplementary file S3**) were similar across epochs, excepting the extra sodium glycerophosphate provided in epoch 2.

Table 3 shows peak plasma Ca^{2+} and nadir plasma PO_4 , K^+ , and peak Na^+ concentrations, and the incidence of hypercalcaemia, hypophosphataemia, hypokalaemia, hypernatraemia, and sepsis within the two epochs. In epoch 2 compared with epoch 1, peak calcium concentrations were lower ($p < 0.0001$) and fewer babies were severely hypercalcaemic (20% vs. 61%). Correspondingly, nadir plasma phosphate concentrations were higher ($p = 0.006$) and there were fewer cases of hypophosphataemia (35% vs. 61%), severe hypophosphataemia (14% vs. 22%), and hypokalaemia (25% vs. 45%). Adjusting for (i) birth weight and gestation, and (ii) small-for-gestational age status (birth weight $< 10^{\text{th}}$ centile) made very little difference to our univariate findings of differing incidences of hypercalcaemia, hypophosphataemia, and hypokalaemia, and of differing plasma calcium and phosphate concentrations, between epochs 1 and 2 (**Table B, supplementary file S4**).

Peak sodium concentrations and proportions with hypernatraemia were not significantly different between epochs (Table 3). Eight babies (16%) in epoch 1 and five babies (10%) in epoch 2 had culture-positive sepsis before discharge.

DISCUSSION

In our region, severe biochemical disturbances of calcium and phosphate occurred frequently in preterm infants given an optimised PN formulation that was introduced with good intentions to better accord with ESPGHAN recommendations for amino acid and calcium content. While this quasi-optimised PN preparation improved provision of calcium, its comparatively low phosphate content led to unanticipated metabolic derangements despite achieving a recommended $\text{Ca}^{2+}:\text{PO}_4$ ratio of 1.3:1. However, after we then increased ad hoc the phosphate concentration of the PN formulation to match calcium in an equimolar ratio, we observed a reduction in the incidence of severe hypercalcaemia, hypophosphataemia, and hypokalaemia. For preterm infants receiving PN with a higher amino acid content in the first postnatal week, an equimolar (1:1) $\text{Ca}^{2+}:\text{PO}_4$ ratio would thus appear preferable to that currently recommended (1.3–1.7:1). Our data support current expert opinion that an adequate $\text{Ca}^{2+}:\text{PO}_4$ molar ratio in neonatal PN in the first postnatal week should be close to or below 1.0 for higher amino acid intakes $>2.5\text{g/kg/day}$ starting from the first postnatal day,[6] and also the suggestion that the current PN guidelines for preterm infants need revision.[7]

In epoch 1, peak Ca^{2+} and nadir PO_4 concentrations had often already occurred by postnatal day 5, highlighting the importance of sufficient early PO_4 provision to achieve a 1:1 $\text{Ca}^{2+}:\text{PO}_4$ ratio and avoid deficit within the first few days. While the additional phosphate supplementation and 1:1 $\text{Ca}^{2+}:\text{PO}_4$ molar ratio in epoch 2 was associated with a reduction in the observed incidence of hypophosphataemia and hypercalcaemia, it did not completely abolish all cases. Thus, some babies in epoch 2 affected by hypophosphataemia needed still further increased PO_4 supplementation to achieve resolution. This was provided this via a higher molar concentration of PO_4

compared to Ca^{2+} (ie a 'reversed' $\text{Ca}^{2+}:\text{PO}_4$ ratio), and was often required for several days.

Several reports have already highlighted the risk of hypercalcaemia and hypophosphataemia with an 'aggressive' PN approach.[4,5,7-11], and that small-for-gestational age infants appear particularly prone.[9-11] The prime cause of these biochemical disturbances is postulated as inadequate early phosphate and potassium supply and/or inadequate proportions of calcium, phosphate, and potassium delivered alongside higher amino acid concentrations.[4] Phosphate is utilised rapidly for production of energy within the cell and for tissue growth in very low birth weight infants with limited reserves. Severe hypophosphataemia accentuates mobilisation of calcium from bone, leading to hypercalcaemia.[4,5] One prospective study showed that severe hypophosphataemia due to a low phosphate content in the PN during the first postnatal week led to increased urinary calcium excretion and a high urinary Ca^{2+} :creatinine ratio, and the associated risks of poor bone mineralisation and nephrocalcinosis.[12] Provision of adequate calcium and phosphorus in PN during the first weeks after birth significantly reduces bone demineralisation in preterm infants and helps preserve short-term bone strength.[13]

Severe hypophosphataemia potentially increases the risk of infection because it inhibits the production of adenosine triphosphate leading to impairment of phagocytic responses, bactericidal activity, and chemotaxis.[14] Recruitment to one RCT of enhanced feeding of very low birth weight infants was curtailed early due to an increased rate of neonatal sepsis associated with enhanced feeding and first-week hypophosphataemia.[5] Others have reported the same association.[10] Yet it remains unclear for preterm infants whether low plasma phosphate is a risk factor for sepsis independent of being extremely low birth weight.

