Impact of an updated electrolytic supply protocol in hydroelectrolytic disorders in very preterm infants receiving optimized parenteral nutrition. A historical cohort study.

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1. ABSTRACT

Background: In the last years recent guidelines of parenteral nutrition for very preterm infants recommend a higher and earlier intake of amino acids (AA) and energy to avoid postnatal catabolism, with the goal of mimicking the AA dose that the foetus would receive *in utero*. However, this new approach can be associated with important metabolic disturbances in the first days of life, particularly influencing the metabolism of potassium, phosphorus and calcium. When phosphorus is taken up by cells, without being adequately provided by the administered nutrition, the level in blood falls. The majority of body phosphate and calcium are located together in the skeleton, and the two minerals are tightly connected to each other, when plasma phosphate levels drop, an increase in bone resorption takes place in order to restore plasma phosphate, as adequate levels are essential for tissue growth and development.

Objective: This study aims to analyze the efficacy of a new parenteral nutrition protocol with updated electrolytic content in reducing the appearance of hydroelectrolytic disorders and its possible impact in associated comorbidities, contributing as a result to audit and improve clinical practice for the optimization of growth and development of very preterm infants.

Design: Retrospective cohort study.

Setting: Hospital Sant Joan de Déu. Barcelona, Spain.

Keywords: Very preterm infants, aggressive parenteral nutrition, amino acid, calcium balance, phosphate balance.

2. ABBREVIATIONS

VPI – Very Preterm Infant.

ROP – Retinopathy Of Prematurity.

CNS – Central Nervous System.

IVH – Intraventricular Haemorrhage.

PN – Parenteral Nutrition.

PPN – Partial Parenteral Nutrition.

EN – Enteral Nutrition.

SPN – Standardized Parenteral Nutrition.

CVC – Central Venous Catheters.

ATP – Adenosine Triphosphate.

AA – Amino Acids.

TBW – Total Body Water.

TEWL – Transepidermal Water Loss.

PROM – Preterm Rupture Of Membranes.

INE – National Statistics Institute.

BPD – Bronchopulmonary Dysplasia.

NEC – Necrotizing Enterocolitis.

VON – Vermont-Oxford Network.

AVP – Arginine vasopressin.

MBD – Metabolic bone disease.

RTC – Randomized clinical trial.

RIG – Restricted intrauterine growth.

3. INTRODUCTION

- **The Very Preterm Infant.**

The neonatal period spans the first 28 days of life, and it is the time with the highest rate of mortality in childhood. Appropriate feeding and care will maximize the child's chances of survival (1).

Preterm birth is defined as a delivery occurring at less than 37 completed weeks or 259 days of gestation and is a major determinant of neonatal morbidity and mortality with short and long-term adverse consequences for health (2–4). Etiology of preterm birth is not completely understood, although it is thought to be multifactorial and the result from the interaction of several pathways (5).
Intra-amniotic infection has been causally linked to spontaneous preterm delivery through the production of chemokines and cytokines (IL-1α, TNFα), as well as inflammatory mediators (prostaglandins and reactive oxygen radicals) and proteases by the stimulation of microbial colonization which ascends into the amniotic cavity from the lower genital tract. These products can initiate myometrial contractility and induce membrane rupture. On the other hand, a subset of patients with preterm labour have placental vascular lesions, including failure of physiologic transformation of the uterine spiral arteries (6), resulting in preeclampsia or intrauterine growth restriction and elective premature delivery.

Children born prematurely have higher rates of cerebral palsy, learning disabilities, sensory deficits, respiratory illnesses and feeding issues compared with children born at term. Most mortality and morbidity affect very preterm neonates, those born at or before 32 gestational weeks and especially those born extremely preterm, at or before 28 weeks of gestation (2,3,7).

Very preterm infants (VPI) may suffer complications during their early postnatal life due to the immaturity of their organs, such as respiratory illnesses (hyaline membrane disease and bronchopulmonary dysplasia), patent *ductus arteriosus*, retinopathy of prematurity (ROP) and central nervous system (CNS) injury, whether due to intraventricular haemorrhage (IVH) or white matter involvement. The risk of complications increases with increasing immaturity and can be associated to different extents of subsequent neurodevelopmental sequelae and motor and cognitive disorders (8). Some reports also suggest that VPIs have an increased risk of adult metabolic diseases such as insulin resistance and hypertension (9), perhaps in relation to the presence of growth recovery, as is the case in low birth weight infants (10). Complications of prematurity can often result in enormous psychological, physical and economic costs in terms of the immediate neonatal intensive care and ongoing long-term complex health needs. They also result in an increased need for special education services and associated costs that will place and additional burden on affected families and governments (11–14).

- **Parenteral nutrition in the Very Preterm Infant.**

Some nutritional concepts are relevant in the management of very preterm infants involved in the present analysis. Parenteral nutrition (PN) is the provision of nutrients through intravenous infusion, usually through central venous catheters placed in the umbilical vein or another location (15,16) in order to cover requirements for normal metabolism and growth. PN can be used in all children that are malnourished or at risk of malnutrition secondary to a digestive or extradigestive, acute or chronic disease in order to meet nutritional needs for health and growth. It is important to keep in mind the possibility of associated complications with the use of central venous catheters (CVC), some of them related to catheter insertion such as pneumothorax, laceration of a vessel, arrythmias, air embolism, nerve plexus injury and also breakage or accidental displacement, occlusion, venous thrombosis and infection. Hence, PN is not indicated in patients with correct bowel function in which nutrition could be carried out orally or enterally. Enteral Nutrition (EN) is a nutritional support technique in which nutrients are administered directly into the gastrointestinal tract through a feeding tube. Strictly speaking, oral administration of artificial formulas is not conceptually accepted as EN. The choice of enteral formula will depend on factors like age, severity of illness and underlying diagnosis, and it needs to be individualized upon assessing the quantity and quality of nutrients (15,17). Partial Parenteral Nutrition (PPN) provides a supplement in contribution with the EN to ensure fulfilling the requirements.

Recommendations for starting a newborn on PN are:
-Predicted fasting period ≥2 days or enteral intake >50% of needs.
-In preterm infants, especially those ≤32 weeks of gestation, from the first hours of life to maintain growth similar to intrauterine rates (18).

Due to the high risk of growth failure and neurodevelopmental impairment in many VPIs, many experts suggest "early and aggressive" nutritional strategies (19). According to the statement of the American Academy of Pediatrics', the goal of postnatal nutrition in preterm infants is to provide nutrition that will duplicate *in utero* growth rate and body composition of the foetus at the same gestational age due to the benefits on growth and developmental outcomes, improving the anabolic state and cellular growth in VPIs (19,20). The first suggestions for the use of parenteral nutrition in preterm babies were extremely cautious, based on the fear that immature metabolic systems would not be able to appropriately process nutrients, exposing the babies to risks of toxicity. This brought about a postnatal situation of cellular catabolism after the abrupt decrease in aminoacids (AA) following the interruption of the umbilical flow of nutrients from the mother through the placenta after birth, resulting in a reduced secretion and activity of insulin with a drop of glucose transport into the cell and a fall of Na^+, K^+-ATPase activity, resulting in intracellular energy failure (20).

