1. SYSTEMATIC REVIEW AND META-ANALYSIS

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Glycerin Enemas and Suppositories in Premature Infants: A Meta-analysis

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Short title: Glycerin Enemas and Suppositories in Premature Infants

Abbreviations: 95% CI – 95% confidence interval, NEC – necrotizing enterocolitis, NICU – neonatal intensive care unit, MD – mean difference, RR – risk ratio, SD – standard deviation

Key words: premature infants, glycerin, enema, suppository, enteral feeding, necrotizing enterocolitis

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Contributors' Statements

Michael H Livingston: Dr. Livingston designed the study, reviewed records, analyzed and interpreted data, drafted the initial manuscript, and approved the final manuscript as submitted.

Anna C Shawyer: Dr. Shawyer reviewed records, extracted and interpreted data, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Peter L Rosenbaum, Connie Williams, Sarah A Jones, J Mark Walton: Drs. Rosenbaum, Williams, Jones, and Walton helped conceptualize the study, interpreted data, reviewed and revised the manuscript, and approved the final manuscript as submitted.

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Abstract

Background and Objective: Premature infants are often given glycerin enemas or suppositories to facilitate meconium evacuation and transition to enteral feeding. The purpose of this study was to assess the available evidence for this treatment strategy.

Methods: We conducted a systematic search of Medline, Embase, Central, and trial registries for randomized controlled trials of premature infants treated with glycerin enemas or suppositories. Data were extracted in duplicate and meta-analyzed using a random effects model.

Results: We identified 185 premature infants treated prophylactically with glycerin enemas in one trial (n=81) and suppositories in two other trials (n=104). All infants were less than 32 weeks gestation and had no congenital malformations. Treatment was associated with earlier initiation of stooling in one trial (2 vs 4 days, p=0.02) and a trend towards earlier meconium evacuation in another (6.5 vs 9 days, p=0.11). Meta-analysis demonstrated no effect on transition to enteral feeding (0.7 days faster, p=0.43) or mortality (p=0.50). There were no reports of rectal bleeding or perforation but there was a trend towards increased risk of necrotizing enterocolitis with glycerin enemas or suppositories (risk ratio=2.72, p=0.13). These three trials are underpowered and affected by one or more major methodological issues. As a result, the quality of evidence is low to very low. Three other trials are underway.

Conclusions: The evidence for the use glycerin enemas or suppositories in premature infants in inconclusive. Meta-analyzed data suggest that treatment may be associated with increased risk of necrotizing enterocolitis. Careful monitoring of ongoing trials is required.

What's Known on this Subject

Premature infants are often given glycerin enemas or suppositories to facilitate the passage of meconium and transition to enteral feeding. There is little evidence to support this practice and guide treatment decisions.

What This Study Adds

The evidence for the use of glycerin enemas or suppositories in premature infants is inconclusive. Meta-analysis suggests that treatment has no consistent benefit and may be associated with increased risk of necrotizing enterocolitis. Careful monitoring of ongoing trials is required.

Feeding and nutrition are significant challenges for premature infants in the neonatal intensive care unit (NICU) [1-8]. These patients often receive glycerin enemas or suppositories to stimulate the passage of meconium and improve feeding tolerance [9]. This practice is based on the observation that many premature infants experience significant delays in the passage of meconium, which is more viscous than normal stool [10,11]. Delays in meconium evacuation appear to be associated with a delay in the transition to enteral feeding [12]. If meconium evacuation could be expedited through the use of glycerin enemas or suppositories, this might lead to faster transition to enteral feeding, decreased reliance on parenteral nutrition, and better clinical outcomes [13]. Unfortunately, there is little evidence to support this practice and guide treatment decisions [9,14].

The objective of the current review was to assess the level of evidence regarding the use of glycerin enemas and suppositories in premature infants by updating the systematic review on this topic. We considered the results from randomized controlled trials of glycerin enemas or suppositories, and meta-analyzed data whenever possible. We also searched the grey literature and trial registries to identify trials that are underway or have not yet been published.

Methods

Search Strategy

We conducted a systematic search of Medline, Embase, and the Cochrane Central Register of Controlled Trials for randomized controlled trials of infants treated with glycerin enemas or suppositories. An experienced medical librarian developed queries for each database to identify studies that mentioned "premature infants" and "glycerin laxatives." These concepts were expanded to ensure that no studies were missed due to variation in syntax and nomenclature (see Appendices 1-3 for search strategies and results).

We also performed manual searches of conference proceedings [15], theses and dissertations [16-18], and trial registries [19-22]. We included all citations up until July 2014. No language limits were placed on the search.

Study selection

Title and abstract screening was completed independently and in duplicate by the first two authors (MHL, ACS). Our default approach when there was disagreement was to automatically include the record. Inclusion criteria were: (1) participants who were premature infants less than 32 weeks gestation and/or birth weight less than 1500 grams, (2) interventions that were glycerin enemas or suppositories used prophylactically or as rescue therapy for jaundice or feeding intolerance, and (3) studies that were randomized

controlled trials. Glycerin enemas and glycerin suppositories were selected as important subgroups a priori. The review protocol is available from the authors upon request.

Data extraction

Studies selected for full text review underwent data extraction independently and in duplicate by the first two authors (MHL, ACS). To ensure consistency, we developed standardized data collection forms prior to use. Participant data included gestational age, birthweight, gender, presence of congenital anomalies, type of feeds (breastfeeding versus formula), age at start of enteral feeds, and calendar years of recruitment. Intervention characteristics included treatment type (glycerin enemas versus suppositories), type of placebo (no intervention versus sham procedure), dose, and treatment duration.

Outcomes of interest were selected a priori. These included (in order of decreasing importance): mortality, necrotizing entercolitis (NEC), rectal perforation, rectal bleeding, feeding intolerance, jaundice, transition to enteral feeding, and meconium evacuation. We selected these outcomes because they are clinically relevant and/or likely to be affected by the use of glycerin enemas or suppositories. Mortality, NEC, and rectal perforation were specified as critical outcomes a priori.

Statistical analysis

Outcome data were pooled whenever possible in Review Manager Version 5.2 [23]. We decided to use a random effects model a priori because we anticipated moderate heterogeneity between studies due to variation in the participants and types of interventions. Summary statistics were reported as risk ratio (RR) for dichotomous outcomes and mean difference (MD) for continuous outcomes. We also reported 95% confidence intervals (CI) and p-values.

Heterogeneity was reported quantitatively using the I^2 statistic: 0-40% might not be important (i.e., low heterogeneity), 30-60% may represent moderate heterogeneity, 50-90% may represent substantial heterogeneity, and 75-100% considerable heterogeneity [24]. The importance of the observed value (e.g., "low" versus "moderate") depends on (1) magnitude and direction of effects and (2) strength of evidence for heterogeneity (e.g., p-value from the chi-squared test) [24].

For the purposes of meta-analysis, we estimated standard deviation (SD) whenever it was not reported explicitly using the following approach: range/4 (when n=15-70) and range/6 (when n>70) [25]. Given the low number of studies meeting our inclusion criteria, we decided not to produce a funnel plot for any of the pooled outcomes [24].

Risk of bias assessment for individuals trials

Risk of bias for individual trials was assessed independently and in duplicate using the Cochrane Collaboration's tool for assessing risk of bias [24]. This instrument consists of six domains that classify risk of bias as low, unclear, or high. Differences between reviewers were resolved through discussion and consensus [24].

Quality of evidence across studies

The quality of evidence for each outcome was reported using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system [26] with GRADEpro Version 3.6 [27]. This system takes into account findings from multiple studies and grades the quality of evidence for each outcome as high (4/4 points), moderate (3/4), low (2/4), or very low (1/4). By default, the quality of evidence is high for results from randomized controlled trials, and low for results from observational studies. Ratings can be downgraded due to risk of bias, inconsistency, indirectness, imprecision, or publication bias, and upgraded due to the presence of a large effect (RR<0.5 or >2), if attempts to control for confounding would change the observed effect, and the presence of a dose-response gradient.

Results

Search Results

Our systematic search of Medline, Embase, and the Cochrane Central Register of Controlled Trials yielded 68 titles and abstracts. This was reduced to 45 records after

duplicates were removed. Titles and abstracts were screened independently and in duplicate.

Six records were selected for full-text review. Two of these were excluded because they were not randomized controlled trials [28,29]. Another study was excluded because the intervention was glycerin enemas as well as oral probiotics three times a day for seven days [30]. Probiotics have been shown to decrease the risk of NEC and all-cause mortality in preterm infants, so combined treatment likely has different effects than using glycerin suppositories alone [31]. This resulted in three randomized controlled trials of glycerin enemas (one trial) or suppositories (two trials) [14,32,33]. An overview of study selection is shown in Figure 1.

Participants

The three randomized controlled trials included 185 premature infants with gestational age less than 32 weeks and/or birth weight less than 1500 grams. One trial included infants between 28 and 32 weeks gestational age only [33]. All three trials specifically excluded infants with major congenital malformations or structural gastrointestinal anomalies. One of the glycerin suppository trials also excluded premature infants with hypoxic ischemic encephalopathy stage 2 or greater [32]. The other excluded those with any signs of hemodynamic instability [33].

Interventions

Study interventions consisted of prophylactic glycerin enemas or suppositories administered once daily for several days. Each trial established slightly different dosing, treatment start date, and duration (Table 1). In the trial of glycerin enemas, study treatments started at 12 hours of life and continued until meconium evacuation was complete (defined as two normal stools free of meconium staining) [14]. No maximum treatment duration was reported. In the two trials of glycerin suppositories, study interventions started at 24 (or 48) hours of life and continued for a total of 10 (or 13) days of treatment, regardless of stooling or meconium evacuation [32,33].

Participants in the control group in two of the trials were assigned to no intervention [14,32]. In Shinde et al., participants in the control group were treated with a sham procedure, which involved opening the diaper and closing it again [33,34].

Mortality

Mortality rates between treatment groups were similar in all three trials and ranged from 5-17% [32,33,35]. Mortality data in the glycerin enema trial was confirmed following personal communication with the principal investigator [35]. Meta-analysis of data from all three trials revealed no difference between treatment groups (RR=1.34, 95% CI 0.58-3.11, I^2=0%, p=0.50) (Figure 2).

Necrotizing enterocolitis

The rates of NEC between treatment groups were similar in all three trails and ranged from 5-9% [14,32,33]. The diagnosis of NEC was based on Bell stage [36] 1 or greater in Shinde et al. [33] and 2 or greater in the other two trials [32,35]. In Shinde et al., the rate of NEC was reported only for infants who survived or were not transferred to another hospital before reaching full enteral feeds [33]. As a result, there were 12 cases of NEC among 179 premature infants (7% overall). 9/93 of these (10%) occurred following the use of glycerin enemas or suppositories and 3/86 (4%) occurred in the control group. Meta-analysis approached statistical significance for increased risk of NEC with the use of glycerin enemas or suppositories (RR=2.72, 95% CI 0.76-9.81, I^2=0%, p=0.13) (Figure 3).

