Reviewer's report

Title: Modification of stool's water content in constipated infants: management by an adapted infant formula

Version: 2 Date: 13 August 2010

Reviewer: Mark Underwood

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The authors present a pilot study of a modified infant formula in 30 constipated infants. The major outcomes are stool water content as determined by near-infrared reflectance analysis (NIRA) and subjective measures of constipation by the parents. The authors address a common problem with a novel proposal: increased magnesium and lactose in the formula. The results are promising. I would characterize the following as major compulsory revisions which require a response from the authors prior to a decision on publication.

1. The abstract should better reflect the scope and results of the study and what is unique about the formula. Be careful with the use of the word “specific” in describing the formula, be consistent with the tense, and put the most important outcome first. Here is an example:

   Background: Constipation is common in formula-fed infants. The aim of this pilot study was to evaluate the impact of a formula with added lactose and magnesium on stool water content and parents' assessment of constipation.

   Material and methods: Thirty constipated term formula-fed infants were included (mean age 4.1 weeks). Stool composition (water, fat, protein, and carbohydrate) was measured by near-infrared reflectance analysis (NIRA) and parental questionnaires were completed at baseline and after 2 weeks of the modified formula diet.

   Results: After 2 weeks of the modified formula, stool water content increased from 71 +/- 8.1% to 84 +/- 5.9%, p<0.02). There was no significant change in the stool content of fat, protein, or carbohydrate. Improvement was noted in parental impressions of constipation with improvement in stool hardness (100% with hard stools at baseline, 10% after 2 weeks), pain on defecation (90% at baseline, 10% after 2 weeks), and requirement for rectal stimulation to achieve defecation (70% at baseline, 30% after 2 weeks, p<0.001 for all three indicators).

   Conclusions: This pilot study suggests that a modified formula with added lactose and magnesium improves stool water content and parental impression of constipation in term formula-fed infants. A larger randomized placebo-controlled trial is indicated.

2. Background: It would be helpful to include more detail about NIRA. For instance, its strengths and weaknesses and how it compares with more traditional methods of determining stool water content (like chemical methods...
and comparisons of wet stool weight to dried stool weight). It would add to the paper to reference the article by Anita Van den Neuker in Clinical Biochemistry 2002 (35:29-33) which offers strong support of the NIRA methodology.

3. More detail in the methods would be helpful. Did you homogenize the stool? How did you calibrate the instrument? Did you measure a coefficient of variation or some other estimate of variability/reliability? This is not the place to include normal values (should be with the results).

4. Results: More detail about the normal NIRA values would be helpful. Reference 12 gives a mean stool water content of 80.7 g/100g (68.7-92.7) for infants < 6 months. Van den Neuker gives a mean of 74.6% (range 58-95%, SD 7.25%) for adults. Please give details about where the quoted normal value of 80-85% comes from. It appears from the text and from Table 3 that these are normal values from infants you have studied. If this is the case, it would strengthen the paper to report the normal values for non-constipated infants using the same technique and instrument. Ideally you could include a group of non-constipated formula-fed infants with mean age of 4 weeks; this would not need to be a large group. These data would allow a meaningful comparison (baseline numbers for the constipated infants vs. the non-constipated infants). At minimum, you should report how many non-constipated infants were included (< 6 months is the most useful, if you have data on at least 10 non-constipated infants < 6 months old, you wouldn’t need the 6-12 month old data). The normal values should be reported as a mean +/- SD (as the study patients are reported), rather than “less than” a given value.

5. Results: The Bristol stool chart should be briefly explained in the text with a reference. The current table 2 should include the mean Bristol chart numbers (ranked data are better than dichotomized data).

6. The tables should be modified to provide more detail. For instance, Table 1 should include the compositional data for the formula most commonly ingested prior to the study formula to allow a direct comparison of the lactose, energy, and magnesium content. If the authors include a non-constipated group of infants for comparison as recommended, the second table would be a good place to compare non-constipated to the baseline data for the constipated group (birth weight, age in weeks at enrollment, % formula fed, and the baseline NIRA data for fat, nitrogen, carbohydrate, and water. The third table would then be for comparison of before and after in the constipated group and should include NIRA data, Bristol stool score, pain with defecation, and need for rectal stimulation.

7. Statistics: The preferred statistical analyses for comparison of two groups are as follows:

   Normally distributed continuous data: T test
   Continuous data that are not normally distributed or ranked data: Mann-Whitney rank sum test
   Categorical data: Chi square test
If the authors include a non-constipated comparison group, these would be appropriate.

The preferred statistical analyses for comparison in one group before and after a treatment are as follows:

- Normally distributed continuous data: paired t-test
- Continuous data that are not normally distributed or ranked data: Wilcoxon signed rank test
- Categorical data: McNemar’s test

For the data in the current table 2, the Chi-square test is not the best test. The Wilcoxon test would be better if the Bristol scores are used and the McNemar test for the pain with defecation and need for rectal stimulation. By my calculations, based on the percentages provided in table 2 (I assumed that none of the infants that did not need external help at inclusion needed external help after 2 week), a McNemar test for the “need for external help” yields a p value of 0.001.

8. Discussion. The amount of magnesium in the modified formula is higher than current formulas available in the U.S. You note in the discussion that this is “close to the maximum level allowed.” It would be helpful to reference that guideline and to note any safety concerns (Did any of the infants develop diarrhea or hypotonia? Did you measure serum Mg levels in any of the infants? Has longer use of this formula resulted in any side effects or evidence of hypermagnesemia?).

9. The discussion is a bit meandering and could be shortened without decreasing the impact of the paper.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.