Peer Review File

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Review comments

The paper titled "Amino acid requirements of total parenteral nutrition (TPN) fed neonates: A review of current knowledge and the basis for a new amino acid solution in neonatal nutrition" is interesting, which summarize current knowledge on amino acid requirements and describe the importance of appropriate composition of amino acids in parenteral nutrition formulas for neonates. The physiological basis for revising current amino acid solutions used in parenteral feeding of neonates is also provided. However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 1. How does gestational age and weight affect urea and mineral excretion in preterm infants receiving total parenteral nutrition?

Reply 1.

It is our assessment that this question posed by the reviewer is not relevant to the current review. Our review is focused on amino acid requirements of the TPN fed neonate and the basis for a new amino acid solution which has not been devised because of lack of data on amino acid requirements in neonates fed TPN. Current amino acid solutions used for TPN contain amino acids whose concentrations are based on approximations. Due to this fact neither of the available commercial solutions are optimal for the neonate. They contain an amino acid profile in which the balance of the amino acids (protein quality) is inadequate. Therefore, any study looking at urea and mineral excretion will be affected by this fact.
Nevertheless, there is data which addresses this issue
(Determinants of urea production and mineral retention in parenterally fed preterm infants Christopher Geoffrey Alexander Aiken. J Clin Diagn Res, 2013 Aug;7(8):1655-8):
Concerning urea excretion:

- TPN providing at least 30 kcal/g amino acids and sufficient protein for normal growth from birth prevent from protein catabolism
- During the first week, urea excretion increases with weight for gestational age (higher rates in above average babies than in below average weight infants).
- Urea excretion also increases with gestational age in above average but not below average weight infants.
  Then, it seems that urea excretion is not affected by the amount of TPN if enough calories and protein are provided. Besides, urea excretion is more related to the weight than to the gestational age of the infants.

Concerning mineral excretion:

- Below average weight infants had lower potassium and phosphate excretion than those above average.

Then, it seems that mineral excretion is more affected by the weight than the gestational age of the infants.

It is however difficult to interpret these results because it was not clear what the amino acid composition (protein quality) of the various TPN solution were or acid base balance of the neonates. In addition, the high calcium TPN had higher sodium which can affect calcium reabsorption at the renal tubule. Therefore, the TPN solutions were not appropriately balance. Also, to discuss mineral excretion, information is needed on vitamin D, PTH and plasma aluminum concentrations. Clearly mineral excretion is not just related to the amino acid solution but to a myriad of factors. We feel strongly that this will distract from the message of our review, hence we have chosen not to include this issue in the manuscript.

Comment 2 The amino acid requirements of newborns receiving total parenteral nutrition are significantly different from those of oral nutrition. How to determine?

Reply 2.
Our group has published a significant body of literature on this issue in the neonatal piglet model which is described on pages 8 and 9 of the review. Using the indicator amino acid oxidation method, we estimated individual amino acid requirements in the same piglets fed enterally or parenterally. The details of the method are provided in the published papers cited on pages 8 and 9 of the review.

Comment 3 Compared with breastfed infants, what kind of net acid excretion rate must be produced by infants receiving TPN to maintain acid-base balance?

Reply 3.
Although we agree acid-base balance in an important issue, the current review is not focused on acid base balance. Inclusion of acid base balance which is an extensive area of research and one that has significant implication in the clinical care of the neonate will distract for the goal of the current review. Therefore we do not see a benefit to including information on acid-base balance in this review.
However, metabolic acidosis occurs frequently in newborns. Chan et al. studied net acid excretion (NAE) in 34 preterm and 12 term infants during the first week of life. Twenty preterm infants received breast milk or formula; the remaining infants received total parenteral nutrition (TPN) -- synthetic amino acids or casein hydrolysate solution. NAE for breast milk vs formula fed infants was 5.4 +/- 0.4 and 7.8 +/- 0.6 muEq/min/m2 (mean +/- SEM). The corresponding values for the two TPN solutions in preterm infants were significantly higher at 12.5 +/- 1.4 and 19.4 +/- 3.5 muEq/min/m2. Term infants produced even greater amount of net acid, 20.6 +/- 2.9 and 35 +/- 3.7 muEq/min/m2 respectively for the two TPN solutions. Milk fed infants are less prone to acidosis because of base generated from milk consumption. Due to its inherent acidogenic effect, TPN solutions induce acidosis more readily. Infants receiving TPN are therefore required to generate a higher NAE rate to

maintain acid-base homeostasis compared to milk fed infants. ***(Net acid excretion during first week of life.Chan LL, Balfe JW, Exeni R, Cifuentes RF, Bryan MH, Atkinson SA. The International Journal of Pediatric Nephrology, 28 Feb 1981, 2(1):37-41PMID: 6800970).***