Later studies showed that “early and aggressive” nutritional support was well tolerated and could normalize postnatal growth and simultaneously improve long term neurodevelopmental outcomes in preterm infants. For this reason, during the past several years, adaptations in PN protocols for the management of preterm infants, have aimed to increase doses of parenteral macronutrients, mostly AA from the first hours of life in order to promote positive nitrogen retention and growth as early as possible (20–24).

However, this new approach can be associated with important metabolic disturbances in the first days of life, particularly influencing the metabolism of potassium, phosphorus and calcium, defining a syndrome that simulates what happens in re-feeding conditions after intense nutritional deprivations such as in Kwashiorkor (16,24,25). This is the hypothesis for the mechanism of Placental Incompletely Restored Feeding syndrome of the preterm infant (PI-ReFeeding syndrome), in which parenteral supply of AA and energy maintains the cell in an anabolic state and promotes its uptake of phosphorus and potassium, causing a decrease of their plasma concentrations in the absence of adequate intake (22–25). Therefore it looks that higher parenteral AA administration has a positive effect on postnatal weight accrual by stimulating protein synthesis, however, a high intake of AA induces a depletion of phosphate due to accelerated protein synthesis transferring phosphate into cells for adenosine triphosphate (ATP) production and this facilitates a condition of hypophosphatemia and hypercalcemia in VPIs at the end of the first week of life due to compensation by increased bone resorption (25,26). Individualization of fluid and electrolyte intakes has been regarded as the best policy to avoid metabolic disturbances in comparison to previous standardized parenteral nutrition (SPN) (21).

For all of these reasons, nutrition in the postnatal period is an important aspect of care in hospitalized children. Both under and overnutrition have negative effects in VPIs, who might be at an increased risk of evolutionary adaptation or “programming” posing them at risk of later metabolic diseases (16). This theory sustains that nutrition during the foetal period and during the first months of life influences the expression of certain genes that establish the functional capacity, the metabolic competence and the future responses to interactions with the environment (27). Although some consequences depend on dietary modifications, the content of micro and macronutrients or the rate of growth of the newborn, a mechanism for “programming” could be an epigenetic modification of critical genes for metabolic homeostasis (16).

- **Hydroelectrolytic disorders in Very Preterm Infants.**

Fluid and electrolyte management in VPIs continues to be a challenge in intensive care. It is relevant for preventing morbidity and mortality, but requirements become difficult to achieve. During the first week of life, healthy neonates lose an average of 5 to 10% of their body weight whereas VPIs can experience a disproportionately large loss because of their large body surface area and the immaturity of their skin. Rapid loss of free water from the interstitial space can produce a hyperosmolar extracellular compartment characterized by hypernatremia and accompanied by hyperglycemia and hyperkalemia (28,29).

Fluid in the foetus and neonate is distributed among three main compartments: plasma, interstitial fluid and cellular fluid. The amount of fluid in each of these compartments changes throughout the neonatal period. In the early foetal period, approximately 95% of the foetus is water, with the proportion gradually decreasing up to 75% at term (29,30). While living within the uterine environment, the neonate is immersed in fluid, the lungs are filled with liquid, the skin is porous and lacks a keratin layer, urine output is high and renal concentrating ability is limited (29).

During labour and delivery, foetal plasma and blood volumes change. Once born, the infant requires adaptation to an abrupt introduction to gaseous and low humidity status (30) followed by systemic changes such as loss of body water and limited sodium excretion (21,29–32). There is a contraction of the extracellular fluid compartment, followed by natriuresis, diuresis and weight loss (29). As the composition of extracellular fluid is mainly sodium and water, sodium and water balance is negative during the postnatal adaptation phase (30,33). All this changes result in a loss of about 10% of total body water (TBW) (29).

This condition is more severe in VPIs, where Transepidermal water loss (TEWL) is the most important cause of postnatal loss of fluids in preterms because of the immaturity of their skin. The highest losses occur during the first days after birth. It is important to consider that skin maturation in preterm infants, unlike the maturation of renal function, is not accelerated by antenatal steroid exposure but is accelerated by birth. According to this, TEWL falls exponentially with increasing postnatal as well as gestational age. In infants born at 24-25 weeks of gestation, TEWL is about 60g/m2/h (about 140ml/kg/day) at a relative humidity of 50% in the first two days after birth, and decreases by day 3 to around 45g/m2/h (105ml/kg/day) and to 24 g/m2/h (55ml/kg/day) by 28 days. By 32 weeks gestation, TEWL has the same magnitude as full term infants at 6-8 g/m2/h (about 12ml/kg/day) (30). Because of all of this, fluid intake should cover insensible water loss and renal loss of about 30ml/kg/day during the weight loss period, and about 60ml/kg/day thereafter. Loss of body water after birth has been analysed in some reports, but few of them have examined the effect on body composition of different regimens of supplementation of sodium and other electrolytes (32).

After birth, hyponatremia and hypernatremia are commonly observed in VPIs and often occur in the same newborn, particularly when receiving diuretic treatment (31,33). Due to the highest losses of water than sodium,

especially through the immature skin, dehydration leads to hypernatremia in the VPI, so high sodium levels in these patients are usually attributable to lack of fluid intake and/or excess loss of water, rather than excess sodium intake. Complications attributable to dehydration include cerebral oedema and thrombosis, intracranial bleeding, cardiovascular collapse, and severe hyperbilirubinemia (31). Current management guidelines recommend delaying sodium supplementation until the infant has experienced the expected weight loss and until sodium serum falls <130mEq/L. (21,29), but it is important to consider that the VPI receives an enormous inadvertent sodium load. Sources of this additional sodium include calcium gluconate, gentamicin, sodium bicarbonate, dopamine, dobutamine, heparin and intravenous fluids used for catheter patency (29).

Hyponatremia is attributable to renal wasting, with impaired reabsorption at the proximal renal tubule, resulting in greater distal sodium delivery, and limited aldosterone responsiveness at the distal tubule. Intestinal absorption is also limited, and oral sodium intake is often low (31,33). The increased natriuresis due to renal tubular immaturity can lead to protracted volume contraction, which can stimulate aldosterone and arginine vasopressin (AVP) release, allowing further water retention and the progression of late hyponatremia. This excessive fluid and water balance with hyponatremia within one week after birth seems to be associated with the development of BPD (34).

Before the “early feeding era” there were some well-known metabolic disturbances, such as hypocalcemia, hypoglycemia, non-oliguric hyperkalemia and hyperphosphatemia, which were the reflection of a catabolic state and represented a neonatal syndrome related to the interruption of the continuous nutritional placental flow after birth (Placental Interrupted Feeding syndrome of the preterm infant) (25).