Rectal perforation

There were no cases of rectal perforation in all three trials. Absence of rectal perforation was reported explicitly in two of the trials [14,32] and was confirmed in the other following personal communication with the principal investigator of the other trial [35].

Rectal bleeding

There were also no cases of rectal bleeding. Absence of rectal bleeding was reported explicitly in one of the glycerin suppository trials [32] and was confirmed in the other two studies via personal communication [34,35].

Feeding intolerance

There were no differences between treatment groups in terms of feeding intolerance. This outcome was reported in the two glycerin suppository trials but was variably defined [7,32,33]. In Khadr et al., there were no differences between groups in terms of incidence of abdominal distension greater than 2 cm, number of bilious residuals, number of feeds withheld, number of feeds reduced or not increased, and percentage of gastric residuals compared to total enteral feed volume [32]. These outcomes were all measured within the first 10 days of life.

In Shinde et al., there was no difference between treatment groups in terms of the number of participants who had feeds withheld: 7/21 versus 4/21 (RR=1.75, 95% CI 0.60-5.10, p-value not reported). Differences in how feeding intolerance was reported in these two trials resulted in data that could not be meta-analyzed.

Jaundice

None of the trials reported outcomes related to jaundice, such as or serum bilirubin level or need for phototherapy.

Transition to enteral feeding

There were no differences between treatment groups in transition to enteral feeding in any of the three trials. Each trial defined full enteral feeding differently: 150 ml/kg/day Haiden et al. [14]; maintaining 180 ml/kg/day for 24 hours in Shinde et al. [33]; and

tolerance of full enteral feeds (prespecified volume not defined) and discontinuation of parenteral nutrition for greater than 48 hours without feeds being reduced or withheld by Khadr et al. [32]. There were also differences in feeding regimens, with feeding initiated on median day number 1 (range 1-1.8) [32], median day number 2 (range 0-9) [14], or mean day 5.5 (SD 2.5) [33].

The two glycerin suppository trials also reported the type of feed used. In the trial by Khadr et al., which included premature infants 24-32 weeks gestation, expressed breast milk was used exclusively in 10/54 infants (19%). In Shinde et al., which included premature infants 28-32 weeks gestation only, 37/50 (74%) received exclusive expressed breast milk. Personal communication with principal investigator of the glycerin enema trial indicated that the use of expressed breast milk when the study was conducted was approximately 70-80% [35].

For the purposes of meta-analysis, we calculated transition to enteral feeding as the difference (in number of days) between the mean or median start of enteral feeding and full enteral feeding. Across all three trials, there was no statistically significant difference between treatment groups (0.7 days faster with glycerin enemas or suppositories, 95% CI 2.4 days faster to 1.0 days slower, p=0.49) (Figure 4). Heterogeneity was substantial (I^2=51%) but there was no statistically significant subgroup difference between glycerin enemas and suppositories (p=0.89).

Completion of meconium evacuation

The trial of glycerin enemas reported a non-significant trend towards earlier completion of meconium evacuation with active treatment (median 6.5 versus 9 days, p=0.11) [14]. The other two trials did not report this outcome [32,33].

Initiation of meconium evacuation

Initiation of meconium evacuation was not affected by the use of glycerin enemas starting at 12 hours of life (median 1 versus 1 days, p=0.68) [14] but a statistically significant effect was observed for the use of glycerin suppositories starting at 24 hours of life (median 2 versus 4 days, p=0.016) [32]. In the glycerin suppository trial, treatment was also associated with lower frequency of delayed initiation of meconium evacuation (24% versus 64%, p=0.003).

Meta-analysis of data for initiation of meconium evacuation did not demonstrate a significant treatment effect (1 day faster with treatment, 95% CI 3.0 days faster to 0.9 days slower, I^2=89%, p=0.30) (Figure 5). The confidence intervals from the two trials did not overlap and the test for subgroup differences was significant (p=0.002).

Other outcomes

There were no differences between treatment groups in terms of intraventricular hemorrhage, retinopathy of prematurity, patent ductus arteriosus, culture-positive sepsis,

or oxygen requirements (Table 2) [32,35]. All three trials also reported no differences between groups for weight gain or length of stay in hospital [14,32,33].

Risk of bias of individual studies

All three trials were at high risk of bias in two or more (of six) domains (Table 3). Sequence generation was created with random number software in two of the trials [34,35] and shuffling of sealed envelopes in Khadr et al. [32]. Allocation concealment was maintained in all three studies using opaque envelopes [33-35]. Two of the three trials were open studies with no blinding [14,32]. Shinde et al. relied on a research nurse to administer study interventions, and all other clinicians, study personnel, and outcome assessors were blinded to treatment allocation [33,34].

Incomplete outcomes were discussed in all three trials. In the most recent glycerin suppository trial, 3 participants in each group (greater than 10% of the total sample size) were transferred to another hospital before complete outcomes could be obtained [33]. We performed a sensitivity analysis for this by imputing missing data for mortality and NEC using the approach of "best and worst-case scenario" [24]. This did not affect the meta-analysis for mortality. For NEC, however, the worst-case scenario (i.e., 3/3 infants in the treatment group developing NEC versus 0/3 in the control group) would have the made our meta-analysis statistically significant (RR=3.68, 95% CI 1.07-12.65, p=0.04). As a result, we concluded that the lack of complete outcome data for this trial resulted in high risk of bias.

The protocol for Khadr et al. was registered and available online, and there was no evidence of selective reporting [32,37]. The other two trials were not registered, so the risk of bias due to selective reporting was unclear [34,35].

All three trials were at high risk of bias due to low power. The glycerin enema trial was powered to detect a "30% difference" in days to complete meconium evacuation [14]. The glycerin suppository trials were powered to detect a reduction in days to full enteral feeding of 3.63 and 3 days, respectively [32,33]. These effect sizes are substantially larger than the magnitude of the true effect size (if one actually exists). Powering these trials for a more moderate effect size would have required substantially larger sample sizes.

The glycerin enema trial was also at high risk of bias due to the number of protocol violations: 15/42 infants in intervention group missed a scheduled glycerin enema, and 8/39 in the control group received at least one enema despite being assigned to no treatment [14]. Outcomes were reported on the basis of intention-to-treat and per protocol but there were no significant differences between these approaches. The other two trials also analyzed results on the basis on intention-to-treat [32,34].

Quality of evidence

The quality of evidence across all three trials for mortality, NEC, transition to enteral feeding, and completion of meconium evacuation was low (2/4 points). Using the

GRADE approach, the quality of evidence was downgraded for all outcomes due to risk of bias (-1 point) (Table 3) [38,39]. Ratings were also downgraded for imprecision by 1 point for low number events (for mortality and NEC) and/or 1 point for confidence intervals that crossed one (for NEC, transition to enteral feeding, and completion of meconium evacuation) [40]. The quality of evidence for NEC was upgraded due to the presence of a large effect (+1 point) [41].

The quality of evidence for rectal bleeding, rectal perforation, and initiation of meconium evacuation was very low (1/4 points) (Table 4). This was due to risk of bias (-1 point) and imprecision due to: (1) low number of events (-1 point), and/or; (2) confidence intervals that crossed 1 (-1 point) [39,40]. The quality of evidence for initiation of meconium evacuation was also downgraded due to inconsistency, since the confidence intervals from the two trials did not overlap [42]. The other possible explanation for this lack of overlap is that glycerin enemas and glycerin suppositories may have truly different effects on initiation of meconium evacuation.

Ongoing trials

Our systematic search of the grey literature and trial registries resulted in 77 records. Three of these were protocols for ongoing randomized controlled trials of premature infants treated prophylactically with glycerin suppositories. The first trial recruited 79 premature infants from 30 to 35 weeks gestation who required phototherapy for physiologic hyperbilirubinemia [43]. The primary outcome was total duration of

phototherapy. Secondary outcomes included duration of initial phototherapy, need to restart phototherapy, peak serum bilirubin, mean serum bilirubin, rate of decline of serum bilirubin, and use of stool softeners after completion of phototherapy. Recruitment was completed in 2013 and results are pending.

We also identified a two-center trial of glycerin suppositories from Saudi Arabia with plans to recruit 220 premature infants with a birthweight less than 1250 grams [44]. The primary outcome is days to full enteral feeding and recruitment began in 2013. In January 2015, our center started recruitment for a pilot randomized controlled trial of 30 premature infants 24 to 32 weeks gestation and/or birthweight 500 to 1500 grams [45]. The purpose of this study is to further assess the feasibility and safety of glycerin suppositories before embarking on a larger, multicenter trial.

Discussion

The quality of evidence for the use of glycerin enemas and suppositories in premature infants is low to very low. Three single-center, randomized controlled trials have been conducted and published to date [14,32,33]. These studies are underpowered and at risk of bias due to a variety of methodological issues. These include problems with blinding, outcome assessment, incomplete follow-up, and/or the possibility of selective reporting [24].

The published data from these three trials suggest that the use of glycerin enemas and suppositories has no effect on transition to enteral feeding or mortality. One trial reported earlier initiation of meconium evacuation with glycerin suppositories [32] and another reported a trend towards earlier completion of meconium evacuation with glycerin enemas [14]. While there were no reports of rectal bleeding or perforation in these three trials, our meta-analysis demonstrated a trend towards increased risk of NEC with the use glycerin enemas or suppositories compared to no treatment. The total number of events in each group was very small (9/92 versus 3/85), and although the risk ratio was 2.72, the accuracy of this estimate remains uncertain.

Given the methodological limitations of randomized trials published to date, we believe that the evidence for the use of glycerin enemas or suppositories in premature infants is inconclusive. Meta-analyzed data suggest that treatment is not associated with any consistent benefits and may result in harm. These conclusions are similar to those of previous systematic reviews but include an additional message of caution given the concerning but non-significant trends demonstrated by our meta-analysis.

A systematic review published in 2011 assessed whether glycerin enemas and suppositories decrease feeding intolerance in premature infants [9]. The authors considered the results from the trial on glycerin enemas by Haiden et al. [14] and a historical cohort study of 83 very low birth weight infants from a single hospital in South Korea [13]. In the cohort study, full enteral feeding was achieved within 16 days during

the treatment period and 22.9 days for the control period ($p<0.001$). Significant improvements were also reported for days to passage of first meconium, duration of central catheter usage, sepsis after 7 days of life, and positive catheter culture. The authors of the systematic review reported that the historical cohort study was of fair quality and reported positive results, while the randomized controlled trial by Haiden et al. was of good quality but reported negative results. They concluded: "The evidence regarding the effectiveness of glycerin [enemas or suppositories] for improving feeding intolerance in very low birth weight infants is inconclusive" [9].

