Author’s response to reviews

Title: 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

Authors:

Reyes Fernández Montes (reyesfmontes@gmail.com)

Javier Urbano Villaescusa (javierurbanovillaescusa@gmail.com)

Ángel Carrillo Álvarez (carrilloalvarez49@gmail.com)

Ana Vivanco Allende (anaviall@hotmail.com)

María José Solana García (mjsolana@hotmail.com)

Corsino Rey Galán (crey@uniovi.es)

Jesús López-Herce Cid (pielvi@hotmail.com)

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Reviewer #1:

The study has a very interesting design and I consider the outcomes very important in the treatment of critically ill children in several clinical situations. However some aspects can be relevant to produce the final text:

1. It's not clear on the third group mainly and seems to be very much protein delivery and hyperproteinemia higher than 8.5 g/dL or serum urea levels elevation higher than 80 mg/dL without evidence of renal function disturbance or hypercatabolism insufficient parameters to diagnose early adverse effects. I suggest to review protein recommendations to avoid complications. ?

According the described protocol of enteral feeding, patients on high-protein enriched group would receive approximately 60 kcal/kg/day, which corresponds to 2.8 g of protein/kg/day. Several trials demonstrated benefits from a protein intake of 2-3 g/k/day in critically ill infants under 2 years old. The current guidelines recommend a minimum of 1.5 g/kg/day, with the
advice that “the optimal protein intake required to attain a positive protein balance may be much higher than this minimum threshold”. There are precedents in the literature in which critically ill infants are provided amounts of protein near 5 g/kg/day, finding serum urea levels elevation and mild gastrointestinal symptoms as the only significant adverse effects.

2. Other aspect is to administrate diets with complete protein (polymeric) by transpyloric tube mainly in children < 1 year old because the immaturity of digestive tract and increased risk of allergy in the future. I think that diets by transpyloric tube should be hydrolyzed, including the supplementary protein of the third group, mainly in that age.

To our knowledge, we have not found studies that support this practice. Based on international recommendations, the site of administration of nutrition does not determine the choice of type of nutritional formula, reserving the administration of oligomeric or monomeric formula for patients with food intolerance or allergy or severely impaired bowel function, regardless of the patient's age.

3. Visceral proteins vary in accordance of inflammatory response and to evaluate in a correct way the results is necessary to monitor procalcitonin or same C-reactive protein at the same time

We agree with this statement. It was not reflected on the original manuscript, but CRP levels will also be monitored, and its variation will be correlated with variation in serum proteins (total proteins, albumin, prealbumin, RBP and transferrin) levels, trying to assess the influence of inflammation on protein synthesis. This has been included in the manuscript.

4. I think that severe hepatic dysfunction should be a exclusion criteria because provokes changes in visceral proteins.

We agree with the reviewer. This criteria was not included because infants with severe hepatic dysfunction are referred to specialized liver-transplant centers different from the centers where the study is going to be performed.
5. Anthropometry is essential for a current nutritional evaluation/classification and the protocol doesn't have many details about this aspect and bioimpedance could be a further good parameter.

Although anthropometry has classically been used to evaluate nutritional status, especially in healthy population, the special characteristics of children admitted to PICU (its severity, connection to devices that make it difficult to mobilize, frequent alterations in the water balance ...) often interfere in the obtention and the reliable interpretation of these measurements. However, whenever it is possible, the patient's weight and length should be measured at enrollment. We consider that a study period as short as 7 days is insufficient to appreciate substantial changes in anthropometry, so serial measurement of patients has not been included in the protocol. Similarly, bioelectric impedance is a noninvasive and safe method of body composition assessment, but its application to the infant population is problematic, resulting in generally poor accuracy.

Reviewer #2: Trials review

Thank you for the opportunity to review this protocol.

I believe the topic of optimal protein dosage during acute critical illness to be ripe for investigation. The authors propose a trial that will add to the feasibility of achieving high protein delivery in the population of interest.

The authors propose their RCT, with 3 arms, to be conducted in 3 centers.

Their main objective is to examine the impact of protein-enriched infant formula on serum levels of total proteins, albumin, prealbumin, transferrin, retinol, nitrogen balance, and energy expenditure; in children under 2 years of age, on enteral nutrition, with expected stay of at least 72 hours in the PICU. A secondary objective is to examine the secondary effects of administering protein-enriched diet.
Major comments:

1. The study proposal was approved by local IRB in 2010. I am not sure why the study has not enrolled any patients until 2019. If there were attempts to enroll, prior study results, changes in protocol since 2010, etc; these findings, observations, experience and rationale for this protocol submission in 2019 must be reported/discussed by the authors.