The importance of adequate early Na⁺ and K⁺ provision when providing higher amino acid concentrations to preterm neonates has been highlighted.[5,7] It is unclear why fewer babies in epoch 2 had hypokalaemia. Better PO₄ and K⁺ retention related to greater nitrogen retention cannot explain the finding because protein and K⁺ intakes remained the same across epochs. Babies coped well with the inadvertent extra sodium provided via the extra phosphate supplementation in epoch 2, because plasma Na⁺ concentrations and proportions with hypernatraemia remained similar.

The NEON RCT compared two regimens of first-week amino acid intakes which provided averaged daily protein intakes from day 1 of either 2.2 g/kg/day or 2.9 g/kg/day.[15] Initial Ca²⁺ and PO₄ provisions from PN were both 1.0 mmol/kg/day (1:1 ratio), and then 1.5 mmol/kg/day Ca²⁺ and 2.0 mmol/kg/day PO₄ from day 3 (1:1.3 ratio) (personal communication, Dr Uthaya). In comparison, infants in the present study received an averaged protein intake of ~2.4 g/kg/day (Table **A, supplementary file S3**). Our epoch 2 infants had a similar incidence of hypophosphataemia (17/49, 35%) as in the NEON study incremental-amino acids groups (33/84, 39%). Yet significantly more infants in our epoch 2 cohort had severe hypercalcaemia (10/49, 20%) than in either of the NEON study amino acid cohorts (vs. NEON incremental-amino acid groups [0/84, 0%], P<0.0001, Fisher's exact test; vs. NEON immediate-recommended daily amino acid groups [5/85, 6%], P =0.02, Chi-squared test. This disparity may predominantly reflect the higher average daily first-week planned calcium provision with our PN regimen compared with that of the NEON study (~1.9 mmol/kg/day [Table 1] versus 1.4 mmol/kg/day), because the intended average daily phosphate provision in epoch 2 was essentially the same as that in the NEON study (~1.9 mmol/kg/day).

Study strengths include this being the first to report the incidence and severity

of biochemical abnormalities associated with neonatal PN formulations that differed only in respect of phosphate (and sodium) content, and that complete data collection was achieved from all eligible infants. Limitations include that this was a retrospective, single-centre review covering two separate albeit essentially-contiguous epochs. Also, as an observational study rather than an RCT, our findings cannot indicate definite causality from the intervention. Nevertheless we believe our data provide useful information to help guide the preferred Ca:PO₄ ratio in early neonatal PN. Our experience also highlights the potential pitfalls of widely introducing standardised PN solutions that have not been subject to the rigorous testing of randomised-controlled trials or careful prospective audit.

Conclusions

For preterm infants given higher concentrations of amino acids (≥ 2.5 g/kg/day) starting on the first postnatal day, increasing the phosphate provision of the PN solution to match Ca²⁺ content was associated with a reduction in the incidence and severity of both hypophosphataemia and hypercalcaemia in the first postnatal week. An equimolar (1.0:1) Ca²⁺:PO₄ ratio therefore appears preferable to a $\geq 1.3:1$ ratio for preterm PN during the first postnatal week. Our study findings support calls to review current international recommendations regarding early phosphate and calcium provision in PN for preterm infants. Our study also emphasises the importance of careful prospective audit of neonatal biochemistry following any changes to PN formulations.

What is already known on this topic

- High amino acid intakes from parenteral nutrition in the first postnatal week may improve growth, but uncertainty remains about optimal intakes, safety, and associated later neurodevelopmental outcomes
- High early amino acid intakes with inadequate accompanying phosphate provision or a sub-optimal calcium:phosphate ratio may lead to severe hypercalcaemia, hypophosphataemia, and hypokalaemia
- Severe biochemical derangements occurred frequently following introduction of a new regional neonatal parenteral nutrition formulation that met current international recommendations for amino acids, calcium, and phosphate

What this study adds:

- Increasing the parenteral nutrition phosphate content to match its higher calcium content mole-for-mole was associated with a reduction in the observed incidence and severity of hypercalcaemia and hypophosphataemia
- For preterm infants receiving higher amounts of amino acids (≥ 2.5 g/kg/day) in the first postnatal week, an equimolar (1:1) $\text{Ca}^{2+}:\text{PO}_4$ ratio appears preferable to that currently recommended (1.3–1.7:1)
- Prospective audit of neonatal biochemistry is important following any changes to PN formulations

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Competing interests/ Conflict of interest statement: PC served as an independent member of the Trial Steering Committee for the NEON study.[15] There are no competing interests and no conflict of interests to declare in relation to this work.

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Ethics approval: This study was undertaken as a clinical audit and service evaluation. Formal ethics approval was not required under the contemporaneous NRES guidance.

