Reviewer’s report

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

Version: 3 Date: 22 December 2010

Reviewer: Mark Underwood

Reviewer’s report:

The manuscript has been significantly improved with response to some of the recommendations of the reviewers. The following are major compulsory revisions that remain and should be addressed prior to publication:

1. The primary outcome is the one that is the most interesting and should be presented and discussed first in each section. Stool NIRA is the outcome that adds to the literature. For instance in the abstract, the last sentence of the material and methods section should be switched to fit the presentation in the results section of the abstract (“Stool composition was measured by near-infrared reflectance analysis (NIRA) and parents answered questions about crying associated with defecation and stool consistency at baseline and after two weeks of the adapted formula.”). In the Patients section, the second paragraph should be divided and the structure changed to put the primary outcome first. Split the paragraph after “modified nutrients or laxatives to treat constipation.” The new paragraph should start with the primary variable: “After enrollment, all 30 infants were fed with Novalac AE (IT) (United Pharamceuticals SA, France) for 2 weeks. The composition of this formula (Table 1) has been adapted, in compliance with EU regulations, to ease constipation. Stools at baseline and after 2 weeks of the adapted formula were assessed for faecal fat, water, carbohydrate, and protein by NIRA. A questionnaire about crying during defecation, stool consistency, and need for help with defecation was filled out by the parents at inclusion and after 2 weeks of the adapted formula. To evaluate stool consistency, the Bristol photographic scale was used ....” The following sentences could then be deleted from this section since they do not add: “The number of stools was not reported here ...” and “A new clinical evaluation and determination of the stool consistency .....” In the Results section, move paragraph two to become the first paragraph and change table 3 to table 2 and table 2 to be table 3.

2. The English is still awkward and should be reviewed by a native speaker. For example the following are corrections of sentences with grammar errors: Abstract conclusion: “This preliminary study suggests that an adapted formula with high levels of lactose and magnesium increases stool water content and improves constipation in term formula-fed infants.” Background first paragraph, fifth sentence: “The term ‘functional constipation’ is used when no specific organic cause can be found.” Patients first sentence, stick with a consistent tense: “In the present study, constipation was characterized ...”(keep the tense the same as that used in the next paragraph). Same section, 2nd paragraph: “All were fed a
standard formula prior to enrollment and visited their paediatrician for constipation. Statistical analysis, first sentence: “Non-parametric tests were used to test for changes in quantitative measures over time (Wilcoxon signed rank test) and categorical changes over time (McNemar test).” Discussion, last paragraph: “In conclusion, this non-randomized non-placebo controlled preliminary study demonstrates that the use of an infant formula with high lactose and magnesium concentrations may increase stool hydration which softens the stools with corresponding clinical improvement.”

3. The discussion has been improved significantly, but could still be better focused. I would suggest starting the second paragraph like this: “Several hypotheses have been proposed for the softer stools associated with breast feeding. First, increased levels of gastric inhibitor polypeptide, motilin, neurotensin, vasoactive intestinal peptide secretion in formula-fed infants compared to breast-fed infants may explain a slower intestinal transit (25). Second, more frequent breast feedings may stimulate the gastro-colic reflex resulting in more frequent defecation. Third, human milk contains large amounts of prebiotic oligosaccharides. Fourth, fat composition may play a role in softer stools. In breast milk and in most infant formulae, fats represent nearly 50% of energy content. The principle saturated fatty acid in breast milk is palmitic acid (C16:0) with more than 70% esterified to the sn-2 position, whereas in regular infant formulae 88-94% of palmitic acid is esterified to the sn-1,3 position. Lipolysis at the sn-1 and sn-3 positions requires pancreatic lipase, which is deficient in the first 6 months of life. The result is relative fat malabsorption, which may react with luminal calcium to form calcium soaps producing hard stools (25,26).” Next paragraph: “Previous NIRA studies have shown lower stool fat in breast fed infants: 2.6% in breast-fed infants versus 7-11% in formula-fed infants younger than 6 months and less than 5% in formula-fed infants older than 6 months (9, 10). Our study confirmed the relative steatorrhea in formula-fed infants (18, 19). Feeding a formula containing high concentration sn-2 palmitic acid caused softer stools but no change in stool frequency in one study (19) and decreased fatty acid soaps in another study (27). We saw no change in stool fat content in the current study, suggesting that improvement in constipation was not related to changes in fat metabolism.” Next paragraph: “A clinical trial by Chao et al of 93 infants (average age 3.8 months) showed that the same formula we used in the current study (Novalac AE/IT) improved infant constipation. The infants receiving this formula had increased stool weight and decreased abdominal distention and irritability compared to infants receiving placebo (28). Our study adds to these results and includes the objective NIRA data.” Next paragraph: “The two additives to the adapted formula likely impact stool water content through different mechanisms. Lactose has prebiotic effects in that it is not completely absorbed in young infants and stimulates the growth of commensal bacteria. Non-hydrolyzed lactose reaches the colon, where it is metabolized by anaerobic microorganisms, producing an osmotic laxative effect as it attracts water into the intestinal lumen (29-32). Magnesium, due to its osmotic properties, increases the laxative effect and stimulates intestinal motility by inducing cholecystokinin secretion (33).”

4. The authors should not imply that the stool fat, nitrogen, and carbohydrate
content decreased with treatment. The last sentence in the results section should be changed to; “Other parameters (fat, nitrogen, carbohydrates) did not change.” The last sentence in the second to last paragraph of the discussion (“The decrease of fat, protein, and carbohydrate content in the stools …”) should be deleted.

5. In my original review, I suggested that the authors add more detail to the manuscript about stool NIRA. In their response they note that they have previously calibrated measurements of fat, nitrogen, water and carbohydrate in 80 stool samples from infants and children. This is helpful information that should be included in the manuscript. For instance the first sentence of the 2nd paragraph of the Methods section could read: “Prior to the current study we compared NIRA measurements of stool fat, nitrogen, water and carbohydrate content in eighty stool samples from infants and children to the same measurements performed by classic methods (6, 8, 15-17).”

The following are minor but essential revisions that will improve the paper;

1. Be consistent with using either commas or periods to represent decimals in the tables and in the text. Switching back and forth is distracting.

2. Be careful about the use of the word “clinical,” which usually implies a judgment by a health care provider rather than the opinions of parents. For instance the title for the current Table 2 (which I suggest should be Table 3) would be more accurate if it were “Parental Assessments.”

3. Be consistent in using either the word carbohydrate or sugar, switching back and forth is distracting.

4. The title of the current Table 3 (which I have suggested should be Table 2 in the revision) should be clearer. For example “Stool NIRA at inclusion and follow-up (2 weeks).” “Biochemical data implies a chemical analysis.

5. The label for the right columns in Tables 2 and 3 should be “p value*” rather than “Wilcoxon test” or “McNemar’s test.” A line can be added below each table: *Wilcoxon signed rank test. or *McNemar’s test.

**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

**Declaration of competing interests:**

I declare that I have no competing interests.