Another study ***(Early optimal parenteral nutrition and metabolic acidosis in very preterm infants. Francesco Bonsante , Jean-Bernard Gouyon , Pierre-Yves Robillard, Béatrice Gouyon , Silvia Iacobelli. Published: November 27, 2017; https://doi.org/10.1371/journal.pone.0186936***), noted "it is currently recognized that an optimized nutritional approach, consisting of an early and substantial supply of protein and energy by parenteral route, may be beneficial for very low birth weight infants and recent guidelines endorse this strategy. However, **the impact of the enhanced parenteral nutrition (PN) on acid-basic balance has never been investigated**. So, the study assessed the effect of nutrient intake on acid-base homeostasis in a large population of preterm infants on PN¨. This observational study described the acid-base profile of very preterm infants (≤29 week's gestation) receiving PN during the first week of life in three different cohorts of infants who received increasing (group 1 to group 3) nutritional intakes were considered. Nutrition data were recorded daily and correlated to acid-base data (pH, base excess, and lactate). The outcome measure to assess metabolic acidosis was the base excess (BE). 161 infants were included in the study. The three groups were different with regard to nutritional intravenous intakes. Group 3 in particular had a higher mean intake of both amino acids (3.3 ± 0.8 g/kg/d) and lipids (2.8 ± 1.4 g/kg/d) during the first week of life. Metabolic acidosis was more severe in the group with the highest parenteral intake of amino acids and lipids: mean BE = -8.7 ± 3.4 (group 3); -6.4 ± 3.4 (group 2); -5.1 ± 3.0 (group 1)]. In summary, the significant risk factors for metabolic acidosis were: gestational age, initial base excess, amino acid and lipid intravenous intakes.
So, acid-base homeostasis was influenced by the nutritional intake. Earlier and higher intravenous amino acid and lipid intakes particularly increased the risk of metabolic acidosis. The nutritional tolerance was different depending on gestational age, and the smaller infants (24–26 week's gestation) displayed greater acidotic disequilibrium and a higher need of bicarbonate.

Comment 4. What is the total sulphur amino acid requirement and metabolism of newborns fed parenterally?

Reply 4. The sulphur amino acids are methionine and cysteine. Methionine is indispensable whereas cysteine can be synthesized from methionine. Cysteine is unstable in solution, so methionine is added in relatively high concentrations in amino acid solution to meet the needs of both methionine and cysteine. In neonates, although cysteine can be performed from methionine, it is metabolically advantageous to provide a balance of the two amino acids as high methionine can be toxic and also lead to high plasma homocysteine. To better understand the sulphur amino acid requirements and metabolism it is important to know the requirement for total sulphur amino acids (methionine only) and minimum methionine (the minimum amount of

methionine required in the presence of excess cysteine). Using the indicator amino acid oxidation method, we previously determined the total sulphur amino acid requirements (as methionine only in the absence of cysteine) to be 49.0 (95% CI: 39.9, 58.0) mg/kg/d in TPN fed neonates. This is significantly lower than what is present in current commercial amino acid solutions. ***(Total sulfur amino acid requirement and metabolism in parenterally fed postsurgical human neonates. Glenda Courtney-Martin, Karen P Chapman, Aideen M Moore, Jae H Kim, Ronald O Ball, and Paul B Pencharz)***

Comment 5. Can indicator amino acid oxidation method determine the restricted amino acids in neonatal parenteral nutrition?

Reply 5. We are not completely sure what the reviewer is referring to by the term "restricted". However, we assume you are referring to dispensable amino acids such as proline. The IAAO method was developed to assess the requirement of indispensable amino which is derived from exogenous sources.

Comment 6. What are the potential adverse effects of excessive or insufficient parenteral amino acid intake?