We agree with the reviewer that there has been a long gap between the IRB approval and the beginning of the enrolment. First of all, we have to admit a mistake that we have acknowledged during the review process. The date of first enrollment is December 2016. This change has also been edited in the clinicaltrials.gov registry.

During this time there have been no attempts to enroll, nor changes in the protocol. The reasons for this delay are several. Firstly, IRB approval is needed prior to the application of a public founding grant. Once the grant is obtained, it takes a minimum of 12 months to receive the funds, so there is a 1- or 2-year gap between the first IRB approval and the beginning of the study. Secondary, there have been administrative issues with local IRB in the partner hospitals. And thirdly we have had repetitive fails to find a stable solution of labeled amino-acid isotopes to be used in order to monitor the protein metabolism, and this outcome was finally abandoned.

Because no medical reasons have influenced in the delay and no changes in the protocol have been made, we consider that this amount of time between IRB approval and the enrolment of patients does not affect the study.

2. As pointed out above, please discuss the differences (if any) between the protocol reported in Clinical trials.gov and the current submission.

As we have explained in the previous paragraph, there are no differences between the protocol reported and the current submission.

I have the following questions about the study design:
3. Please provide a clear hypothesis for each aim.

We agree with the reviewer and have added the hypothesis in the manuscript.

4. Please explain the rationale for limiting the study to children under 2 years of age.

Even though actual requirements of protein on critically ill children are not yet well known, based on previous studies, there is evidence that there is an association between age and protein intake, with the need of higher protein intake per weight in infants and younger children. In fact, previous recommendations use three different age groups to establish the recommended protein delivery in critically ill children. We wanted to evaluate the optimal protein intake in the first of those groups, infants under 2 years old, since they represent most of the patients admitted to our units. One more reason is that beyond 18-24 months of age, the enteral formulas needed to achieve a complete nutrition have a different composition, and this would add more groups to be compared, increasing the complexity of the study and the analysis.

5. I understand that patients on PN were excluded, as the study is only exploring the impact of protein supplementation enterally. Do the authors have enough patients in the 3 PICUs who would qualify for the study and therefore complete the study in time? Was this relevant to the delay in study starting after 9 years of initial study protocol approval?

As we have explained before, the delay has been due to administrative and technical reasons. However, we agree with the reviewer that completing the study in time is going to be challenging because of the high number of patients that are receiving exclusive breastfeeding or in a need of special enteral formula.

6. Please explain what is meant by Protein levels

We used the term “protein levels” referencing levels of serum proteins: total proteins, albumin, prealbumin, RBP and transferrin.
7. Please explain how protein metabolism will be defined. Currently the authors state that protein levels and nitrogen balance will be measured. How will this equate to metabolism? How will serum levels of albumin, prealbumin and RBP equate to protein metabolism. These concepts and definitions for metabolic states must be clarified. Also, important to state these in the form of the hypothesis.

We agree with the reviewer that the calculation of nitrogen balance and the measurement of specific serum proteins such as albumin, prealbumin, etc do not reflect completely the protein or aminoacid turnover. In clinical practice, there is no method that can evaluate directly the protein metabolism. Our first attempt was to use labeled amino acid isotopes to estimate protein metabolism, however due to technical reasons this was not possible for us. On the other hand, NB and serum protein levels have been used in other 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. This explanation has been added in the manuscript.

8. Enrollment: When will enrollment be completed? Is there a limit after admission, before which enrollment should be achieved?

There is no fixed time limit in which the patient must be recruited, as long as it is before the enteral feeding is initiated, which usually happens in the first 24 to 48 hours of admission in our PICUs. Despite it has not been described in the manuscript, the time from PICU admission until the initiation of enteral feeding would be recorded. The establishment of enteral nutrition should never be delayed by achieving enrollment. Enrollment would be completed in December 2020.

9. I appreciate the inclusion of pts with LOS>72 hrs. What is the average/median length of stay for patients in these PICUs? Will there be meaningful exposure to study treatments? Again, do the authors think they have the numbers needed to complete this trial?