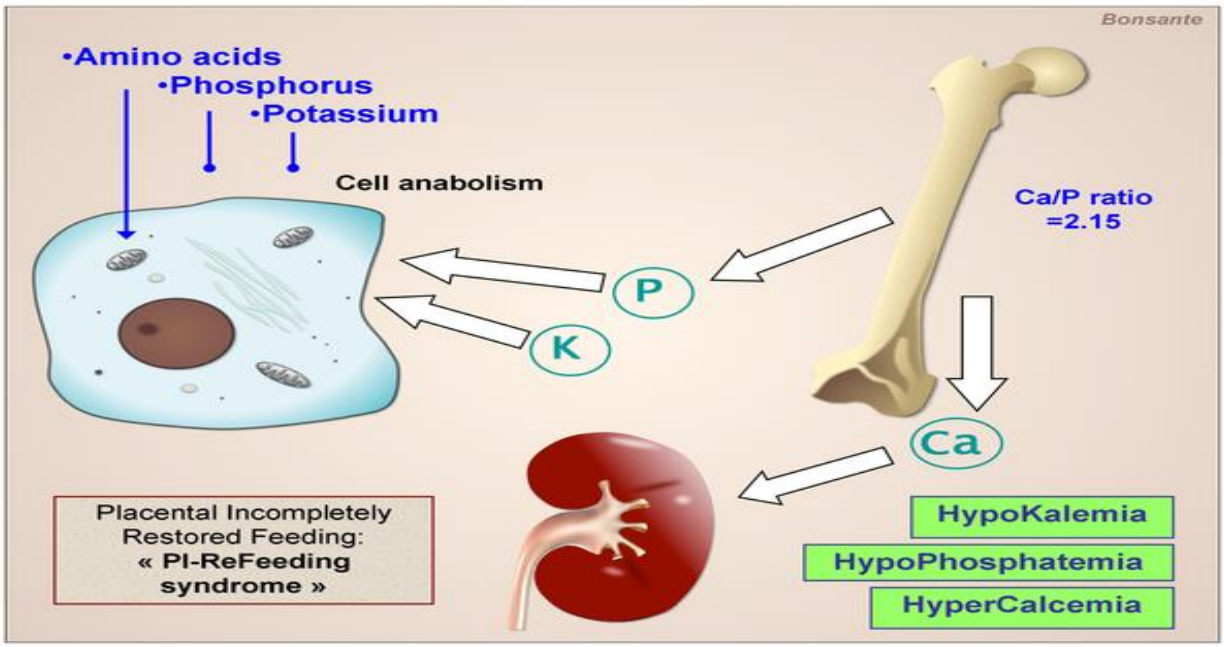
During the last trimester of gestation, phosphate, calcium and AA are transferred from the mother to the foetus in higher amounts than at any other time during pregnancy, in order to facilitate growth and mineralisation of the skeleton (23,25,26). The intracellular phosphorus to nitrogen ratio is not stable and it increases in rapidly growing tissues. Also, phosphate content represents the main anion in the intracellular space and it is part of the composition of nucleic acids, ATP and the cell membrane (25,26). When phosphorus is taken up by cells without being adequately provided by the administered nutrition, the level in blood falls. The majority of body phosphate and calcium are located together in the skeleton, and the two minerals are tightly connected to each other, when plasma phosphate levels drop, an increase in bone resorption takes place in order to restore plasma phosphate, as adequate levels are essential for tissue growth and development (25,26). At birth, preterm infants are deprived of this provision of nutrients and it is important to restore the supply to ensure a postnatal growth rate that corresponds to the intrauterine rate of growth (23,25,26). Due to metabolic interactions of phosphate and calcium, phosphate concentration disturbances will also induce variations in calcium plasma levels and urinary excretion during the bone resorption and formation process (22,23). The drop in plasma calcium levels after birth

makes PTH levels increase in response. In the kidney, PTH enhances calcium reabsorption while decreasing phosphate reabsorption, leading to urinary phosphate wasting, and in bone, PTH stimulates resorption and subsequent release of calcium and phosphate resulting in a disbalance in calcium and phosphate levels in the form of hypercalcemia and hypophosphatemia. With insufficient calcium intake over time and the high degree of skeletal growth occurring in the first weeks after birth, these biochemical changes can persist and accompany metabolic bone disease (MBD) (35). Nowadays, the disturbances observed are related to a suboptimal provision of electrolytes after the Placental Feeding Disruption, since the restoration of a continuous flow of AA and energy is not accompanied by the intake of micronutrients (22,24). In this sense, it looks that higher AA intake immediately after birth, is effective to modify K^+ metabolism, by reducing non-olyguric hyperkalemia and urinary K^+ loss (33).

This depletion of ATP secondary to hypophosphatemia inhibites chemotaxis, initial phagocytosis with acquired phagocyte dysfunction and bactericidal activity, explaining the relation between hypophosphatemia and septicemia (36).

This phenomenon of phosphorus deprivation may also be observed in preterm infants enterally fed when a supplementation of protein is not accompanied by a modification of the Ca^{2+}/P ratio. Therefore, the association of hypokalemia, hypercalcemia and hypophosphatemia may effectively describe a new syndrome in premature infants, linked to early aggressive parenteral nutrition (22).

FIGURE 1: Hypothesis for the mechanism of Placental Incompletely Restored Feeding syndrome of the preterm infant (PI-ReFeeding syndrome) (25).

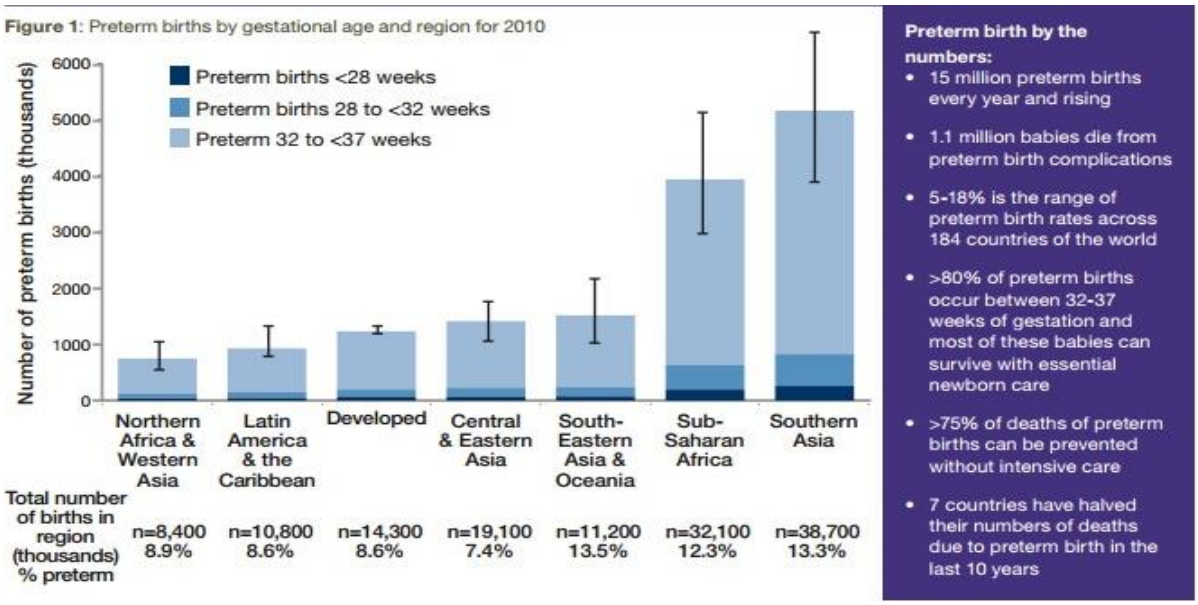


- **Epidemiology.**

Rates of preterm birth have been reported to range from 5% to 7% of live births, and estimated to be higher in developing countries (6). Of those, approximately 45 to 50% are idiopathic, 30% are related to preterm rupture of membranes (PROM) and another 15 to 20% are attributed to medical indicated or elective preterm deliveries. Premature babies represent 28% of all early neonatal deaths (those within the first 7 days of life) (5).