In 2014, an unpublished Cochrane Review was conducted that considered the results from the trials by Haiden et al. and Shinde et al. [14,32,46]. The authors concluded that the available evidence does not support the use of glycerin enemas or suppositories in clinical practice, and that further studies are needed [46].

Previous trials and systematic reviews have not assessed the effect of glycerin enemas or suppositories on jaundice in premature infants. Results are pending from a recently completed trial of 79 premature treated with glycerin suppositories to reduce jaundice [43]. Three other randomized controlled trials have also been conducted in healthy term infants [47-49]. A systematic review of these three studies was published in 2011 and concluded that early meconium evacuation using glycerin suppositories was not associated with any clinical benefit in healthy term infants [50].

Our search strategy identified three trials on the use of glycerin suppositories in premature infants that are underway or have not yet been published. One of these was recently completed and results are pending [43,11]. This trial did not specify NEC as a primary or secondary outcome a priori, but communication with the principal investigator confirmed that none of the 79 infants were diagnosed with NEC [51]. This was likely because this trial enrolled late (30 to 35 weeks gestation) rather than early preterm infants (less 32 weeks gestation), who are at higher risk for developing NEC.

The other two ongoing trials of glycerin suppositories in premature infants have started recruiting participants and are ongoing. These trials should be closely monitored for increased risk of NEC. The nature of the association between glycerin medications and NEC will become clearer as additional data become available. One possibility is that the apparent relationship is nothing more than a spurious correlation that will disappear with more data. The other explanation is that there is a real effect that has not yet become statistically significant. Our center has elected to proceed with a trial as a pilot study limited to 30 premature infants [45,52]. This trial and others like it should be stopped if it becomes clear that glycerin suppositories are associated with increased harm.

Conclusions

The quality of evidence for the use of glycerin enemas and suppositories in premature infants is low to very low. Previous randomized controlled trials are underpowered and at risk of bias due to a variety of methodological issues. As a result, the evidence for this

treatment is inconclusive. Meta-analyzed data suggest that glycerin enemas and suppositories have no consistent effect on meconium evacuation, transition to full enteral feeding, or mortality. Previous trials have assessed the effect of these medications on jaundice in term but not premature infants. There have been no cases of rectal bleeding or perforation in any of the trials published to date, but meta-analyzed data indicates a non-significant trend towards increased risk of NEC when glycerin enemas or suppositories are used on a daily basis. We recommend that ongoing trials be monitored carefully since treatment may be associated with increased harm.

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References

1. Su B. Optimizing Nutrition in Preterm Infants. Pediatr Neonatol 2013;55:5-13.
2. Belfort MB, Rifas-Shiman SL, Sullivan T, Collins CT, McPhee AJ, Ryan P, Kleinman KP, Gillman MW, Gibson RA, Makrides M. Infant growth before and after term: effects on neurodevelopment in preterm infants. Pediatrics. 2011;128:e899.
3. Sharma R, Hudak ML. A clinical perspective of necrotizing enterocolitis: past, present, and future. Clin Perinatol. 2013;40:27-51.
4. Raval MV, Hall NJ, Pierro A, Moss RL. Evidence-based prevention and surgical treatment of necrotizing enterocolitis-a review of randomized controlled trials. Semin Pediatr Surg. 2013;22:117-21.
5. Gardener VA, Walton JM, Chessel L. Refeeding technique in a Premature infant with Short Bowel Syndrome. Adv Neonatal Care. 2003;6:258-71.
6. Al-Harbi K, Walton JM, Gardner V, Chessel L, Fitzgerald P. Mucous fistula Refeeding in Neonates with Short Bowel Syndrome. J Pediatr Surg. 1999;34:1100-3.
7. Lucchini R, Bizzarri B, Giampietro S, De Curtis M. Feeding intolerance in preterm infants. How to understand the warning signs. J Matern Fetal Neonatal Med 2011;24 Suppl 1:72-4.

8. Morris BH, Miller-Loncar CL, Landry SH, Smith KE, Swank PR, Denson SE. Feeding, medical factors, and developmental outcome in premature infants. Clin Pediatr (Phila). 1999;38:451-7.
9. Shah V, Chirinian N, Lee S. Does the use of glycerin laxatives decrease feeding intolerance in preterm infants? Paediatr Child Health. 2011;16:e68-70.
10. Loening-Baucke V, Kimura K. Failure to pass meconium: Diagnosing intestinal obstruction. Am Fam Physician. 1990;60:2040-50.
11. Meetze WH, Franz AR, Linder W, Pohlandt F. Meconium passage in very-low birthweight infants. J Parenter Enteral Nutr. 1993;17:1537-40.
12. Mihatsch WA, Franz AR, Lindner W, Pohlandt F. Meconium passage in extremely low birthweight infants and its relation to very early enteral nutrition. Acta Paediatr. 2001;90:409-11.
13. Shim SY, Kim HS, Kim DH et al. Induction of early meconium evacuation promotes feeding tolerance in very low birth weight infants. Neonatology. 2007;92:67-72.
14. Haiden N, Jilma B, Gerhold B et al. Small volume enemas do not accelerate meconium evacuation in very low birth weight infants. J Pediatr Gastroenterol Nutr. 2007;44:270-3.
15. Conference Proceedings Citation Index. Accessed 1 August 2014. Thomas Reuters: New York, New York.
16. Index to Theses. Accessed 1 August 2014. ProqQuest: Cambridge, United Kingdom.
17. ProQuest Dissertations & Theses Database. Accessed 1 August 2014. ProQuest: Ann Arbor, Michigan.

18. OpenGrey. Accessed 1 August 2014. Institut de l'Information Scientifique et Technique: Vandoeuvre-lès-Nancy Cedex, France. http://www.opengrey.eu/
19. ClinicalTrials.gov. Accessed 1 August 2014. http://clinicaltrials.gov/
20. International Clinical Trials Registry Platform. Accessed 1 August 2014. http://apps.who.int/trialsearch/
21. European Union Clinical Trials Register. Accessed 1 August 2014. https://www.clinicaltrialsregister.eu/
22. International Standard Randomised Controlled Trial Number Registry. Accessed 1 August 2014. http://www.isrctn.org/
23. Review Manager (RevMan) Version 5.2. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012.
24. Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.
25. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. *BMC Med Res Methodol* 2005;5:13.
26. Guyatt GH, Oxman AD, Schünemann HJ, et al. GRADE guidelines: A new series of articles in the Journal of Clinical Epidemiology. J Clin Epidemiol 2010;64:380-2.
27. Grading of Recommendations Assessment, Development and Evaluation (GRADE) profiler (GRADEpro) Version 3.6. © GRADE Working Group, 2011.
28. Zenk KE, Koeppel RM, Liem LA. Comparative efficacy of glycerin enemas and suppository chips in neonates. Clinical Pharmacy. 1993;35:358-60

29. Shim SY, Kim HS, Kim DH et al. Induction of early meconium evacuation promotes feeding tolerance in very low birth weight infants. Neonatology. 2007;92:67-72.
30. Wang G, Li H, Huang Y, Huang C, Tan H. Effect of early intervention with glycerine enema and golden diplococci on feeding intolerance in very low birth weight infants. Journal of Applied Clinical Pediatrics. 2008;14:1110-35.
31. AlFaleh K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. 2014;4:CD005496.
32. Khadr SN, Ibhanesebhor SE, Rennix C et al. Randomized controlled trial: impact of glycerin suppositories on time to full feeds in preterm infants. Neonatology. 2011;100:169-76.
33. Shinde S, Kabra NS, Sharma SR, Avasthi BS, Ahmed J. Glycerin suppository for promoting feeding tolerance in preterm very low birthweight neonates: a randomized controlled trial. Indian Pediatr. 2014;51:367-70.
34. Nandkishor K. Personal communication 8 January 2015.
35. Haiden N. Personal communication. 7 January 2015.
36. Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Ann Surg. 1978;187:1–7.
37. Ibhanesebhor S. Tolerance of milk feeds in preterm, very low birth-weight babies: the use of glycerine suppositories. International Standard Randomised Controlled Trial Number Registry. ISRCTN47065764. http://www.isrctn.com/ISRCTN47065764. Accessed 1 August 2014.

38. Guyatt G, Oxman AD, Akl EA, et al.; GRADE Working Group. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. Clin Epidemiol 2011;64:383-94.
39. Guyatt GH, Oxman AD, Vist G, et al.; GRADE Working Group. GRADE guidelines: 4. Rating the quality of evidence--study limitations (risk of bias). J Clin Epidemiol 2011;64:407-15.
40. Guyatt G, Oxman AD, Kunz R, et al.; GRADE Working Group. GRADE guidelines 6. Rating the quality of evidence--imprecision. J Clin Epidemiol 2011;64:1283-93.
41. Guyatt GH, Oxman AD, Sultan S, et al.; GRADE Working Group. GRADE guidelines: 9. Rating up the quality of evidence. J Clin Epidemiol. 2011;64:1311-16.
42. Guyatt G, Oxman AD, Kunz R, et al.; GRADE Working Group. GRADE guidelines: 7. Rating the quality of evidence—inconsistency. 2011;64:1294-302.
43. D'Angio C. Glycerin Suppositories to Reduce Jaundice in Premature Infants. NCT01746511. https://www.clinicaltrials.gov/ct2/show/NCT01746511
44. Khadawardi EM. Efficacy of Prophylactic Glycerin Suppositories for Feeding Intolerance in Very Low Birth Weight Preterm Infants: a Randomized Trial. NCT01799629. https://www.clinicaltrials.gov/ct2/show/NCT01799629
45. Walton JM. Glycerin Suppositories Used Prophylactically in Premature Infants (SUPP): a Pilot Randomized Placebo-controlled Trial. NCT02153606. https://clinicaltrials.gov/ct2/show/NCT02153606

46. Anabrees J, Shah VS, AlOsaimi A, AlFaleh K. Glycerin laxatives for prevention or treatment of feeding intolerance in very low birth weight infants. Cochrane Database Syst Rev. CD010464. (in press)
47. Weisman LE, Merenstein GB, Digirol M, Collins J, Frank G, Hudgins C. The effect of early meconium evacuation on early-onset hyperbilirubinemia. Am J Dis Child 1983;137:666-668.
48. Chen JY, Ling UP, Chen JH. Early meconium evacuation: Effect on neonatal hyperbilirubinemia. Am J Perinatol 1995;12:232-4.
49. Bader D, Yanir Y, Kugelman A, Wilhelm-Kafil M, Riskin A. Induction of early meconium evacuation: Is it effective in reducing the level of neonatal hyperbilirubinemia? Am J Perinatol 2005;22:329-33.
50. Srinivasjois R, Sharma A, Shah P, Kava M. Effect of induction of meconium evacuation using per rectal laxatives on neonatal hyperbilirubinemia in term infants: a systematic review of randomized controlled trials. Indian J Med Sci. 2011;65:278-85.
51. D'Angio CT. Personal communication. 6 January 2015.
52. Thabane L, Ma J, Chu R, Cheng J, Ismaila A, Rios LP, Robson R, Thabane M, Giangregorio L, Goldsmith CH. A tutorial on pilot studies: the what, why and how. BMC Med Res Methodol. 2010;10:1.
53. Moher D, Liberati A, Tetzlaff J, et al.; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol 2009;62:1006-12.

