Comparison of the effect of three different protein content enteral diets on serum levels of proteins, nitrogen balance and energy expenditure in critically ill infants: study protocol for a randomized controlled trial

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Reyes Fernández Montes
Hospital Universitario Central de Asturias
ORCiD: 0000-0002-3586-2466

Javier Urbano Villaescusa Javierurbanovillaescusa@gmail.com
Corresponding Author
ORCiD: 0000-0002-3511-2905

Ángel Carrillo Álvarez
Hospital General Universitario Gregorio Maranon

Ana Vivanco Allende
Hospital Universitario Central de Asturias

María José Solana García
Hospital General Universitario Gregorio Maranon

Corsino Rey Galán
Hospital Universitario Central de Asturias

Jesús López-Herce Cid
Hospital General Universitario Gregorio Maranon

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Abstract

Background Nutritional support is essential in the care of critically ill children, since malnutrition in this population is associated to increased morbidity and mortality. Injury in patients admitted to pediatric intensive care units (PICU) results in a catabolic state, with augmented protein breakdown, leading to a negative protein balance. Current recommendations about protein prescription in PICU are fundamentally based on expert opinions, with a minimum threshold of 1.5 g/kg/day of protein, although protein needs could be higher in certain subgroups of patients. The main objective of the present study is to examine if the administration of a protein-enriched infant formula increases the serum levels of total proteins, albumin, prealbumin, transferrin, retinol, and improves nitrogen balance; and to analyze the effect of the high-protein diet on energy expenditure. A secondary objective is to register possible secondary effects of the protein-enriched diet. Methods a multicenter prospective randomized controlled trial (RCT) will be performed in three hospitals. Patients meeting inclusion criteria will be randomly allocated to one of three enteral feeding formula with different protein content. Blood and urine test, nitrogen balance assessment and energy expenditure testing by indirect calorimetry will be performed at the beginning of nutrition regimen and at 24 hours, 72 hours and 5-7 days after initiation. The sample size for this trial is estimated as 90 participants, with approximately 30 participants in each group. The data analysis will be by intention to treat. Discussion this RCT will provide new data about the amount of protein needed to improve levels of serum protein and nitrogen balance, surrogate of protein balance, in critically ill infants receiving enteral nutrition.

Background

Nutritional support is an essential aspect in the care for children with critical illness.
Malnutrition has been reported with a prevalence between 24 and 70 % of critically ill children, depending on series (1-5). It can be present before admission or develop and increase during the hospital stay (6), due to several factors, as metabolic stress response, inaccurate estimation of energy requirements and inappropriate nutrient delivery (7).

Protein-caloric malnutrition, with an incidence of 15-20 % (8), is the most important type of malnutrition in Pediatric Intensive Care Units (PICU). It is associated to poor outcomes in critically ill children: malnourished patients present an increased physiologic instability and quantity of care (9), with higher duration of mechanic ventilation and length of stay and increased mortality (2, 3, 10).

Injury in the pediatric population admitted to intensive care units result in a catabolic state and increased protein turnover. Hepatic protein synthesis is enhanced, but there is an even more augmented muscle protein breakdown, leading to negative net protein balance (PB), which conduces to a loss of lean body mass (11-13). The progressive degradation of muscle mass can affect the diaphragm and other muscles involved in respiration, which may contribute to the onset or worsening of respiratory failure, and even loss of heart muscle (14). These changes are observable within the first two weeks of admission (6), but the most pronounced deficit of calories and proteins occurs during the first few days. Infants under the age of two are particularly susceptible, due to an intrinsic lack of endogenous stores and greater baseline requirements (12).

Current recommendations about protein prescription in critically ill children are fundamentally based on expert opinions (15), since studies on protein supplementation are scarce, with small sample sizes and heterogeneous patient populations, doses of protein and route of administration. The American Society of Enteral and Parenteral Nutrition (ASPEN) recommends in their latest guidelines a minimum intake of protein of 1.5 g/kg/day for children admitted to PICU (16), a minimum threshold supported by a
systematic review including 347 mechanically ventilated PICU patients (17) and another cohort study with 76 subjects (18).

Higher protein intake has been associated to early achievement of positive nitrogen balance (NB), a surrogate of PB (19-21), promoting protein anabolism (22). Moreover, higher protein delivery has been related to lower mortality and higher ventilator-free days in PICU patients (23, 24). However, the exact and safe amount of protein needed to avoid negative PB remains unclear. Protein intakes greater than 3 g/kg/day have been associated to elevated serum urea nitrogen levels and metabolic acidosis (19, 20, 25, 26), whilst other studies have reported higher incidence of lower intelligence quotient in very low birth weight infants receiving over 6 g/kg/day of protein (27, 28).