In 2005, about 12.9 million births occurred before term. From this burden, 10.9 million births were concentrated in Africa and Asia, while 0.9 million occurred in Latin America and the Caribbean and 0.5 million preterm births occurred in Europe and North America (37). In the poorest countries, on average, 12% of births are preterm infants compared to 9% in higher income countries. Within countries, poorer families are at higher risk (5). It is also important to notice that preterm births have a tendency to recur in subsequent pregnancies, with the recurrence risk appearing the greatest when the preterm birth in the first pregnancy occurred at <28 weeks of gestation, and progressively declining with advancing gestation (38).

FIGURE 2: Preterm births by gestational age and region for 2010 (5).



In Spain, rates of prematurity have increased in the last 20 years. On data available for the last 10 years from the National Statistics Institute (INE), the overall rate of prematurity between 1996 and 2006 ranged from 5.84% to 6.84%. There are differences between Spanish regions and hospitals, in some cases exceeding 10% of total births. Preterm births with gestational age less than 32 weeks remained relatively stable, being between 1 and 2% of all births (39).

As stated in our Hospital database, approximately 80 VPI of ≤32 weeks of gestation are admitted each year at our Neonatal Unit.

There is a variable survival rate between centres. In a multicentre database, tertiary referral centres participating in the Oxford-Vermont network reported in 2011 a survival of 34% at 23 weeks of gestational age, from 61% at 24 weeks, 79% at 25 weeks and 87% to 26 weeks. Data from the Spanish Society of Neonatology in 2013 show that mortality varies according to weight and gestational age, reaching numbers above 82.9% in the extreme for preterms with <24 weeks of gestational age and descending as gestational age advances, being from 9,7% at 28 weeks of gestation and less than 5% at 29-30 weeks of gestation (16,40).

- **Cost of prematurity.**

The adverse effects resulting from preterm birth impose a considerable burden on finite resources on fields such as healthcare, education, social services and families. For that reason, while talking about costs, we are referring to an ambitious term which encompasses from direct medical costs including outpatient and inpatient cost areas; direct-non medical costs for education, travelling, accommodation and child care; indirect costs such as parental time and/or wage losses and also, intangible costs, associated with the birth and caring for preterm infants (12–14).

Preterm infants are significantly more likely to be re-hospitalized than infants born at full term and re-hospitalization together with outpatients visits, pharmaceuticals and medical aids are one of the most relevant cost components after the neonatal period (12–14). Preterm birth is accompanied by broad emotional and financial costs and lost opportunities for families. This often makes a negative overall impact associated with the loss of quality of life which adds physical and emotional burden to the process of caring for the infant. Mothers of preterm infants are at a particularly great risk of psychological distress (11,32).

According to statistics, the burden associated with preterm birth in the United States was in excess of 26.2 billion USD in 2005, or 51.600 USD per infant. The share that medical care services contributed to the total cost was approximately 16.9 billion USD (33.200 USD per preterm infant), maternal delivery costs contributed another 1.9 billion USD (3.800 USD per preterm infant), special education services associated with a higher prevalence of disabling conditions among preterm infants added 1.1 billion USD (2.200 per preterm infant). Newborns who weighed <2500g at birth were almost 50% more likely to be enrolled in any type of special education than newborns who were of normal weight at birth, resulting in an estimated incremental cost to the United States education services of 322.9 million GBP per year. Whereas lost household and labor market productivity associated with such disabling conditions contributed 5.7 billion USD (11.200 per preterm infant) (11,12,14).

According to the gestational age, the range of costs per surviving newborn is considerable. Hospital costs for preterm infants born during the 25th week reach 202.700 USD, and 46.400 USD for preterm infants born during the 30th week, decreasing to only 1.100 USD for a 38-week newborn (14).

In the European context, the average overall two year costs are 104.635 EUR for surviving preterm infants, compared to 3.135 EUR for at term infant. It is estimated that families spend from 2 to 4% of their gross annual income on non-reimbursed payments attributable to their infant's condition (14).

4. STUDY JUSTIFICATION

Current nutritional practices for preterm infants, particularly for those ≤32 weeks of gestation, are suboptimal because recommended nutritional supplies do not achieve a pattern of growth and organ maturation similar to the intrauterine model (41).

At the beginning, immediately after birth, most of the nutrients must be administered parenterally because of the immaturity of the gastrointestinal tract and, at times, because of their clinical severity. In the past, fear of overloading an immature metabolic system translated into a limitation on doses of parenteral nutrients, forcing premature babies to degrade their own structural proteins, leading to a catabolic state, especially during the first weeks of life (42). In the last decade, optimized nutritional protocols have been proposed to increase the caloric and protein intake (43,44).

In keeping with these guidelines, the Neonatal Unit at Hospital Sant Joan de Déu in Barcelona, Spain, updated the proposed parenteral nutritional supplies for VPIs in 2005, with a higher basal macronutrient content from day one and a progressive increase over the first week. Following these changes, an increase in electrolytic disturbances in this population during the first week of life was noted by the clinical staff, mostly in the form of hypercalcemia and also hypokalemia. In the last five years, several publications have reflected the same situation in other neonatal units throughout the world, whether within updates on clinical practice or in the context of clinical trials regarding nutritional optimization (19,25). These findings seem the result of the "Placental Incompletely Restored Feeding syndrome of the preterm infant", in which a successful induction of an anabolic state by the parenteral supply of sufficient AA and energy, results in a depletion of phosphate for the synthesis of cellular mass and adenosine triphosphate (ATP) and results in hypophosphatemia and hypercalcemia (22–26).

In June 2015, the suggested parenteral nutrition management of these very preterm infants was changed again, mainly in the form of more adequate ionic contributions to try and prevent hydroelectrolytic derangements.

This project aims to analyze the efficacy of the new parenteral nutrition protocol in reducing the appearance of hydroelectrolytic disorders and its possible impact in associated comorbidities, contributing as a result to audit and improve clinical practice for the optimization of growth and development of very preterm infants. Although ion and mineral disturbances have been described in the context of RCTs on improved nutrition in this population, or reported as a side effect of parenteral nutrition, no study to date has compared the efficacy of a specific change in the electrolyte supply to prevent this undesired consequence of nutrition in VPIs.

5. HYPOTHESIS

The early addition of phosphorus in the electrolytic supply in the new parenteral nutrition protocol will reduce the incidence and severity of hydroelectrolytic disorders in VPIs, while maintaining appropriate macronutrient doses. This will associate an improvement in comorbidities potentially associated with hydroelectrolytic balance in VPIs, like BPD (bronchopulmonary dysplasia), nosocomial sepsis or bone metabolic disease of prematurity.

6. OBJECTIVES

- **Main objective**

– To compare the incidence of hypercalcemia (hypophosphoremia when possible) in the VPI of ≤32 weeks of gestation admitted to Hospital Sant Joan de Déu between the different parenteral nutrition protocols in two periods, before and after June 2015.

- **Secondary objectives**

– To assess the implementation rate of the PN protocol changes.

– To compare the incidence of other electrolytic disturbances (hypokalemia and hyper/hyponatremia) in the VPIs between the two PN protocols.

– To compare the incidence of clinical outcomes potentially related to hydroelectrolytic management between VPIs treated under each of the two PN protocols.

-Initial postnatal weight loss.
-Growth during admission.
-Nosocomial sepsis.
-Bronchopulmonary Dysplasia (BPD).
-Necrotizing enterocolitis (NEC).
-Intraventricular haemorrhage (IVH).
-Retinopathy of Prematurity (ROP).