Table 1.1: Characteristics of randomized controlled trials of premature infants treated with glycerin enemas or suppositories

Trial	Participants	n	Years recruited	Country	Interventions	Protocol
Haiden 2007	**Inclusion criteria**: • Gestational age 24-32 weeks • Birth weight ≤1500 g **Exclusion criteria**: • Major congenital anomalies • Gastrointestinal anomalies	81	2000-2001 [35]	Austria	1. **Daily glycerin enema** (n=42) 10 ml/kg (0.8 g glycerin in 10 ml of normal saline) 2. **No intervention** (n=39)	Not registered [35]
Khadr 2011	**Inclusion criteria**: • Gestational age 24-32 weeks **Exclusion criteria**: • Major congenital anomalies • Gastrointestinal anomalies • Hypoxic ischemic encephalopathy stage >2	54	2006-2008	United Kingdom	1. **Daily glycerin suppository** (n=29) 250 mg (24-28 weeks) 500 mg (28-32 weeks) 2. **No intervention** (n=25)	International Standard Randomised Controlled Trial Number 47065764 [37]
Shinde 2014	**Inclusion criteria**: • Gestational age 28-32 weeks • Birth weight 1000-1500 g **Exclusion criteria**: • Major congenital anomalies • Gastrointestinal anomalies • Hemodynamic instability	50	2010-2011	India	1. **Daily glycerin suppository** (n=25) 1000 mg (28-32 weeks) 2. **Sham procedure** (n=25) Open and closing diaper [34]	Not registered [34]

Table 1.2: Outcomes reported among included trials (primary outcome highlighted in **bold**)

Outcomes reported	**Haiden 2007**	**Khadr 2011**	**Shinde 2014**
Relevant outcomes[1]			
Mortality	Yes[2]	Yes	Yes
Necrotizing enterocolitis	Yes	Yes	Yes
Rectal perforation	Yes	Yes	Yes[2]
Rectal bleeding	Yes[2]	Yes	Yes[2]
Feeding intolerance		Yes	Yes
Transition to enteral feeding	Yes	**Yes**	**Yes**
Completion of meconium evacuation	**Yes**[3]		
Initiation of meconium evacuation	Yes	Yes[4]	
Other outcomes			
Intraventricular hemorrhage	Yes[2]	Yes	
Retinopathy of prematurity		Yes	
Patent ductus arteriosus	Yes[2]	Yes	
Culture-positive sepsis		Yes	
Oxygen requirements		Yes	
Length of stay	Yes	Yes	Yes
Weight gain	Yes	Yes	Yes

[1] Clinically relevant and/or likely to be affected by use of glycerin enemas or suppositories
[2] Confirmed following personal communication with principal investigator [34,35]
[3] Trend towards faster completion of meconium evacuation with glycerin enemas (median 6.5 vs 9 days, p=0.11)
[4] Faster initiation of meconium evacuation with glycerin suppositories (median 2 vs 4 days, p=0.016)

Table 1.3: Risk of bias of individual studies according to the Cochrane Collaboration risk of bias tool [24]

Domain	**Risk of Bias**		
	Haiden 2007	**Khadr 2011**	**Shinde 2014**
Random sequence generation	Low	Low	Low
Allocation concealment	Low	Low	Low
Blinding of patients and personnel	High	High	Low
Blinding of outcome assessment	High	High	Low
Incomplete outcomes addressed	Low	Low	High
No selective reporting	Unclear	Low	Unclear
No other bias	High[1,2]	High[1]	High[1]

[1] Low statistical power

[2] Frequent protocol violations

Table 1.4: Grading of Recommendations Assessment, Development and Evaluation (GRADE) evidence profile [36]

Quality assessment							Number of participants		Effect		Quality	Importance
No of studies	**Design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Glycerin laxatives**	**No Treatment**	**Relative (95% CI)**	**Absolute**		
Mortality												
3 (n=179)	randomized trials	serious[1]	none	none	serious[2]	none	12/93 (13%)	8/86 (9%)	RR 1.34 (0.58-3.11)	32 more per 1000 (39 fewer to 196 more)	⊕⊕OO LOW	CRITICAL
Necrotizing enterocolitis												
3 (n=179)	randomized trials	serious[1]	none	none	very serious[2,3]	large effect[4]	9/93 (10%)	3/86 (4%)	RR 2.72 (0.76-9.81)	60 more per 1000 (8 fewer to 307 more)	⊕⊕OO LOW	CRITICAL
Rectal perforation												
3 (n=185)	randomized trials	serious[1]	none	none	very serious[2,3]	none	0/96 (0%)	0/89 (0%)	-	-	⊕OOO VERY LOW	CRITICAL
Rectal bleeding												
3 (n=185)	randomized trials	serious[1]	none	none	very serious[2,3]	none	0/96 (0%)	0/89 (0%)	-	-	⊕OOO VERY LOW	IMPORTANT
Jaundice												
0 (n=0)	-	-	-	-	-	-	-	-	-	-	-	IMPORTANT
Transition to enteral feeding												
3 (n=177)	randomized trials	serious[1]	none	none	serious[2]	none	92	85	-	0.7 days faster (2.4 faster to 1.0 slower)	⊕⊕OO LOW	IMPORTANT
Completion of meconium evacuation												
1 (n=81)	randomized trials	serious[1]	none	none	serious[2]	none	42	39	-	2.5 days faster (p=0.11, CI not reported))	⊕⊕OO LOW	IMPORTANT
Initiation of meconium evacuation												
2 (n=135)	randomized trials	serious[1]	serious[5]	none	serious[2]	none	71	64	-	1 day faster (3.0 faster to 0.9 slower)	⊕OOO VERY LOW	IMPORTANT

[1] See Table 3
[2] 95% confidence intervals for relative risk include 1 [38] or p>0.05
[3] Small number of events (less than 300) [38]
[4] Risk ratio >2 [39]
[5] No overlap between confidence intervals from different studies [40]

Figure 1.1: Search results and study selection as per Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [53]

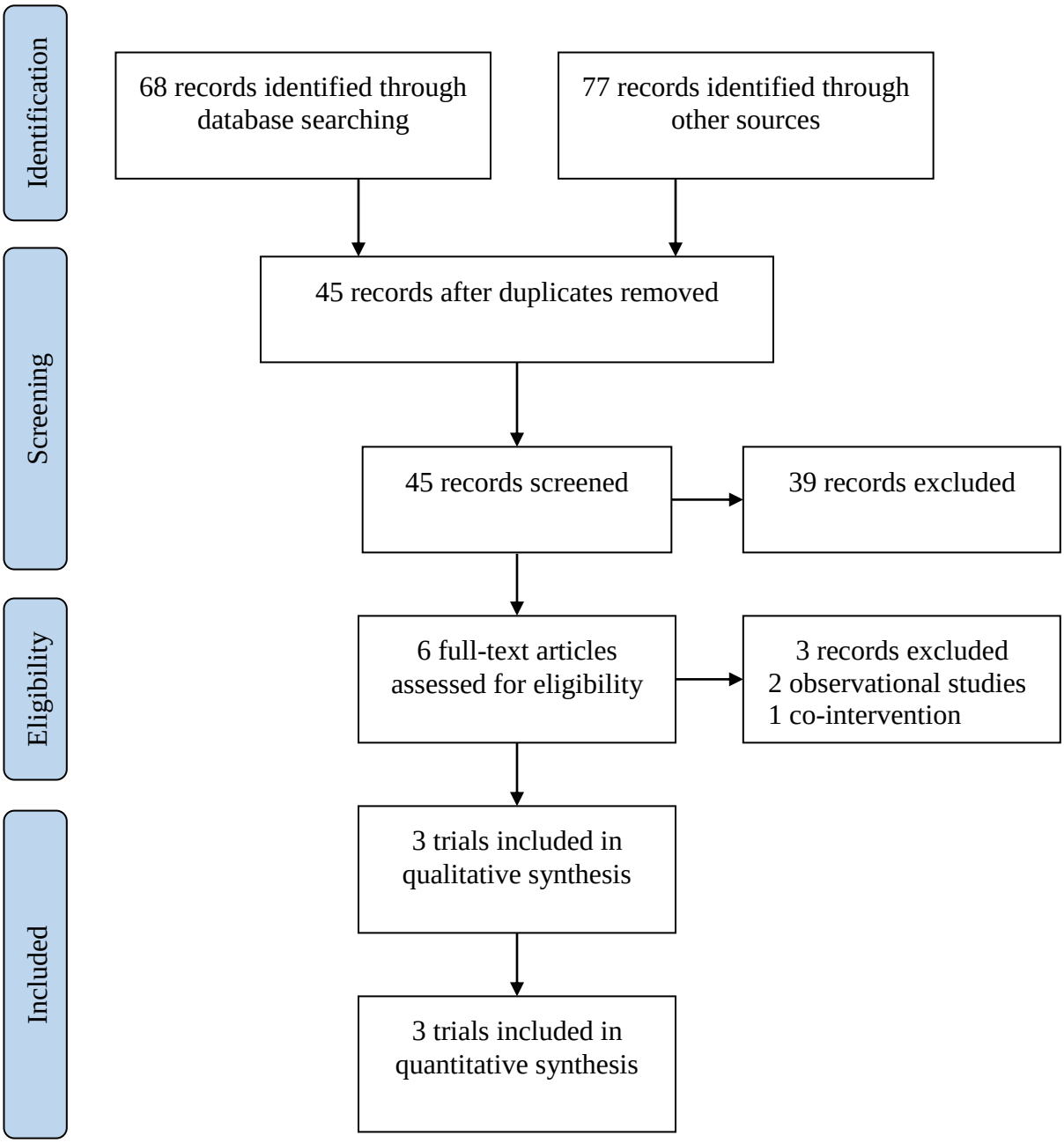


Figure 1.2: Risk of mortality in premature infants treated with glycerin enemas or suppositories versus no treatment.