The aim of the present study is to analyze whether the administration of a high-protein diet improves protein metabolism (serum protein levels and NB) on critically ill infants, without increasing energy expenditure assessed by indirect calorimetry. A secondary objective is to register possible secondary effects of the protein-enriched diet.

**Methods**

**Hypothesis and aims of the study**

The hypothesis of this study are:

1) Critically ill infants receiving a higher amount of proteins on enteral feeding will experience a higher increase on serum protein levels (total proteins, albumin, prealbumin, transferrin and retinol binding protein) and NB than children receiving a standard enteral diet. 2) The administration of an enriched protein and high-protein enriched diet does not increase energy expenditure on critically ill infants. 3) Protein-enriched and high-enriched protein enteral diets are well tolerated, with mild gastrointestinal side effects and tolerable serum urea and total proteins elevation as adverse events.

The corresponding objectives are: 1) To analyze if the administration of a higher amount
of protein on enteral diet improves protein metabolism observed by the increase of specific serum protein levels (total proteins, albumin, prealbumin, transferrin and retinol binding protein (RBP)) and NB in critically ill infants. 2) To evaluate the effect of protein supplementation on energy expenditure on these patients. 3) To register possible secondary effects of the administration of protein-enriched and high-enriched protein diet.

**Study design**

This is an open randomized controlled clinical trial. The study was conceived and designed in 2010. It has been retrospectively registered on the Clinical Trials Database (ClinicalTrials.gov) with the registry number NCT03901742. This protocol, version 1.0, was approved on September 30th, 2010. Central ethical approval has been confirmed from the Institutional Review Board of Hospital General Universitario Gregorio Marañón (ref approval no. 2010-022851-47) and recruiting will not begin at other centers in the trial until local ethical approval has been obtained. Informed consent will be obtained from parents before their children are enrolled in the study. A report releasing study results will be submitted for publication in an appropriate journal. This protocol has been written in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT). A SPIRIT checklist is provided in Additional file 1.

**Study setting**

PICUs from three hospitals in Spain will participate: Hospital General Universitario Gregorio Marañón (Madrid), Hospital Clínico Universitario de Santiago (Santiago de Compostela) and Hospital Universitario Central de Asturias (Oviedo).

**Participants**

Inclusion criteria

The following are the inclusion criteria:

1. Children aged 1 month to 2 years.
2. Children admitted to PICU.
3. Children receiving enteral nutrition with an estimated length of over 72 hours.

Exclusion criteria

Children who met any of the following criteria will be excluded:

1. Age less than 1 month or over 2 years.
2. Diabetes mellitus or any inborn metabolic error.
3. Parenteral nutrition.
4. Bicarbonate infusion.
5. Renal replacement therapy.
6. Children receiving exclusive breastfeeding or in a need of special enteral formula.

**Recruitment, randomization, and study development.**

Once an eligible patient is admitted to PICU, written informed consent will be requested from parents or legal representative of the child by the physician responsible of the patient. They will be made aware that participation is voluntary, and they will be allowed to refuse further participation in the trial whenever they want.

After enrolment, the patient will be allocated randomly, in order of recruitment, into one of the three diet groups using a randomized data table generated with EPIDAT 3.1 software. A copy of the randomization list will be securely stored in an envelope located at PICU working area desk drawer, which will be opened after the patient enrollment on the study. Physicians, care givers and investigators will know the allocation prior to the start of enteral feeding.

All patients will receive exclusively enteral nutrition via nasogastric or transpyloric tube.

Group Standard Enteral Nutrition (SEN) would be fed exclusively with cow's milk based infant formula (Nidina 1; Nestlé, Barcelona, Spain). Group Protein-enriched Enteral Nutrition (PEN) would be fed exclusively with a polymeric infant formula (Infatrini;
Nutricia, Madrid, Spain). Group High Protein-enriched Enteral Nutrition (HPEN) would receive a polymeric infant formula (Infatrini; Nutricia, Madrid, Spain) supplemented with 2.6 g of protein/100 mL of formula. The source of the protein supplement would be a nonhydrolyzed protein cow's milk-based formula (Resource Protein Instant; Nestlé, Barcelona, Spain) (table 1).