7. METHODOLOGY

7.1. STUDY DESIGN

This study is designed as a retrospective cohort study. Cohort 1 will comprise patients born before June 2015 who received PN under protocol 1 and cohort 2 patients admitted after the date of protocol change. Data will be collected by retrospective revision or clinical charts, records of blood test results and data entered in the electronic databases of the benchmarking networks in which the unit participates (SEN1500 and VON).

7.2. STUDY POPULATION

- **Inclusion criteria:**

-Infants born at ≤32 weeks of gestation receiving parenteral nutrition in the Neonatal Unit at Hospital Sant Joan de Déu in Barcelona before and after June 2015.
-Infants born in other centres and admitted to the Neonatal Unit of Hospital Sant Joan de Déu within 24 hours of birth, receiving parenteral nutrition before and after June 2015.

- **Exclusion criteria:**

-Major congenital malformation.
-Chromosomal abnormalities.
-Survival for less than 48 hours (PN-associated electrolytic disturbances usually appear after this period).
-Any underlying disease that justifies a modified nutritional protocol (for example: inborn errors of metabolism).

7.3. SAMPLE SELECTION

The sample will be selected, based upon inclusion and exclusion criteria, from the admission records of the Neonatal Unit. A non-probabilistic consecutive sampling method will be used. Subjects that fulfil the above mentioned criteria will be recruited.

7.4. SAMPLE SIZE

The sample size and power calculator "GRANMO" (45) was used to calculate sample size. By accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-side test and using the POISSON approximation, 64 exposed subjects (PN protocol 1) and 64 non-exposed (PN protocol 2) subjects are necessary to recognize as statistically significant a relative risk greater than or equal to 0.3. The incidence in the non-exposed group (PN protocol 2) has been estimated to be 0.3 based on our hospital database. A dropout rate of 0% is anticipated because data collection is restricted to information obtained during admission, being readily available in the Hospital records.

PN protocol 2 was implemented in June 2015. Approximately 80 VPI of ≤32 weeks of gestation are admitted each year at our Neonatal Unit, so recruitment would cover a period of 10 months before and 10 months after the change of protocol.

7.5. STUDY VARIABLES

- **Independent variable:**

-Parenteral Nutrition Protocol 1 vs 2

This variable defines the two cohorts, one with VPIs of ≤32 weeks of gestation who were treated with Protocol 1 of PN, and the second one with VPIs of ≤32 weeks of gestation who were treated with Protocol 2 of PN.

In Protocol 1, AA intake in VPIs was started on day one (2.5g/Kg/day) and increased daily up to 3g/kg/day at day three, decreasing at the end of the first week up to 2g/Kg/day, Ca^{2+} infusion was started on the 1st day of life at 2mEq/Kg/day and P infusion was started on the 2nd day of life. In protocol 2, the initial amount and the rate of AA increase in VPIs was of 2.5g/Kg/day on the 1st day, increasing 0.5g/Kg/day up to 4g/Kg/day at day 4th, Ca^{2+} infusion was started on the 1st day of life at 2mEq/Kg/day and P infusion was started on the 1st day of life at 0.4 Mm/Kg/day. Being the main difference between the protocols the addition of phosphorus on day one.

FIGURE 3: Parenteral Nutrition Protocol 1 (before June 2015). Adapted from: Nutrición parenteral para el prematuro < 1500g (46).

	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**
Recommended total liquids (ml/kg/d)	80-90	90-100	100-110	110-120	120-130	130-140	140-150	150-160
Proteins (g/kg/día)	2,5	3	3,5	3,5	3,5	3,5	3,5	3,5
Glucose (g/kg/d)[1]	8,6	10	11,5	12,9	14,4	15,8	17,2	17,2
Lipids (g/kg/día)	2	2,5	3	3	3	3	3	3
Kcal/kg/día	64,4	77	90	95,6	102	107	113	113
g N^2/Kcal no prot	1/136	1/135	1/135	1/145	1/156	1/166	1/175	1/175
Minimal iv peripherical liquids for PN (ml/kg/d)	92	65	73	80	88	95	102	102
Minimal iv central liquids for PN (ml/kg/d)	53	105-115	127-138	137-146	145-155	153-162	161-172	161-172
Na^+ (mEq)	0	0-3	1-3	1-3	1-3	1-3	1-3	1-3
K^+ (mEq)	0	0	0-3	1-3	1-3	1-3	1-3	1-3
Cl^- (mEq)	0	0-3	1-3	1-3	1-3	1-3	1-3	1-3
Ca^{2+} (mEq)	2	2	1-2	1-2	1-2	1-2	1-2	1-2
Mg^{2+} (mEq)[2]	0	0,5	0,5	0,5	0,5	0,5	0,5	0,5
P (mmol)	0	0-1	0,5-1	0,5-1	0,5-1	0,5-1	0,5-1	0,5-1
Carnitine[3] (mg/kg/day)	10	10	10	10	10	10	10	10
Heparin[4] (mg/100 ml)	1	1	1	1	1	1	1	1
Vitamins/ Trace elements	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

1- If maintained hyperglycemia >150mg/dl appears, start insulin perfusion according to pattern.

2- Not in children of mothers treated with magnesium sulphate.

3- In preterm <34 gestational weeks.

4- In preterm <26 gestational weeks or difficulty in lipid clearance.

FIGURE 4: Parenteral Nutrition Protocol 2 (from June 2015). Adapted from: Nutrición parenteral en el recién nacido (18).

	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**
Glucose (g/kg/day)[1]	7,2-8,6	8,6	11,5	12,9	14,4	15,8	17,2	17,2
Proteins (g/kg/day)	2,5	3	3,5	3,5-4*	3,5-4*	3,5-4*	3,5-4*	3,5-4*
Lipids (g/kg/day)[2]	2	2,5	3	3	3-3,5*	3-3,5*	3-3,5*	3-3,5*
Kcal/kg/day	59-64,4	71	90	95-98	102-108	107-114	111-119	111-119
Na^+ (mEq)[3]	0,4	0	1-3	2-4	3-4	4-6	4-7	4-7
K^+ (mEq)[4]	0	0,8!!	1-3	2-4	3-4	4-6	4-7	4-7
Cl^- (mEq)[5]	0	0,2						
Ca^{2+} (mEq)[6]	2	2	1-4	1-4	1-4	1-4	1-4	1-4
Mg^{2+} (mEq)[7]	0	0,5	0,5	0,5	0,5	0,5	0,5	0,5
P (mmol)[8]	0,4 (inorgan.)	0,8 (inorgan.)	1-1,5	1-1,5	1-1,5	1-1,5	1-1,5	1-1,5
Vitamins/ Trace elements	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Illustrative volume[9] **(and minimum)** (ml/kg/d)	70-80 (60)	90-100 (85)	100-110 (73)	110-120 (80)	120-130 (88)	130-140 (95)	140-150 (102)	150
Rel cal no prot/N2	122-136	124	135	146	156-144	166-153	182-176	182-176
Osm. Aprox. Mosm/L	1263 (1516)	1063 (1136)	1035	1022	1000	1091-1136	1150-1102	1102

*In VPI <28 gestational weeks these are ideally contributions to achieve: [1]glycemia 80-125mg/dl/ [2]triglycerides (in sepsis, thrombocytopenia, hyperbilirubinemia) <250mg/dl/ [3]natremia 135-145mEq/L/ [4]Kalemia 4,5-5,5 mEq/L in non hemolyzed serum/ [5]cloremia 95-110 mEq/L/ [6]ionic calcium 1,1-1,4 mmol/L, total calcium 7,5-10 mg/dl/ [7]magnesemia 1,8-2,7 mEq/L/ [8]phosphoremia 5,5-10,9 mg/dl/ [9]volume to achieve a weighy loss <10% except in edema, hydrops and avoid hydration (hyponatremia or edema in the first days of life). !!To start potassium only when established diuresis.