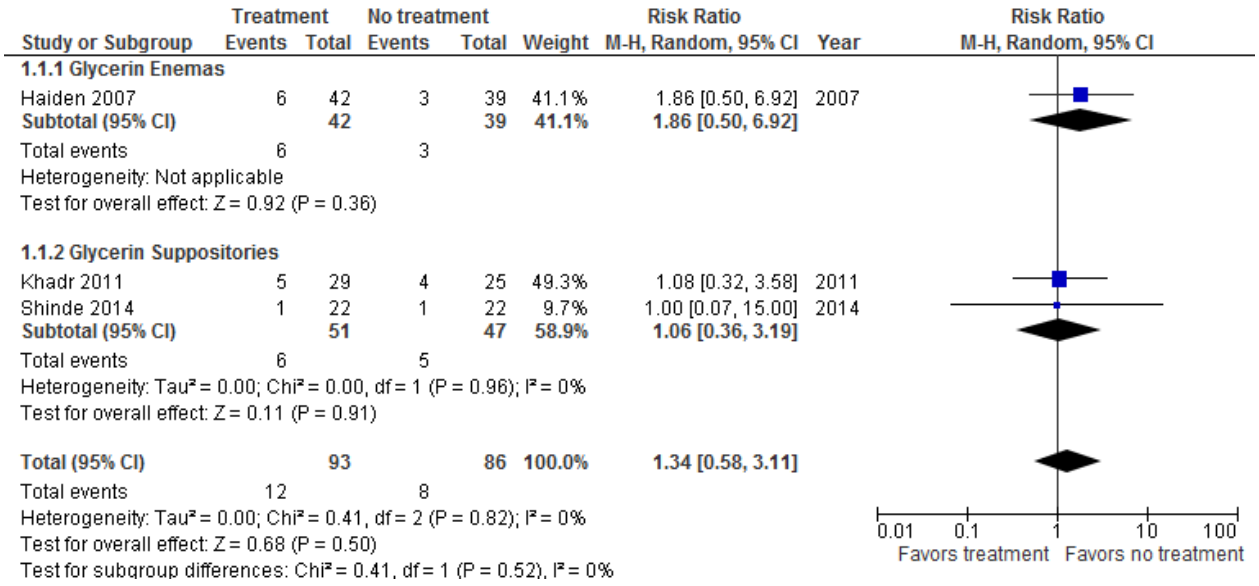


Figure 1.3: Risk of necrotizing enterocolitis in premature infants treated with glycerin enemas or suppositories versus no treatment.

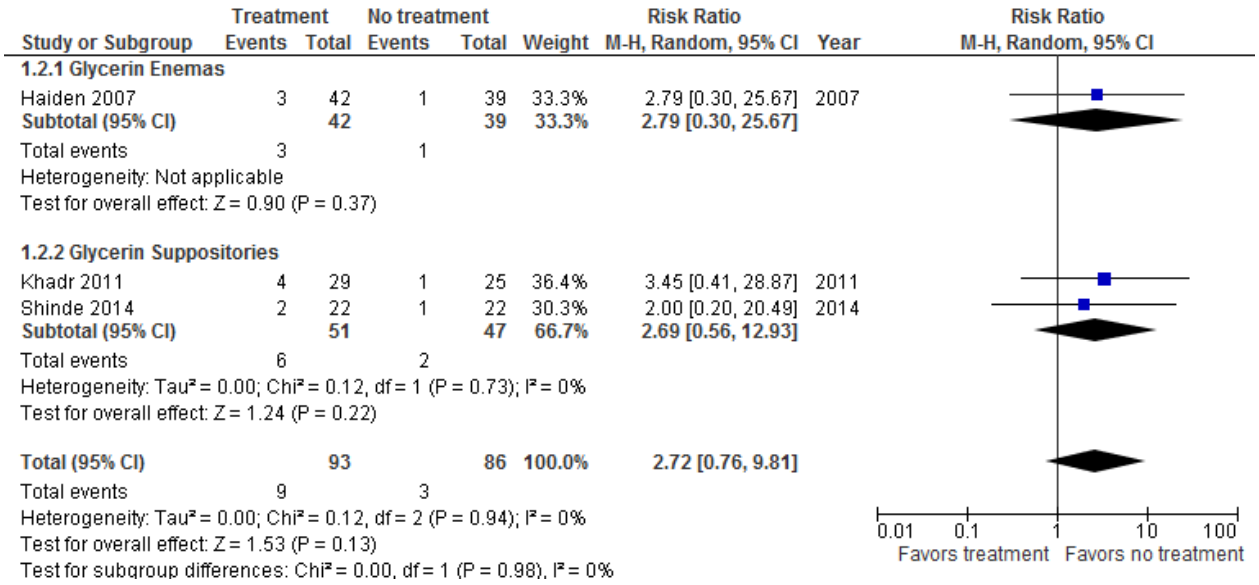


Figure 1.4: Transition to enteral feeding (mean difference in days to full enteral feeding) in premature infants treated with glycerin enemas or suppositories versus no treatment.

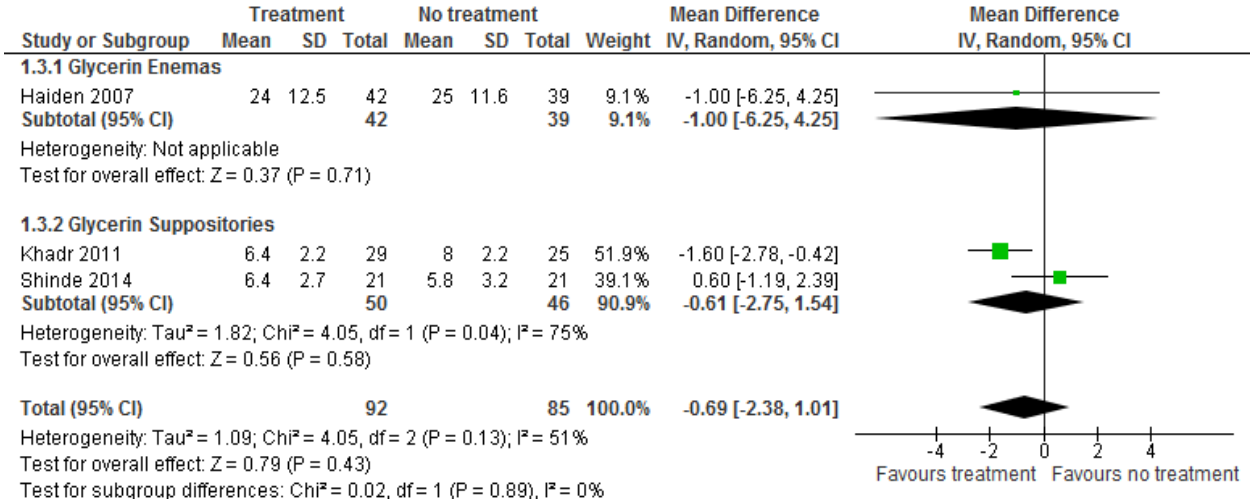
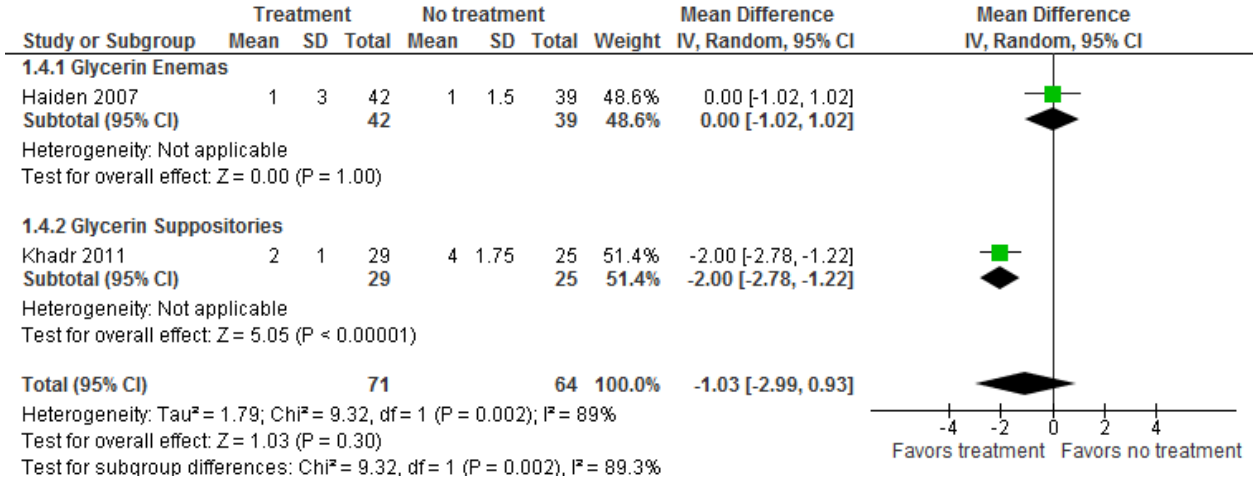


Figure 1.5: Mean difference in days to initiation of meconium evacuation in premature infants treated with glycerin enemas or suppositories versus no treatment.



Appendix 1.1: Medline search strategy and results

#	*Searches*	*Results*
1	exp glycerol/	34379
2	(glycerol$ or glycerin$ or glyrol or microglycerin or ophthalgan or osmoglyn or rovi or vilardell).tw.	42071
3	or/1-2	62979
4	suppositories/	3686
5	(suppository or suppositories or suppositorium$).tw.	3900
6	or/4-5	5276
7	enema/	6413
8	(enema$ or clysma$ or clyster$ or enteroclysis).tw.	9365
9	or/7-8	11995
10	3 and (6 or 9)	15
11	exp Infant/	942470
12	((premature$ or pre-mature$ or prematuritas or preterm$1 or pre-term$1 or (low adj$ weight$) or low-birth-weight$ or underweight$) adj5 (infant$ or neonate$ or neonatal$ or baby or babies or birth or child or children or childbirth or newborn$)).mp.	99818
13	(infant$ or neonate$ or neonatal$ or baby or babies or newborn$).tw.	544867
14	or/11-13	1164297
15	**10 and 14**	**20**

Appendix 1.2: Embase search strategy and results

#	*Searches*	*Results*
1	glycerol/	34923
2	(glycerol$ or glycerin$ or glyrol or microglycerin or ophthalgan or osmoglyn or rovi or vilardell).tw.	50661
3	or/1-2	61942
4	suppository/	5168
5	(suppository or suppositories or suppositorium$).tw.	5979
6	or/4-5	7596
7	exp enema/	8498
8	(enema$ or clysma$ or clyster$ or enteroclysis).tw.	14226
9	or/7-8	17341
10	3 and (6 or 9)	353
11	prematurity/ or exp low birth weight/ or newborn/ or infant/	954355
12	((premature$ or pre-mature$ or prematuritas or preterm$1 or pre-term$1 or (low adj3 weight$) or low-birth-weight$ or underweight$) adj5 (infant$ or neonate$ or neonatal$ or baby or babies or birth or child or children or childbirth or newborn$)).mp.	115604
13	(infant$ or neonate$ or neonatal$ or baby or babies or newborn$).tw.	711577
14	or/11-13	1221298
15	**10 and 14**	**38**