Reply 6.
Adverse effects of insufficient and excessive amounts of parenteral amino acids are discussed on page 6 of the review and again on pages 10-11. In addition, have discussed amino acid intake in the context of balance or protein quality to extend the focus beyond just total intake.
Others have discussed this. Recently, a Cochrane Systematic Review (***Osborn DA, Schindler T, Jones LJ, Sinn JKH, Bolisetty S. Higher versus lower amino acid intake in parenteral nutrition for newborn infants. Cochrane Database of Systematic Reviews 2018, Issue 3. Art. No.: CD005949. DOI: 10.1002/14651858.CD005949.pub2.)*** assessed the effects of higher *versus* lower amino acid intake in parenteral nutrition for newborn infants. In the review, the authors defined the amino acid intake at maximal infusion of parenteral nutrition as following:
•Very low amino acid intake (≤ 2 g/kg/d)
•Low amino acid intake (> 2 to ≤3g/kg/d)
•High amino acid intake (> 3 to≤4g/kg/d)
•Very high amino acid intake (> 4 g/kg/d).
The authors pointed out that "the potential benefits of higher amino acid intake during parenteral nutrition of improved nitrogen balance, growth, and infant health may be outweighed by the infant's ability to utilize high intakes of parenteral amino acid, especially in the days after birth, resulting in high concentrations of amino acids, ammonia, and urea, and an exacerbation of metabolic acidosis". The authors also addressed "the importance of determining the optimal amount of each indispensable amino acid intake via parenteral nutrition for the adequate growth and health of newborn infant".

Adding to that, Embleton and Van Den Akker stand out that "high early amino acid intakes from PN might be harmful, since studies show that higher amino acid intakes are associated with higher plasma amino acid levels but no RCTs show clear negative impacts on infant neurodevelopment. At which level higher amino acid, ammonia or urea concentrations become toxic is yet unknown. While data are limited, they emphasize that current PN formulations and practices require further consideration." (***Early parenteral amino acid intakes in preterm babies: does NEON light the way? Nicholas D Embleton, Chris HP Van Den Akker. Arch Dis Child Fetal Neonatal Ed March 2018 Vol 103 No 2).***

Besides that, Embleton and McGuire recently discussed the results of the above mentioned Systematic Review and said that "it is not possible to determine the optimal regimen from the studies included in these meta-analyses. Higher intake may be beneficial or harmful. Populations of pre-term infants vary markedly. It is plausible that a relatively stable 24-weeks'-gestation infant may benefit from an amino acid intake closer to 4 g/kg while an unstable 30-weeks'-gestation infant with severe sepsis may be harmed by an intake >2 g/kg. We urgently need adequately powered trials with long-term functional outcomes to determine optimal amino acid intake." (***Nicholas D. Embleton, William McGuire. Commentary on "Higher versus Lower Amino Acid Intake in Parenteral Nutrition for Newborn Infants". Neonatology 2019;116:92–96DOI: 10.1159/000495913).***

In summary, not only the total amount of amino acid provided in PN should be considered in follow-up studies, but the amino acid profile and the amount of each amino acid (mg/kg/day) provided, because some of them may be harmful when their parenteral supply are considered insufficient or in excess. This is the main reason why not only the total amino acid requirement, but each indispensable amino acid requirement shall be defined urgently.

Comment 7. What effect will early parenteral nutrition have on the prevention of jaundice in term and recent newborns who cannot be fed by enteral?

Reply 7.
Many factors contribute to neonatal jaundice. Neonatal jaundice is not only due to TPN cholestasis. The purpose of this review is not to discuss neonatal jaundice but rather to focus on amino acid requirement of TPN fed neonates. A discussion of neonatal jaundice could potentially distract from the goal of this review. From an amino acid perspective- high amounts of methionine intake has been shown to produce cholestatic changes in animal models. This was mentioned on page 13 of the review.

Comment 8 How to determine the tyrosine requirement of parenteral-fed newborns who receive graded intake of glycyl-L-tyrosine as a source of tyrosine?

Reply 7. The tyrosine requirement of TPN fed neonates was determined by our group using the indicator amino acid oxidation method. The details of the method are described in the published paper. ***Roberts SA, Ball RO, Moore AM, Filler RM, Pencharz PB. The effect of graded intake of glycyl-L-tyrosine on phenylalanine and tyrosine metabolism in parenterally fed neonates with an estimation of tyrosine requirement. Pediatric research. 2001;49(1):111-119.***