- **Dependent variables and assessment.**

-Hydroelectrolytic disorders:

A blood gas analyzer (Radiometer®ABL90) is available within the Neonatal Unit allowing for the analysis of electrolytic parameters, including serum sodium, potasium and calcium in a small capillary blood sample (0.2ml). The study of phosphatemia requires a greater volume of blood (0.6 ml) and the test is performed in the clinical laboratory. Repeated blood sampling is the most important cause of anemia needing transfusion in VPIs (47), who have an average blood volume of 80ml/kg. Hypercalcemia results from hypophosphoremia due to compensatory mechanisms, and will be used as a proxy for hypophosphoremia in those patients in which phosphorus has not been directly measured.

Mean daily values of sodium, potassium, chloride, calcium and phosphorus will be collected for the first two weeks of life, as well as daily enteral and parenteral supply of total fluid (ml/kg/day) and each of the electrolytes, urine output (ml/kg/day) and the humidity in incubator (%) if needed.

-Hyponatremia will be defined as blood sodium level of <130mmol/L in blood and it will be classified as **severe hyponatremia** if the blood sodium level is <125mmol/L.

-Hypernatremia will be defined as blood sodium level of >145mmol/L in blood and it will be classified as **severe** if the blood sodium level is >150mmol/L.

-Hypokalemia will be defined as a blood potassium level of <2.5mmol/L.

-Hypercalcemia will be defined as a blood calcium level of >1.35mmol/L and will be considered **severe** if the blood calcium level is >1.75mmol/L.

-Hypophosphatemia will be defined as blood phosphate level of <1.0 mmol/L.

A patient will be classified as presenting an electrolyte disturbance if the mean value of the electrolyte in blood falls within each definition. Incidence of electrolyte disturbances will be calculated per day and globally.

- Implementation of new protocol:

Given that the main difference between the protocols is the addition of phosphorus on day 1, implementation will be measured as “0=No” or “1=Yes” for prescription on phosphorus on day 1.

- Initial postnatal weigh loss will be assessed by the following variables:

Growth failure will be defined during the immediate postnatal period according Z-Scores loss: As “moderate”, when loss >1.34 of Z-Score, which corresponds to 2 bands percentiles or a decrease, for example, from P50 to P10; and as “serious”, when loss >2 of Z-Score or, for example, decreasing from P50 to P3 with respect intrauterine curves. In addition, it will be done a comparative analysis between patients who are at the top and

bottom quartile of growth upon reaching the age of term and a separate analysis of children with and without restricted intrauterine growth (RIG), defined as birth weight below the 3rd percentile or fetal weigh at birth below P10, plus evidence of fetal hemodynamic alterations by flow Doppler umbilical artery and middle cerebral artery on prenatal ultrasounds.

-Maximum percentage of weight loss with respect to birth weight.
-Age at maximum weight loss.
-Age at recovery of birth weight.

-Growth during admission:
Will be assessed by the following variables: Weight, length and head circumference at 28 days of life and 36 weeks postmenstrual age.

-Growth failure:
Defined during the immediate postnatal period according to loss of Z-Scores being "moderate", when loss of Z-score is >1.34, which corresponds to 2 bands percentiles or a decrease, for example, from P50 to P10; and "severe", when loss or Z-score is >2 or, for example, decreasing from P50 to P3 with respect to intrauterine curves.

-Other clinical outcomes:
Other neonatal complications potentially associated with hydroelectrolytic imbalances, will be defined by the presence or absence of the following items:

-**Time of admission to the NICU:** Defined by days of hospitalization (length of stay).
-**Nosocomial infection:** Defined by culture-proven sepsis, meningitis or pneumonia.
-**Survival to discharge.**
-**BPD:** Defined by the severity of lung disease as mild, moderate and severe, according to the need of oxygen and/or ventilator support at the time of diagnosis according to the NICHD (Eunice Kennedy Shriver National Institute of Child Health and Human Development) (48).

FIGURE 5: Bronchopulmonary Dysplasia (BPD) classification. Adapted from: Displasia broncopulmonar: definiciones y clasificación (48).

Grade/Severity	Definition
1/mild	Need for supplemental O_2 for ≥28 days but breathing room air at 36 weeks of post-menstrual age or at discharge, whichever occurs earlier, in less than 32 weeks gestational age or 56 days postnatal age or at discharge, whichever occurs before, in 32 weeks or more weeks gestational age
2/moderate	Need for supplemental O_2 for ≥28 days and FiO_2 < 30 % at 36 weeks of post-menstrual age or at discharge , whichever occurs earlier, in less than 32 weeks gestational age or 56 days postnatal age or discharge, which whichever occurs first , in 32 weeks or more weeks gestational age.
3/severe	O_2 need for ≥ 28 days and FiO_2 > 30 % and / or continuous positive airway pressure (nasal CPAP) or mechanically at 36 weeks of post-menstrual age or high ventilation , whichever occurs earlier, in less than 32 weeks of gestational age or 56 days postnatal age or at discharge, whichever occurs earlier, in the 32 or more weeks gestational age.
1.Ph*/mild	O_2 need for ≥28 days and document $SatO_2$ >90% with room air at 36 weeks of post-menstrual age or at discharge, whichever occurs earlier, in less than 32 weeks gestational age or 56 days postnatal age or at discharge, whichever occurs before, in 32 weeks or more weeks gestational age.
2.Ph*/moderate	O_2 need for ≥28 days and documented needed of FiO_2 < 30 % based on incapacity of maintain $SatO_2$ >90% after a reducing O_2 test at 36 weeks of post-menstrual age or at discharge, whichever occurs earlier, in less than 32 weeks gestational age or 56 days postnatal age or discharge, which whichever occurs first , in 32 weeks or more weeks gestational age.
3.Ph*	O_2 need for ≥ 28 days and FiO_2 > 30 % based on SaO_2 and / or continuous positive airway pressure (nasal CPAP) or mechanically at 36 weeks of post-menstrual age or high ventilation, whichever occurs earlier, in less than 32 weeks of gestational age or 56 days postnatal age or at discharge, whichever occurs earlier, in the 32 or more weeks gestational age.

*Ph: Physiologic.

-**NEC:** Defined as disease of stage IIA or higher from the modified Bell staging criteria for NEC (49).

FIGURE 6: Modified Bell Staging Criteria for NEC (49)..