Appendix 1.3: Cochrane Central Register of Controlled Trials search strategy and results

#	*Searches*	*Results*
1	exp glycerol/	2232
2	(glycerol$ or glycerin$ or glyrol or microglycerin or ophthalgan or osmoglyn or rovi or vilardell).tw.	1083
3	or/1-2	2870
4	suppositories/	537
5	(suppository or suppositories or suppositorium$).tw.	1119
6	or/4-5	1231
7	enema/	444
8	(enema$ or clysma$ or clyster$ or enteroclysis).tw.	854
9	or/7-8	959
10	3 and (6 or 9)	37
11	exp Infant/	23208
12	((premature$ or pre-mature$ or prematuritas or preterm$1 or pre-term$1 or (low adj3 weight$) or low-birth-weight$ or underweight$) adj5 (infant$ or neonate$ or neonatal$ or baby or babies or birth or child or children or childbirth or newborn$)).mp.	7946
13	(infant$ or neonate$ or neonatal$ or baby or babies or newborn$).tw.	24480
14	or/11-13	35991
15	**10 and 14**	**10**

eLetter to the Editor

Published June 9, 2015

I read with interest the meta-analysis of glycerin suppository and/or enema studies by Livingston et al. (peds.2015-0143). The authors have adequately acknowledged the major methodological issues with the 3 studies that were included in the meta-analysis (1-3). The study indicates that the evidence for routine prophylactic glycerine enemas/suppository in preterm infants is inconclusive.

I have major concerns with the conclusion that 'glycerine enemas/suppository treatment may be associated with increased risk of necrotising enterocolitis (NEC). The NEC incidence in their meta-analysis was 9/93 in treatment group and 3/86 in the control group (RR 2.72[0.76 to 9.81], p=0.13). However in Haiden et al, (1), all 3 cases of NEC happened in patients with protocol violations (i.e. all 3 NEC cases did not receive any glycerine enema). In light of the high rate of protocol violation (~25%), it would be more appropriate to include the per-protocol (PP) analysis results of their study in the meta-analysis (1). In Shinde et al (2), the 2 NEC cases reported in the treatment arm were 'stage 1' NEC which is traditionally excluded in most NEC studies because of its poor specificity. If we consider these factors in the analysis, the results would be as follows:

	NEC events (Treatment)	NEC events (Controls)
Haiden et al, (PP analysis) (1)	0/27	0/31
Khadr et al, (2)	4/29	1/25
Shinde et al, (3)	0/21	1/21
Total NEC events	4/77	2/77

This analysis will give a RR 2.00 [0.38 to 10.6], p=0.68. This suggests that the use of glycerine enema/suppository has no effect on the incidence of NEC.

Also, NEC was not the primary outcome in any of these studies. The meta-analysis shows primary outcome (time to reach full enteral feeds) was shorter in the treatment group, though not statistically significant. The studies also did not report any major adverse events related to treatment.

Pietz et al, reported one of the lowest incidence of NEC in very low birth weight infants in United States NICU (4). Their feeding protocol included regular use of glycerin suppositories if there is no bowel movement for 24 hours. The rationale was to prevent the development of intestinal distension, with has the potential to alter intestinal blood flow (5).

The occasional use of glycerine suppository in preterm infants with feeding intolerance associated with no bowel movements or no rectal gas shadows is very common in our

practice, often with good results. I see no reason to change this practice based on the current available evidence. I think, the news of further RCT's in the pipeline is encouraging, and may help to address these issues in a more definitive way.

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References

1. Haiden N, Jilma B, Gerhold B, et al. Small volume enemas do not accelerate meconium evacuation in very low birth weight infants. J Pediatr Gastroenterol Nutr. 2007;44(2):270-273
2. Khadr SN, Ibhanesebhor SE, Rennix C, et al. Randomized controlled trial: impact of glycerin suppositories on time to full feeds in preterm infants. Neonatology. 2011;100(2):169-176
3. Shinde S, Kabra NS, Sharma SR, Avasthi BS, Ahmed J. Glycerin suppository for promoting feeding tolerance in preterm very low birth weight neonates: a randomized controlled trial. Indian Pediatr. 2014;51(5): 367-370
4. Pietz J, Achanti B, Lilien L, Stepka EC, Mehta SK. Prevention of necrotizing enterocolitis in preterm infants: a 20-year experience. Pediatrics. 2007 Jan;119(1):e164-70

5. Meyers RL, Alpan G, Lin E, Clyman RI. Patent ductus arteriosus, indomethacin, and intestinal distension: effects on intestinal blood flow and oxygen consumption. Pediatr Res. 1991; 29:569-574

eLetter Response to the Editor

Published June 29, 2015

Many thanks to Dr. Viswanathan for his letter and interest in our study (1). Glycerin suppositories are used to treat premature infants in many neonatal intensive care units, including our own. These interventions are low-cost, appear to be low-risk, and anecdotally, appear to facilitate meconium evacuation and the transition to full enteral feeding in this patient population (1).

At the bedside, the passage of meconium following the administration of glycerin suppositories suggests to many of us that this must be "a good thing" and associated with superior clinical outcomes. Our review demonstrated that the evidence for this practice is limited and inconclusive. The three trials published to date are small and affected by one or more other major methodological issues (2-4). As a result, these interventions may or may not be associated with either clinical benefit or harm, such as necrotizing entercolitis. This is especially important to consider since glycerin suppositories have never been formally approved for use in this patient population.

We agree with Dr. Viswanathan that the numbers in this meta-analysis are small and do not carry enough statistical power to make specific recommendations. This has important implications that we acknowledged explicitly in our review: "The nature of the association between glycerin medications and necrotizing enetercolitis will become

clearer as additional data become available. One possibility is that the apparent relationship is nothing more than a spurious correlation that will disappear with the inclusion of more data. The other explanation is that there is a real effect that has not yet become statistically significant."

In general, we would advise against meta-analyzing outcomes on a per protocol basis. This skews data such that treatment groups are no longer equivalent at baseline. Furthermore, protocol violations often do not occur with the same frequency in each treatment arm, such as in the study by Haiden et al., where protocol violations were almost twice as frequent in the intervention group compared to controls (36% vs 21%) (2). Finally, even with the multiple post hoc modifications made by Dr. Viswanathan, the risk ratio in the revised analysis is still 2.0, which translates into a doubling of the background risk of necrotizing enterocolitis with the use of glycerin medications. If this trend is eventually shown to be a statistically significant treatment effect, we suspect most clinicians would stop using these interventions.

We are now in the process of conducting a pilot study for a multicenter randomized trial of glycerin suppositories among premature infants less than 32 weeks gestation (5). We have randomized 22/30 participants to date and look forward to sharing our results in the near future. Based on the findings of this meta-analysis, we plan on assessing rates of necrotizing entercolitis on an interim basis in the full-scale trial. If there is evidence that treatment is associated with increased harm, then we would plan to stop that trial early.

The findings of our review should not change clinical practice. We do think, however, that all of us involved in the care of premature infants should think more critically about our beliefs and treatment paradigms, and be wary of developing treatment protocols that are not based on sound evidence. We would also suggest that the "trends" highlighted in our meta-analysis should inform future studies on the use of glycerin medications in this patient population.

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References

1. Livingston MH, Shawyer AC, Rosenbaum PL, et al. Glycerin Enemas and Suppositories in Preterm Infants: A Meta-analysis. Pediatrics. 2015;135(6):1093-1106
2. Haiden N, Jilma B, Gerhold B, et al. Small volume enemas do not accelerate meconium evacuation in very low birth weight infants. J Pediatr Gastroenterol Nutr. 2007;44(2):270-273

3. Khadr SN, Ibhanesebhor SE, Rennix C, et al. Randomized controlled trial: impact of glycerin suppositories on time to full feeds in preterm infants. Neonatology. 2011;100(2):169-176
4. Shinde S, Kabra NS, Sharma SR, Avasthi BS, Ahmed J. Glycerin suppository for promoting feeding tolerance in preterm very low birth weight neonates: a randomized controlled trial. Indian Pediatr. 2014;51(5):367-370
5. Walton JM. Glycerin Suppositories Used Prophylactically in Premature Infants (SUPP): a Pilot Randomized Placebo-controlled Trial. NCT02153606. https://clinicaltrials.gov/ct2/show/NCT02153606

2. RESULTS FROM THE PILOT STUDY

Pending submission to the *Journal of Perinatology*

Glycerin Suppositories Used Prophylactically in Premature infants (SUPP): a pilot study for a multicenter randomized controlled trial

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Running title: Glycerin Suppositories Used Prophylactically

Abbreviations: NEC – necrotizing enterocolitis, NICU – neonatal intensive care unit, SD – standard deviation

Key words: premature infants, glycerin, suppository, enteral feeding

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Conflict of Interest: The authors declare no conflict of interest.

Abstract

Objective: The evidence for the use of glycerin suppositories in premature infants is inconclusive.

Study Design: We conducted an external pilot study for a multicenter randomized controlled trial of premature infants randomized to glycerin suppositories or placebo procedure. Outcomes included cost, recruitment, and treatment-related adverse events.

Result: Twenty-two infants less than 32 weeks gestational age and/or 1 500 grams were randomized between January and June 2015. Sixty-one were screened, 46 (75%) were eligible and approached for consent, 25 (54%) consented, 22 (48%) were randomized, and 19 reached full enteral feeds. Three infants (14%) experienced rectal bleeding 5 to 43 days after completing study treatments. An anal fissure was also noted in two of these patients (9%). There were no cases of rectal perforation or necrotizing enterocolitis. Protocol violations occurred during 14 of 130 (11%) treatment days.

Conclusions: Conducting a multicenter randomized controlled trial of glycerin suppositories in premature infants is feasible.

Introduction

Adequate nutrition is a significant challenge for premature infants in the Neonatal Intensive Care Unit (NICU) [1-6]. These patients have immature digestive tracts and may take several days to pass meconium and have normal stools. Infants who take longer to achieve complete meconium evacuation also appear to experience delays in the transition to enteral feeding. As a result, many are given glycerin enemas or suppositories to stimulate the passage of meconium and improve feeding tolerance [7,8].