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Table 1: Standardised regimen for incremental fluid, PN, and electrolyte intakes over the first 5 days postnatal

	Day 1	Day 2	Day 3	Day 4	Day 5*
Fluids, mL/kg/day	80	100	120	135	150
Aqueous phase, mL/kg/day	75	90	105	120	130
Lipid phase, g/kg/day [†]	0.9	1.8	2.7	2.7	3.6
Aminoacid, g/kg/day	2.5	3.0	3.5	3.8	4.3
Nitrogen, g/kg/day	0.35	0.42	0.49	0.54	0.61
Protein, g/kg/day	2.2	2.7	3.1	3.4	3.8
Calcium, mmol/kg/day	1.3	1.5	1.8	2.0	2.2
Phosphate, mmol/kg/day	0.8	1.0	1.2	1.3	1.4
Ca ²⁺ :PO ₄ ratio [‡]	1.5:1	1.3:1	1.3:1	1.3:1	1.3:1
Potassium, mmol/kg/day	1.4	1.7	2.0	2.3	2.5
Sodium, mmol/kg/day	2.3	2.7	3.2	3.6	3.9

Data shown are for epoch 1; intakes for epoch 2 were identical apart from an increased daily phosphate supplementation given to match the daily calcium intakes (Ca²⁺:PO₄ ratio 1.0:1), plus the resultant extra sodium

*Prescribed provision on days 6 and 7 was same as for day 5

†Each 1 g/kg/day of lipid (5 mL/kg/day Intralipid) contributed an additional 0.08 mmol/kg/day of phosphate to overall intake

‡Overall Ca²⁺:PO₄ ratio from PN when including lipid phase phosphate content

Further nutritional details of the aqueous phase content and the common daily parenteral provision of vitamins, trace elements, and micronutrients provided across the study period are available in **supplementary file S1**

Table 2: Baseline characteristics of preterm infants who received parenteral nutrition in two epochs of different phosphate supplementation

	Epoch 1 N = 51	Epoch 2 N = 49	P – value***
Gestational age, weeks	29.9 (29.0 – 30.8)	28.7 (27.8 – 29.6)	0.07
Birth weight, g*	1182 (1075 – 1299)	1061 (945 – 1192)	0.16
Male gender, n (%)	23 (45)	22 (45)	0.98
Small for gestational age, n (%)	20 (39)	13 (27)	0.18
Postnatal day PN started **	1 (1 – 2)	1 (1 – 1)	0.46
Postnatal age PN commenced, hours**	12 (6 – 41)	9 (6 – 23)	0.18
PN days in first week**	6 (5 – 7)	7 (6 – 7)	0.20

PN, parenteral nutrition

Data are arithmetic mean (95% CI) or n (%) unless otherwise stated

* Geometric mean (95% CI) presented for birth weight data

** Median (IQR)

*** t-tests, Mann-Whitney U and Chi-squared tests used, as appropriate.

Table 3: Plasma calcium, phosphate, potassium and sodium concentrations and proportions of infants affected by electrolyte derangement and sepsis in the two epochs of different phosphate supplementation

	Epoch 1 N = 51	Epoch 2 N = 49	P-value***
Calcium			
Peak Ca ²⁺ concentration, mmol/L*	3.09 (3.02 – 3.17)	2.83 (2.78 – 2.89)	<0.0001
Postnatal day of peak Ca ²⁺ concentration**	5 (4 – 7)	6 (5 – 7)	0.73
Ca ²⁺ >3.0 mmol/L, n (%)	31 (61)	10 (20)	<0.0001
Phosphate			
Nadir PO ₄ concentration, mmol/L	1.32 (1.21 – 1.43)	1.54 (1.42 – 1.65)	0.006
Postnatal day of nadir PO ₄ concentration**	5 (4 – 6)	5 (2 – 7)	0.29
PO ₄ <1.5 mmol/L, n (%)	31 (61)	17 (35)	0.009
PO ₄ <1.0 mmol/L, n (%)	11 (22)	7 (14)	0.34
Potassium			
Nadir K ⁺ concentration, mmol/L	3.6 (3.4 – 3.7)	3.7 (3.5 – 3.8)	0.25
K ⁺ <3.5 mmol/L, n (%)	23 (45)	12 (25)	0.03
Sodium			
Peak Na ⁺ concentration, mmol/L	146 (145 – 147)	148 (146 – 149)	0.13
Na ⁺ >150 mmol/L, n (%)	9 (18)	14 (29)	0.19
Sepsis, n (%)	8 (16)	5 (10)	0.42

Data are arithmetic mean (95% CI) or n (%) unless otherwise stated

* Geometric mean (95% CI) presented for peak Ca²⁺ concentration data

** Median (IQR) or n (%)

*** t-tests, Mann-Whitney U and Chi-squared tests used, as appropriate