Since this is an open-label trial, the assigned diet will be written down on the medical prescription of each patient and will be prepared by the PICU staff at the own unit, using for it the branded bottles where the different formulae are commercialized.

Continuous enteral nutrition will be typically initiated within the first 24 hours of PICU admission, by transpyloric or nasogastric tube, at a rate of 0.5-1 ml/kg/hour, with increases of 0.5-1 ml/kg every 3-4 hours, if well tolerated, to reach a caloric intake of 60-65 kcal/kg/day, or as needed based on resting energy expenditure measured by indirect calorimetry. There is no limit of time before the patient must be recruited, as long as it is before the enteral feeding is initiated. The establishment of enteral nutrition should never be delayed by achieving enrollment.

Demographic data will be recorded at inclusion: gender, age, weight, height, and diagnosis on admission. The risk of mortality at admission will be calculated using pediatric scales: Pediatric Index of Mortality 2 (PIM2), Pediatric Risk of Mortality (PRISM), and Pediatric Logistic Organ Dysfunction (PELOD).

Blood concentrations of urea, creatinine, total proteins, albumin, prealbumin, transferrin, RBP levels, urinary concentration of urea in 24-hours or isolated urine sample, and energy expenditure, oxygen consumption (VO2) and carbon dioxide production (VCO2) by indirect calorimetry (Datex S5 monitor, E-COVX; GE Healthcare/Datex-Ohmeda, Helsinki, Finland) will be measured at admission and at days 1, 3 and 5-7 after initiation of enteral feeding. Air leaks will be measured using the mechanical ventilator. Calorimetry-derived data will
be collected only in patients with tracheal intubation, when air leakage is <10 \%, FiO2 less than 80 \%, absence of inhaled nitric oxide, sevofluorane or heliox, or connection to ECMO. The collection of indirect calorimetry data will be done over 30 to 120 minutes. NB will be calculated as: nitrogen intake minus total nitrogen losses. Total nitrogen losses will include total urinary nitrogen and fecal/miscellaneous losses estimated according to the World Health Organization recommendations (29).

Another blood biochemical parameters as glucose, cholesterol, triglycerides, procalcitonin, C-reactive protein, ions and blood gas will also be recorded.

Time between PICU admission and the initiation of enteral nutrition, total daily enteral energy and protein delivery, intravenous albumin infused, and other treatments such as vasoactive drugs, neuromuscular blockers, sedatives and analgesic drugs, diuretics and steroids would be registered.

**Protein-enriched diet safety**

Enteral complications (constipation, diarrhea, abdominal distension, gastric residue), serum urea and total protein levels, as well as any unexpected adverse event occurring during the trial will be recorded to evaluate the safety of the protein-enriched diet. The discontinuation of the enteral nutrition would be decided on the judgment of the physician looking after these patients.

**Study ending**

The study will be ended:

1. After 7 days of enteral feeding.

2. At PICU discharge.

3. If hyperproteinemia higher than 8.5 g/dL is present.

4. If serum urea levels elevate higher than 80 mg/dL without evidence of renal function disturbance (according to KDIGO criteria) or suspected hypercatabolic state.
Study outcomes

The primary outcome of the study will be the variation of NB from baseline to the study ending and the incidence of hyperproteinemia or uremia causing the need to stop the study.

Secondary outcomes include the variation of plasma protein levels (total proteins, albumin, prealbumin, transferrin and RBP), expenditure energy measured by indirect calorimetry, and the incidence of gastrointestinal complications (abdominal distension, vomiting, diarrhea and excessive gastric residue) and metabolic acidosis.

Data management

Trial data will be extracted from the medical history of the patient, registered by investigators on a data collection form, and subsequently recorded on a central database. Each patient will be identified by number of medical history and subject number, so confidentiality of the patient will be kept.

Statistical analysis

As there are not previous studies reporting expected standard deviations, we use the standardized difference of means for computing the optimal minimum number of patients to include in the trial. The calculation of the sample size has been done with EPIDAT 3.1 software. Considering a significance level of 5% (Type I error), a power of 80% (complementary of the Type II error) and a minimum detectable standardized difference of means of 0.9, we need 30 patients per group (Bonferroni correction included).

We will use an intention-to-treat approach. A descriptive analysis will be performed where quantitative variables will be described by their means and standard deviations or their medians and interquartile ranges, as appropriate. The quantitative ones will be summarized by their absolute and relative frequencies. Parametric and non-parametric
tests will be employed for contrasting equality among groups.