Stage	Classification	Systemic signs	Intestinal signs	Radiologic signs
IA	Suspected NEC	Temperature instability, apnea, bradycardia, lethargy	Increased pregavage residuals, mild abdominal distention, emesis, guaiac-positive stool.	Normal or intestinal dilatation, mild ileus.
IB	Suspected NEC	Same as above	Bright red blood from rectum	Same as above
IIA	Proven NEC – mildly ill	Same as above	Same as above, plus absent bowel sounds, with or without abdominal tenderness	Intestinal dilation, ileus, pneumatosis intestinalis
IIB	Proven NEC – moderately ill	Same as above, plus mild metabolic acidosis, mild thrombocytopenia	Same as above, plus absent bowel sounds, definite abdominal tenderness, with or without abdominal cellulitis or right lower quadrant mass	Same as IIA, plus portal venous gas, with or without ascites
IIIA	Advanced NEC – severely ill, bowel intact	Same as IIB, plus hypotension, bradycardia, severe apnea, combined respiratory and metabolic acidosis, disseminated intravascular coagulation, and neutropenia	Same as above, plus signs of generalized peritonitis, marked tenderness, and distention of abdomen	Same as IIB, plus definite ascites
IIIB	Advanced NEC – severely ill, bowel perforated	Same as IIIA	Same as IIIA	Same as IIB, plus pneumoperitoneum

-**IVH:** Standard images will be taken at 3 days of life and weekly or biweekly day after. Injury will be classified according to the lesion with the most severe grading.

Haemorrhage:

-H0: No haemorrhage.

-H1: Localised Haemorrhage (subependymal or choroidal).

-H2: Intraventricular Haemorhage with no ventricular dilatation.

-H3: Intraventricular Haemorrhage with ventricular dilatation.

-H4: Parenchymal/Periventricular Lesions.

Ventricles:

-V0: No ventricular dilation.

-V1: Ventricular dilation not requiring any surgical intervention (including taps).

-V2: Dilation requiring surgery or taps.

Parenchymal Cysts:

-P0: No cysts.

-P1: Porencephalic cysts.

-P2: Cystic leukomalacia.

-ROP: Screening will be done in all participating infants. First examination will take place between 4 and 6 weeks postnatal age. Any subsequent examinations will be at the ophthalmologists discretion but all infants should be seen a minimum of fortnightly until they reach 36 weeks corrected gestational age. If there is no evidence of ROP by 36 weeks corrected gestational age no further follow up is necessary. If ROP has been identified the infants are followed up until it is clear regression has occurred OR that progression to the point of treatment has been reached.

Staging:

-Stage 1: Demarcation line.

-Stage 2: Ridge.

-Stage 3: Extraretinal fibrovascular proliferation

-Plus disease: Dilation and tortuosity of posterior retinal vessels.

-Stage 4: Retinal Detachment. (50).

7.6. VARIABLE DESCRIPTION AND STATISTICAL ANALYSIS

Univariant analysis

- Independent variable:
  - **Parenteral Nutrition Protocols:**

  Values will be defined as 0: PN Protocol 2 and 1: PN Protocol 1.

  It will be presented as a nominal qualitative variable.

- Dependent variables:
  - **Hydroelectrolytic disorders:**
    - Mean daily value of each electrolyte in blood. Continuous quantitative values. Defined by mean and standard deviation.
    - Mean daily supply of fluid and each electrolyte. Continuous quantitative values. Defined by mean and standard deviation.
    - Urine output. Continuous quantitative values. Defined by mean and standard deviation.
    - Humidity of incubator. Continuous quantitative values. Defined by mean and standard deviation.
    - Incidence of each electrolyte disturbance. Dichotomous nominal qualitative variable (Values: 0: No, 1: Yes). Described by frequencies.

  - **Implementation of new protocol**:

  Dichotomous nominal qualitative variable (Values: 0: No, 1: Yes).

  - **Initial postnatal weigh loss:**

  Defined by the following continuous quantitative variables:

  -Maximum percentage of weight loss with respect to birth weight. Defined by mean and standard deviation.

  -Age at maximum weight loss. Continuous quantitative variable. Defined by mean and standard deviation.

  -Age at recovery of birth weight. Continuous quantitative variable. Defined by mean and standard deviation.

- **Growth during admission**:

Weight, length and head circumference at 28 days of life and 36 weeks postmenstrual age, as continuous quantitative variables. Defined by mean and standard deviation.

- **Growth failure:**

Defined as nominal qualitative variable (Growth failure: 1: No, 2: Moderate, 3: Severe).

- **Other clinical outcomes:**
  - **Length of admission to the NICU:** Continuous variable. Describe by mean and standard deviation.
  - **Nosocomial infection:** Dichotomous nominal qualitative variable (0: No, 1: Yes). Described by proportions.
  - **Survival to discharge.** Dichotomous nominal qualitative variable (0: No, 1: Yes). Described by proportions.
  - **BPD:** Dichotomous nominal qualitative variable (0: No, 1: Yes). Described by proportions.
  - **NEC:** Dichotomous ordinal qualitative variable. Describe by proportions.
  - **IVH:** Dichotomous ordinal qualitative variable. Describe by proportions.
  - **ROP:** Dichotomous ordinal qualitative variable. Describe by proportions.

Patients will be followed up until death or discharge from the Neonatal Unit or until 40 weeks of postmenstrual age, whichever happens first.

Bivariate analysis

-The comparison of a continuous normal-distributed variable with a qualitative one will be realized with a Student's t-test. The comparison of two qualitative variables will be realized with a Chi-squared test.

Multivariate analysis

-For the association between PN protocol over time and hydroelectrolytic disorders (hypercalcemia), we will use a Generalized Linear Mixed Model (GLMM) adjusted for the co-variables (the ones with probability values of $p<0.05$ obtained during the bivariate analysis).

-For the association between hydroelectrolytic disorders (hypercalcemia) over time and other clinical outcomes, we will use a Logistic Regression Model adjusted for the co-variables. Hydroelectrolytic disorders (hypercalcemia) will be expressed as a dichotomous nominal qualitative variable and "Other clinical outcomes" will be expressed as qualitative variables except "length of admission to the NICU" which is a continuous variable, and will be evaluated during the whole hospitalization in time.

8. ETHICAL AND LEGAL ASPECTS

The PN solution was a standard medical prescription that was changed to improve quality of clinical care and the nutritional protocol, record keeping and blood and urine sampling were part of the standard care used in our Unit. As a retrospective review and analysis of pre-existing clinical data, individual written informed consent of parents or legal surrogates is not needed according to our institutional review board. In any case, the research protocol will be presented for approval to the ethics committee, Comité Ético d'Investigació Clínica – CEIC, held in Sant Joan de Déu' Foundation and accredited since 2006. Previous projects of a similar nature have previously achieved favourable consideration from the CEIC.

Anonymity of patient's data, by using an assigned correlative number instead of a medical record number or the patient's name will be guaranteed during the review and analysis of clinical data introduced in the database. In addition, it is important to keep in mind that as a retrospective cohort study no direct interventions are performed in our patients.

The detailed project will be conducted according to national and international ethics laws and guidelines, such as:

- WHA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects from June 1964.
- Ley Orgánica 15/1999, del 13 de diciembre, de Protección de Datos de Carácter Personal.
- Ley Orgánica 41/2002, del 14 de noviembre, de Autonomía del Paciente, Derechos y Obligaciones en Materia de información y Documentación Clínica.

Investigators of the current project declare that there are no conflicts of interests.

9. STUDY LIMITATIONS

Due to it is a retrospective cohort study, it is difficult to control the confounding variables. To avoid this, we will collect data for those variables that we expect could influence the results, based in those collected in previous studies and adjusting the results with a multivariate analysis.