We recently conducted a systematic review and meta-analysis on the use of glycerin enemas and suppositories in premature infants [8]. We identified a total of 185 participants from three single-center, randomized controlled trials. These studies focused on the prophylactic use of glycerin enemas (one trial) or suppositories (two trials) [9-11]. Across all three trials, there were no differences between participants in the intervention group (daily glycerin enemas or suppositories) and control groups (no intervention or placebo procedure) in terms of meconium evacuation, transition to full enteral feeding, or mortality. There were no reports of rectal bleeding or perforation, but our meta-analysis revealed a trend towards increased risk of necrotizing enterocolitis (NEC) with active treatment. This trend was observed in all three trials but did not cross the threshold of statistical significance in the meta-analysis (risk ratio=2.72, 95% confidence interval 0.76-9.81, I^2=0%, p=0.13).

The results from our systematic review were complicated by the fact that all three trials were underpowered and affected by one or more major methodological issues. For each outcome, the quality of evidence was therefore low to very low. We reported that

the evidence for the use of glycerin suppositories or enemas in premature infants is inconclusive and that further research is required. As a result, we designed an external pilot study to assess the feasibility of a multicenter randomized controlled trial of prophylactic glycerin suppositories in premature infants [12-13]. Outcomes for the pilot included recruitment rate, treatment-related adverse events, and cost. We also reported protocol violations, post-randomization exclusions, and other methodological challenges.

Methods

Study Protocol

The Glycerin Suppositories Used Prophylactically in Premature infants (SUPP) Trial is an external pilot study for a placebo-controlled, parallel-design, multicenter randomized controlled trial. The study protocol was registered on clinicaltrials.gov (NCT02153606) and a more complete description is available elsewhere [12-13].

Participants

Premature infants were recruited from the Level 3 NICU at McMaster Children's Hospital over a 6-month period (January to June 2015). This unit has 42 beds and admits approximately 1 000 infants per year of all gestational ages. Approximately 150 of these patients would be eligible for the SUPP Trial.

Parents or guardians were approached for consent after delivery and within the first 48 hours of life. Inclusion criteria were gestational age of 24 to 32 weeks and/or birth weight of 500 to 1 500 grams. We excluded infants who had life-threatening

congenital anomalies, were clinically unwell, had contraindications to receiving a glycerin suppository (i.e., coagulopathy or neutropenia), or were unlikely to benefit from treatment (i.e., because they had already achieved complete meconium evacuation and were having normal stools). Inclusion and exclusion criteria are listed in full in Table 1.

Interventions

Active treatment consisted of 250 mg glycerin suppositories administered once daily starting between 48 and 72 hours of life (i.e., day 3). This smaller suppository was created by cutting the tip off a 1 440 mg glycerin suppository. In order to maintain consistent dosing, we designed a plastic measurement guide that creates a glycerin "tip" weighing approximately 250 mg [13]. In keeping with usual practice in our unit, this medication was covered with a water-based lubricant and placed in the infant's rectum.

Participants in the control group received 250 mg glycerin suppositories placed in the diaper once daily. In usual practice, partially dissolved suppositories are often ejected from the rectum either with or without stool. Leaving a suppository in the diaper (but not in the rectum) makes it ambiguous as to whether it was placed in the rectum and ejected, or simply placed in the diaper. Furthermore, ensuring that the charge nurses administered study treatments to all participants helped maintain blinding.

All participants received study treatments once daily until they passed two bowel movements free of meconium staining. This treatment duration was also used in a randomized controlled trial of glycerin enemas [9]. In the SUPP trial, maximum treatment duration was set at 12 days, such that all treatments stopped on day 14 of life,

regardless of stooling pattern. All infants were made "nil per rectum" while receiving study treatments and bedside nurses were advised not to reinsert suppositories found in the diaper.

Randomization

Infants were allocated to treatment groups via web-based, stratified blocked randomization. Previous studies have shown that the size of the infant is highly predictive of the time to full enteral feeds [14]. In order to maintain prognostic balance between groups, we stratified participants by gestational age: (1) 24 weeks – 27 weeks 6 days; or (2) 28 weeks – 31 weeks 6 days. This strategy has been used in other randomized controlled trials of feeding intolerance in premature infants [9,10,15,16]. Further details regarding sequence generation, treatment codes, and block size are described in the study protocol [13].

On day 3 of life, one of the co-investigators discussed the infant's status with the bedside nurse and reviewed recent bloodwork. If there were any relevant changes (e.g., need for vasopressors or development of thrombocytopenia), then the infant was not randomized. If the infant continued to meet all inclusion and exclusion criteria, then he or she was randomized to active treatment or placebo. This typically occurred before noon, which left ample time for the charge nurse on duty to administer study treatments during routine handling between noon and 6:00 PM.

Outcomes

Outcomes for the pilot study included recruitment rate (percentage of eligible infants randomized), completion rate (percentage of infants reaching the primary endpoint of full enteral feeds), treatment-related adverse events, and cost. We also assessed frequency and type of protocol violations and post-randomization exclusions.

The primary outcome for the multicenter trial will be days to full enteral feeding (defined as 150 mL/kg/day). Advancing the rate of enteral feeds is typically based on a standardized NICU feeding protocol [17-19]. Deviations from this protocol occur when infants become unwell, develop signs of feeding intolerance, or if there are other clinical concerns. Secondary outcomes for the multicenter trial include feeding volume on day 14 of life (in mL/kg), days to complete meconium evacuation, days of parenteral nutrition, incidence of NEC, incidence of line sepsis, compliance with treatment regimen, and mortality.

Blinding

Parents and guardians, investigators, physicians, bedside nurses, allied health professionals (e.g., dieticians), research assistants, outcome assessors, and statistical analysts were blinded to treatment allocation. The only individuals who were not blinded were the charge nurses who administered study treatments. These individuals are experienced neonatal nurses who are not routinely involved in the bedside care of infants in the NICU.

Data Safety and Monitoring Board

Members of the Data Safety and Monitoring Board (DSMB) included one pediatric surgeon and two neonatologists. This group met after the first 5 participants were enrolled and every three months thereafter to discuss treatment-related adverse events. There were no a priori rules regarding stopping the pilot study. The members of the DSMB were able to reveal the assigned treatment to determine if adverse events were related to treatment. Unblinding of participants was facilitated by a second research assistant who was not otherwise involved in the trial.

Statistical Analysis

All data were analyzed in the Statistical Package for the Social Sciences (SPSS) version 22 (Chicago, IL, USA). Counts of days were reported using median and standard deviation (SD). Missing data were not imputed. Participants were analyzed on the basis of intention-to-treat (i.e., analyzed as randomized).

We chose a sample of size of 30 participants because previous methodological reviews of pilot studies recommend using at least 12 participants per group [20]. Other studies suggest that 30 may be more appropriate, especially if the data will be used to perform a sample size calculation [21-23].

Results

Recruitment

The SUPP Trial opened to enrollment in January 2015. From January to June 2015, we recruited 22 participants who were 28-32 weeks gestation. We used this approach for two reasons: (1) we wanted to demonstrate safety and feasibility among these older and less fragile infants prior to enrolling infants in the 24-28 weeks gestation age group; and (2) competing studies prevented us from enrolling infants in the younger stratum.

Over a six-month period, 61 infants were screened, 46 (75%) were eligible and approached for consent, and 25 (54%) consented to participate. Three of the infants who consented were ineligible at the time of randomization (i.e., day 3 of life) because their platelet count had dropped below 100×10^9/liter. As a result, 22 of 46 (48%) were randomized and 19 reached the primary endpoint of full enteral feeds (Figure 1). Some of the reasons for not providing consent included: belief that the infant was already clearing meconium and did not require a suppository; not wanting to expose their child to risk from suppository administration if it wasn't necessary; or simply not wanting to be involved in research. Recruitment rate was 22/46 (48%) and completion rate was 19/22 (86%). Baseline characteristics of participants who were randomized are summarized in Table 2.

Treatment-related Adverse Events

Three of 22 infants (14%) experienced a treatment-related adverse event. The first of these was an infant who received the placebo procedure once daily for six days. A rescue suppository was administered on day 5 of life, complete meconium evacuation was achieved on day 9, and study treatment was stopped at that point. On day 13, the infant passed a few drops of bright red blood per rectum and was noted to have an anal fissure. The Pediatric Surgery service was consulted but no further investigations or treatment was recommended. Given the delay in time of treatment to the adverse event, the DSMB judged this outcome as "possibly" related to study treatment.

The second infant received active study treatments (i.e., glycerin suppositories per rectum) once daily for 5 days. Complete meconium evacuation was achieved on day 8 of life. Twenty-eight days after receiving study treatments, the stool was found to be streaked with blood and an ulcerated perianal rash and anal fissure were located. The anal fissure healed but the infant continued to have excoriated buttocks for a month until the infant was diagnosed with cow's milk protein allergy. The mother started a dairy-free diet and the skin healed with the assistance of a zinc-based cream.

Finally, the third infant received active study treatments for four days and complete meconium evacuation was achieved on day 7 of life. Forty-three days after completing study treatments, streaks of blood were noted in the stool. This patient was also determined to have a cow's milk protein allergy and the stools cleared after the mother commenced a dairy-free diet. These last two events were judged to be "unlikely

related" to study treatment. There were no cases of rectal perforation. Safety and feasibility data are summarized in Table 3.

Protocol Violations

Seven of the 22 participants (32%) experienced protocol violations. One participant (5%) received their first study treatment after 72 hours of life, which was six hours later than indicated in the study protocol. This occurred because the charge nurse was unavailable to administer the study treatment at the usual time because of a contamination issue which required some infants in the NICU to be temporarily moved to a new area.

Five participants (23%) missed between one and two days of treatment, with a total of nine missed study treatments. With the exception of one day, where a charge nurse mistakenly believed study treatment should have been stopped for two patients, these instances were attributed to exceptionally busy days in the NICU. In most instances, the charge nurse was working alone with no other personnel available to assist with administering study treatments.

Four participants (18%) received treatments one or two days longer than required. The first case occurred over the first weekend of the study. The infant passed two normal stools (free of meconium staining) on two separate days and this was not recognized prior to the infant receiving an additional day of treatment. The second and third cases occurred several months into the study on very busy days. The charge nurse was managing a heavy workload and forgot to confirm meconium evacuation prior to giving

an extra study treatment. One infant also received two extra days of treatment. This patient had already received 12 days of treatment without meconium evacuation, and study treatment should have stopped as per protocol.

Cost

The main costs of the pilot study were: (1) salary for the part-time research assistant responsible for obtaining consent and collecting outcome data ($22.60/hour × 15 hours/week × 36 weeks = $12 204); (2) honorariums to compensate charge nurses to administer study treatments ($100.00 per nurse × 26 nurses = $2 600); and (3) administrative costs for printed materials (approximately $1 000) and server space (approximately $500). As a result, the total cost was approximately $16,000 and the cost for each participant randomized was approximately $700.