Univariate and multivariate mixed models will be used in order to assess the effects size of the different diets (fixed effects) on the patients (random effects) unadjusted and adjusted by potential confounders, respectively. P-values under 5% will be considered statistically significant.

Discussion

Current recommendations about protein prescription in critically ill children are not based on RCT results. Data regarding protein supplementation are lacking. Studies with small sample sizes and heterogeneous patient populations are the only source for this information. A minimum intake of protein of 1.5 g/kg/day for children admitted to PICU (16) is the current recommendation of the American Society of Enteral and Parenteral Nutrition (ASPEN) in their latest guidelines. This recommendation is only supported by a systematic review (17) and a cohort study (18).

The exact and safe amount of protein needed to avoid negative PB remains unclear. Higher protein intake has been associated to early achievement of positive nitrogen balance (NB) (19-21), and higher protein delivery has been related to lower mortality and higher ventilator-free days in PICU patients (23, 24). On the other hand, enriched-protein diets have been associated to elevated serum urea nitrogen levels (19, 20, 25, 26)

This RCT is designed to obtain new data about the amount of protein needed to avoid negative PB, as well as the amount of protein that is safe without producing secondary effects. The results of the study will be important to decide if a protein-enriched diet will be or not be useful in critically ill infants. Moreover, the results will be clinically significant to know the exact amount of proteins we can recommend to critically ill infants.

In clinical practice, there is no method that can evaluate directly the protein metabolism.
On the other hand, NB and serum protein levels have been used in most of previous studies to estimate and adequate protein intake. We will use NB, considering a positive NB as indicator of protein anabolism and negative NB as protein catabolism. Moreover, we will evaluate as an indirect indicator of a protein metabolism improvement the increase of total proteins, albumin, prealbumin, RBP and transferrin serum levels from baseline to the study endpoint.

The main problems we will face during the collection of data are: presence of secondary effects in any of the groups that recommends to stop the study and difficulty to include enough valid patients because of problems with urine collection.

In summary, this multi-center, prospective RCT will compare the effect of three different enriched-protein diets on serum levels of proteins, nitrogen balance and energy expenditure in critically ill infants, as well as their possible secondary effects.

**Trial Status**

The trial began recruitment at Hospital Gregorio Marañón in December 28th, 2016. Participants will be recruited until December 2020, if necessary. During the period of time between the protocol version approved (2010) and the first patient enrolled (2016) no protocol amendments, nor enrollment attempts have occurred.

**Abbreviations**

PICU: pediatric intensive care unit; PB: protein balance; NB: nitrogen balance; RBP: retinol binding protein.

**Declarations**

**Acknowledgements**

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**Availability of data and materials**

The final datasets generated and analyzed in the current study will be available from the corresponding author on reasonable request, and in ClinicalTrials.gov, NCT03901742.

**Authors’ contributions**

ACA, CRG and JLC participate design of the study. RFM, JUV, AVA and MJSG participated in the design and development, including the statistical analysis plan. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

Ethics: This study has been approved by the Ethics Committee of the Institutional Review Board of Hospital General Universitario Gregorio Marañón. The approval number is 2010-022851-47. Informed consent: Written informed consent will be collected from each study participant prior to enrolment by the physician responsible of the patient. They will be made aware that participation is voluntary, and they will be allowed to refuse further participation in the trial whenever they want.

**Consent for publication**

Not Applicable

**Competing interests**

The authors declare that they have no competing interests.
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Tables

Table 1. Composition of diets used in the study.

|                                      | Protein (g) | Carbohydrate (g) | Lipids (g) |
|--------------------------------------|-------------|------------------|------------|
| Standard Enteral Nutrition           | 1.7         | 7.4              | 3.4        |
| Protein-enriched Enteral Nutrition  | 2.6         | 10.3             | 5.4        |
| High protein-enriched Enteral Nutrition | 5.1         | 10.5             | 5.5        |

Content per 100 ml.

Figures
**Figure 1**

SPIRIT diagram for this protocol.
Flowchart for this protocol, comparing the effect over protein metabolism of three different amount of protein delivery. PICU: pediatric intensive care unit; EN: enteral nutrition; NB: nitrogen balance.
This is a list of supplementary files associated with the primary manuscript. Click to download.

SPIRIT_checklist 8_12_18.doc