Parenteral nutrition guidelines between centres are different in terms of electrolytic supply, and this could affect the external validity of our project, being difficult to extent the results to other situations and patients with the same condition.

As a retrospective cohort study, this project has some strong points such as feasibility in terms of cost and length. We can analyse and compare the incidence of the event in a short period of time and in a cheap way, by assessing multiple outcomes (risk and benefits) that might be related to exposure. Also, all patients were treated in a single centre, which will probably minimize heterogeneity in other aspects of care that might not have been taken into account.

10. WORK PLAN AND CHRONOGRAM

- **1st Stage: Accomplishment of protocol plan and coordination** (3 months)

  -Activity 1: Sending proposal protocol to the Ethics Committee for its approval.

  -Activity 2: Hypothesis and objectives approach.

  -Activity 3: Bibliography research.

  -Activity 4: Informative meeting: Francisco Vílchez (medical student) and Dr. Isabel Iglesias (main investigator) will have different meetings in order to monitor the process and discuss doubts. In addition, objectives will be explained to the rest of the research team. The chronogram of the study will be done and tasks will be distributed.

  -Activity 5: Design of data collection sheet.

  -Activity 6: Design of database.

- **2nd Stage: Data collection** (12 months)

  -Activity 7: Research team meeting with the aim of discuss if there are any doubt about data collection.

  -Activity 8: Population identification and sample selection.

  -Activity 9: Data collection and data entry in the performed database according to the inclusion and exclusion criteria.

  -Activity 10: Quality control of the data entered in the database.

- **3nd Stage: Statistical analysis and interpretation of the results** (2 months)

  -Activity 11: Data will be analyzed in collaboration with a qualified statistician.

  -Activity 12: Results will be interpreted and discussed by the research team.

- **4th Stage: Publication and dissemination of the results** (6 months)

  -Activity 13: Dissemination of the findings by assisting to national or international meetings about paediatric nutrition.

  -Activity 14: Manuscript results.

  -Activity 15: Publication of the results in specialized journals.

	Personnel	**2015**				**2016**												**2017**						
		Sep	Oct	Nov	Dec	Jan	Feb	Mar	Ap	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Ap	May	Jun	Jul
Stage 1																								
Proposal protocol to the Ethics Committee.	MI																							
Hypothesis and objectives approach.	MI, MS																							
Bibliography research.	MI, MS																							
Informative meeting.	MI, MS																							
Design of data collection sheet.	MS, CI																							
Design of database.	MS																							
Stage 2																								
Research team meeting.	MI, MS, CI																							
Population identification and sample selection.	MS																							
Data collection and data entry.	MS, CI																							
Quality control.	MI																							
Stage 3																								
Data analyze.	MI, MS, ST																							
Interpretation and discussion of results.	MI, MS, CI																							
Stage 4																								
National or international conferences.	MI																							
Manuscript results.	MS, CI																							
Publication of results.	MI																							

MI: Main investigator, MS: Medical student, CI: Research collaborator; ST: Statician.

11. BUDGET

Hospital Sant Joan de Déu has specific research laboratories and research support units, and the corresponding Institutional Review Board (Comité Ético d'Investigació Clínica). Researchers are supported by previous experience in conducting similar studies in paediatric patients. The Neonatal Unit will be the physical framework to analyze and monitor patient's data.

The investigator team working for the project will not receive a specific compensation for their work in this study. The budget does not include material as office supplies and software such as IBM – Software SPSS® and Microsoft Windows®, because our Unit holds the correspondent licences.

STUDY BUDGET	COST (EUROS)
1. Staff costs	
2. Statistical consulting and analysis of study data - Qualified statistician: -35€/h x 2h/day x 3days/week x 8weeks.....	1.680€
3. Travel and subsistence arrangements • International Conference on Nutrition and Growth. March 2017. -Inscription (early rate)..... -Travel costs..... -Accommodation..... • Annual Meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN). May 2017. -Inscription (non-member)..... -Travel costs..... -Accommodation.....	450€ 250€ 400€ 750€ 250€ 400€ Subtotal: 2.500€
4. Specialized journal publication - Publication and diffusion fees.....	1.500€
TOTAL AMOUNT OF AID CLAIMED	**5.680€**

12. EXPERIENCE OF THE INVESTIGATION TEAM

The research team in our Neonatal Unit has a long career of investigation experience in the area of paediatric nutrition and associated disorders. Most of the members work in a research program based on monitoring risk of newborns which is one of the oldest in Spain, and has low rates of patient loses, contributing to guarantee the reliability in the analysis of long-term results.

The lead researcher, Dr. Isabel Iglesias Platas, is particularly interested in the metabolic characteristics of the perinatal period, the regulation of growth at this stage of life and its impact on later health, especially in VPIs. For this reason, she served as principal investigator in the European multicenter study NIRTURE (EudraCT 2004-002170-34), which investigated the utility of using insulin early in order to avoid postnatal catabolism in VPIs and has demonstrated the usefulness of systems of continuous glucose monitoring at this population, showing the causes and consequences of abnormal carbohydrate metabolism in newborns with less than 1500g at born. In addition, she was invited as adviser to the NIH (National Institute of Health) of the United States of America at the meeting of "Neonatal Hypoglycemia" for planning research objectives in this field, in September 2008. Her doctoral thesis, read in 2012 at the University of Barcelona, deals with the regulation and expression of the locus of PLAGL1 in the placenta and its relationship with foetal growth and was partially funded by a grant from the Health Foundation in 2000. She has contributed to the description of epigenetic mechanisms of regulation of this and other regions, and has participated in the description of the first full "methylome" of healthy newborns and their evolution with aging. She has also collaborated on a project funded (MEC-2008 (SAF2008-01578)) that explored epigenetic stability in pregnancies conceived using assisted reproductive techniques and the results are in press.

In addition, because of their long experience in paediatric clinical nutrition, the results of our proposed study will be also discussed by the committee for Nutrition of our hospital constituted by a nutritionist, a gastroenterologist, a pharmacist, a neonatologist and a paediatric intensivist.

13. PROJECT IMPACT ON THE NATIONAL HEALTH SYSTEM.

According to the report "Born Too Soon" published by the European Union in May 2012, prematurity is increasing in Europe and is still the leading cause of infant morbidity and mortality. This report recommends investment in treatment strategies that can improve prognosis and reduce long-term adverse consequences associated with prematurity.

The aim of this project is to determine the role of nutrition and its association with short adverse consequences in VPIs. This is a necessary step in optimizing treatment for preventing the development of chronic conditions that will mean an increased consumption of health, educational and social resources during childhood and often a lifetime of those who were born very prematurely. Our study addresses these objectives with a clinical methodology that is indispensable in translational research in order to introduce changes in a short and medium-term to improve patient management. The successes in the identification of biochemical markers of good prognosis allow individual adjustment of the nutritional intake during the period of hospitalization, adjusting care attention to the paradigm of personalized medicine also advocated in the strategic plan.

This study represents the consolidation of a line of research with potential for leadership in the definition of metabolic and nutritional care of VPIs, both nationally and internationally. The field of hydroelectrolytic disbalances in VPIs associated with the different doses of AA intake during the first days of life is very novel and, despite some studies pointing at their relevance for the improvement of nutritional management and associated comorbidities, more research is necessary to confirm the new paradigm.

For this reason, studies like the present proposal are essential for seeking solutions to real problems of everyday clinical practice in order to optimize and individualize patient management.

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