We agree with the reviewer that these observations are some of the key points of the study. In our opinion, it is feasible because this research group have published a similar study, performed

We have already started enrollment and have 51 patients enrolled.

We believe that with the partnership of two more centers, these objectives should be achievable.

10. Thank you for including the details of EN initiation and advancement. Is this protocolized in all 3 institutions? Are definitions of intolerance uniform across the units?

The enteral nutrition initiation and advancement was protocolized in the study because of the lack of protocols in the three centers. Intolerance is not uniformly defined as it is assessed by clinical judgement. The discontinuation of the enteral nutrition is decided on the judgment of the physician looking after these patients. We acknowledge that this could be a limitation.

11. What is the current expectation of indirect calorimetry at these sites? All 3 have the capability? What do the authors expect in terms of percentage with IC versus estimated caloric goals?

In the present time, only one of the three participating centers has the possibility to perform indirect calorimetry, so we are aware that the energy expenditure will not be measured in all patients. Energy expenditure has shown to be widely variable and hardly predictable among critically ill children. Estimates of energy expenditure using available standard equations are often unreliable, leading frequently to both under or overfeeding in PICU. Previous studies performing indirect calorimetry in critically ill children (Nutrition. 1998 Sep;14(9):649-52.; J Pediatr. 2011 Jul;159(1):27-32.e1. doi: 10.1016/j.jpeds.2011.02.001. Epub 2011 Mar 22.; Clin Nutr. 2016 Apr;35(2):460-467. doi: 10.1016/j.clnu.2015.03.015. Epub 2015 Apr 2.; An Pediatr (Barc). 2007 Mar;66(3):229-39.) found, despite variability, a median measured energy expenditure around 55-60 kcal/kg/day, so we have chosen this goal, knowing that in many occasions it can be discordant with the real energy expenditure.
12. Please explain the rationale for aim # 3; impact of protein supplementation on energy expenditure.

Because protein synthesis is an energetically costly process, we can think that improving protein metabolism would increase energy expenditure. However, several previous studies (Clin Nutr. 2009 Jun;28(3):249-55. doi: 10.1016/j.clnu.2009.03.005. Epub 2009 Apr 8.; J Pediatr. 2011 Jul;159(1):27-32.e1. doi: 10.1016/j.jpeds.2011.02.001. Epub 2011 Mar 22.) did not find differences on measured energy expenditure (MEE) between infants receiving protein enriched or standard formula, reflecting the fact of MEE trends to be stable along PICU stay, past the first few hours after injury.

13. Please explain study end criteria # 4. What is hypercatabolism? How is renal function disturbance defined?

Renal function disturbance was not previously defined when the study was designed on purpose because criteria of acute kidney injury (AKI) were changing in the past years. As we are starting the enrollment of patients, all the centers agree to follow the KDIGO criteria to diagnose AKI. This information is added in the protocol.

We agree with the reviewer that a hypercatabolic state is difficult to define and to diagnose, and no single parameter could be enough to do so. A protein hypercatabolic state would be suspected in the case of a patient with elevated serum urea levels, absence of acute kidney injury and negative nitrogen balance, with an underlying stressful or inflammatory condition. If this combination is present, enteral diet with or without protein supplements would be maintained, with a special vigilance of serum urea levels.

Statistical analysis:

14. I believe there are prior trials in infants where protein dosage is examined for its impact on nitrogen balance. These trials also report blood urea levels. Please explain why these trials were not used to derive sample size estimates? (REFERENCES: JPEN J Parenter Enteral Nutr. 2018 Jan;42(1):196-204. doi: 10.1002/jpen.1031. Epub 2017 Nov 3.; Clin Nutr. 2009 Jun;28(3):249-
There were only two previous studies published before the design of our study. These studies include only mechanically ventilated patients with SRV infection. Both groups had a small size (8 and 10 patients), and this studies only reflect a specific patient population. Our study would include a more heterogeneous population, and therefore needs a larger sample size. However, the 2018 study including children after cardiac surgery comprises 24 and 26 patients in each group, which is a similar size of the groups in our study.

Minor comment:

Please change A.S.P.E.N. to ASPEN through the manuscript.

Done.

Overall, this is an important study and the results will add significantly to literature on a subject that deserves further investigations. The current proposal requires addition of further details to clarify study methodology.

Thank you.