Sample size for multicenter trial

The mean time to full enteral feeds across the 19 participants who reached this endpoint was 7.3 days (SD=3.0 days). Power was set at 0.80 and type 1 error was 0.05. Using these parameters, detecting a difference between treatment groups of 1 day would require 284 participants to be randomized, 2 days would require 72, and 3 days would require 34 [24].

Other methodological issues

None of the infants in the SUPP Trial experienced post-randomization exclusions causing them to stop treatment early. Three infants (14%) were transferred to a Level 2 NICU at a community hospital prior to reaching full enteral feeds. Eight infants (36%) received a rescue suppository prior to completing study treatments (2/11 in the active treatment group and 6/11 among those randomized to placebo procedure). Two of these (9%) received rescue suppositories prior to starting study treatments because they were judged to have signs of feeding intolerance by the medical and nursing team. One received a rescue suppository on day 2 of life (prior to randomization) and the other on day 3 (six hours before the first study treatment was administered). The most common reason given for the other six infants was that no significant stooling had occurred for at least 48 hours.

Discussion

This external pilot study assessed the feasibility and safety of a multicenter randomized controlled trial of glycerin suppositories among premature infants. One of the issues that surprised us was the number of infants who initially met the inclusion criteria but had to be excluded prior to randomization on day 3 of life. Fortunately, we set up our electronic database with two inclusion and exclusion criteria "checkpoints": one at the time of consent (usually within the first 48 hours of life) and one immediately prior to randomization (before noon on day 3 of life). By minimizing the length of time from randomization to receiving the first treatment, we experienced no post-randomization

exclusions. If we had randomized participants at the time of consent, then 3 of 25 infants (12%) would have stopped treatment early due to post-randomization exclusions.

The most common reason for an infant being ineligible was thrombocytopenia. Seven of 61 screened participants (11%) were not approached for consent and three others (5%) were not randomized for this reason (16% total). The mean platelet count for these infants was 81×10^9/liter (range 67-90×10^9/liter), which was only slightly below our cut-off of less than 100×10^9/liter. If the exclusion criterion had been set at 50×10^9/liter, then none of these infants would have been excluded.

Randomizing participants at noon each day was helpful. Each morning, the research assistant had plenty of time to obtain consent, liaise with nursing and medical staff, review bloodwork with one of the co-investigators, and randomize new participants. In the afternoon, the charge nurse was able to administer study treatments between noon and 6:00 PM (whenever the infant was awake for routine care). This approach meant that fewer study treatments were missed, resulting in an acceptable rate of protocol violations.

Randomizing participants on day 3 of life also meant that all potentially eligible participants could be approached for consent on a weekday. For example, an infant born on a Monday could be approached for consent on Tuesday and randomized on Wednesday. An infant born on a Saturday could be approached for consent on Monday morning and randomized at noon later that day. The other benefit of randomizing infants on day 3 of life was that it selected out infants who were likely to be excluded for clinical reasons once they started treatment. In the 28-32 week group, infants were given approximately 72 hours to achieve complete meconium evacuation prior to receiving

treatment. Similarly, infants in the 24-28 week group will have plenty of time to declare themselves as clinically unwell (e.g., needing vasopressors or developing culture-positive sepsis) before being randomized. These factors also contributed to a low rate of post-randomization exclusions (0%). From a clinical perspective, the infants who were ultimately randomized tended to be the "feeders and growers" who were most likely to benefit from prophylactic glycerin suppositories because they were: (1) not overly sick and; (2) did not achieve complete meconium evacuation until at least day 4 of life.

Infants who were born on a Thursday or Friday had to be randomized on a Saturday or Sunday, respectively. In these cases, parents were approached for consent by the research assistant on a weekday and had baseline data entered into the web-based electronic database before each weekend. On a weekend morning, one of the co-investigators reviewed bloodwork from home, reviewed the infant's clinical status with the bedside nurse by telephone, randomized the participant using the web-based system, and then asked the nurse to record the treatment code on the bedside chart. This process took approximately 15 minutes to complete and could be performed remotely.

A significant challenge from a feasibility standpoint was the need to assess participants each day and decide whether study treatments should continue or be stopped. When we designed the protocol for the SUPP Trial, we believed that the only potential benefit of administering glycerin suppositories prophylactically was to facilitate meconium evacuation. Thus, giving glycerin suppositories beyond the point of complete meconium evacuation would likely have no benefit and only expose participants to the small but non-zero risk of rectal perforation, rectal bleeding, or anal fissure. On a

practical level, however, stopping study treatments after complete meconium evacuation meant that our research assistant had to spend considerable time each day reviewing bedside charts and determining if infants should continue to receive study treatments. This involved carefully reviewing the nursing record of each participant and deciding whether they had passed two normal bowel movements free of meconium staining.

On days where the research assistant was not present to review the bedside chart (such as weekends), it became the responsibility of the charge nurse to confirm complete meconium evacuation prior to administering study treatment. This resulted in several protocol violations where study treatments should have been stopped but were not. This close monitoring was time-intensive and may not have been necessary. In fact, the two previous randomized controlled trials of glycerin suppositories in premature infants used fixed treatment lengths: (1) 10 days starting at 24 hours of age [11] or; (2) 13 days starting at 48 hours of age [10]. None of the infants in these trials (n=104) experienced a treatment-related adverse event and determining the length of treatment duration was greatly simplified.

An even better option might be to use predefined for each age group (i.e., stratum) based on the median treatment duration in the pilot study (e.g., 5 days total for infants 28-32 weeks gestation). This would minimize the need for a long course of treatment in the older age group and ensure that infants in the younger group received sufficient therapy to clear meconium in most cases. Using this approach in the multicenter trial would be far more feasible than using a variable duration of treatment as we did in this pilot. This

approach would also likely decrease the rate of protocol violations. Furthermore, stooling pattern would not have to be assessed on a daily basis.

Another challenge was the number of participants (14%) transferred to a Level 2 NICU in a community hospital prior to reaching full enteral feeds. In the study protocol for the SUPP Trial, we anticipated that some infants would be transferred prior to reaching term (i.e., 37 weeks gestation) but not likely before reaching full enteral feeds [13]. In practice, however, this turned out not to be not the case and some participants were effectively lost to follow-up.

We were concerned to see that rescue suppositories were used more frequently in the control group (6/11 infants) compared to those randomized to active treatment (2/11 infants). This may have been a simple consequence of these patients not receiving glycerin suppositories and not having regular stools. The other possibility is that some element of unblinding and bias played a factor in the decision to administer rescue suppositories. For example, some of the bedside nurses or medical personnel may have become unblinded (by secretly observing which study treatments were administered, overhearing treatment assignments being discussed by the charge nurses, opening the infant's diaper immediately after study treatments were administered, etc.). If these clinicians realized that certain infants were assigned to receive the placebo procedure only, then they might have been more likely to advocate for the use of rescue suppositories.

One possible solution would be to set up more stringent criteria for the use of rescue suppositories (e.g., only if infants have not stooled in 48 hours and there is

ongoing clinical concern). We did not do this in the pilot study because we wanted this study to be simple and pragmatic. We also clinicians to feel free to use their best judgement and not give the impression that infants in the SUPP Trial were "not allowed" to receive non-study glycerin suppositories. Setting up specific rules for initiating rescue treatment makes the study less pragmatic and may make the multicenter trial less feasible.

Another potential solution would be to modify the blinding strategy. Some clinicians suggested that infants randomized to the placebo procedure should have suppositories discarded rather than placed in the diaper. This approach was used in the trial by Shinde et al., where the control intervention was simply opening and closing the diaper [11]. While we agree that this option is better in principle, we were concerned that in practice, this might lead to some charge nurses not performing the placebo procedure at all. Leaving suppositories in the diaper acts as a reminder for the charge nurses to go to the bedside and participate in the treatment ritual on a daily basis. This may have helped maintain blinding and compliance with the study protocol.

Finally, a philosophical issue that came up during the course of the pilot study was whether the term "placebo" was appropriate to use in reference to glycerin suppositories left in the diaper. Some clinicians felt that this intervention was actually a form "sham" therapy. A Cochrane Review from 2010 reported that there is no formal definition of placebo that clinicians and researchers agree upon [25]. They noted that: "It is generally assumed that any effect of a placebo intervention is unrelated to its essential component… but caused by the special interaction between patient and healthcare

provider associated with the treatment ritual." Similarly, a recent systematic of sham interventions noted these procedure are "… characterized by physical change of bodily tissue through manual or robotic operation and thereby inherently imply physical harm and/or risks" [26]. Thus, we believe the term "sham" implies a level of risk that does apply to the control procedure in this trial.

Over the next 8 months, we plan to recruit 8 infants in the 24-28 week gestation age group. This will not only ensure that a multicenter trial is feasible in this younger and more fragile patient population, but will also allow us to reach the sample size of 30 participants. We look forward to sharing our results and recognize that this may lead to additional changes to the study protocol for the multicenter trial.

Conclusion

Our recent systematic review on the use of glycerin enemas and suppositories in premature infants demonstrated that the evidence for this treatment strategy is inconclusive. The three trials published to date are small, underpowered, and affected by one or more other major methodological issues. The published literature on this topic demonstrates "absence of evidence" rather than "evidence of absence" [27]. The results from this pilot study suggest that conducting a multicenter randomized controlled trial is feasible and safe, so there is good reason to conduct a larger study and improve the quality of evidence. The study protocol for the multicenter trial will need some minor modifications to improve feasibility (and require interim analyses to assess for harm) but

these issues should not prevent us from conducting a more definitive study on this important clinical issue.

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Conflict of Interest: The authors declare no conflict of interest.

References

1. Meetze WH, Franz AR, Linder W, Pohlandt F. Meconium passage in very-low birthweight infants. *J Parenter Enteral Nutr* 1993; **17**: 1537-1540
2. Morris BH, Miller-Loncar CL, Landry SH, Smith KE, Swank PR, Denson SE. Feeding, medical factors, and developmental outcome in premature infants. *Clin Pediatr (Phila)* 1999; **38**: 451-457.
3. Mihatsch WA, Franz AR, Lindner W, Pohlandt F. Meconium passage in extremely low birthweight infants and its relation to very early enteral nutrition. *Acta Paediatr* 2001; **90**: 409-411.
4. Belfort MB, Rifas-Shiman SL, Sullivan T, Collins CT, McPhee AJ, Ryan P, Kleinman KP, Gillman MW, Gibson RA, Makrides M. Infant growth before and after term: effects on neurodevelopment in preterm infants. *Pediatrics* 2011; **128**: e899.
5. Lucchini R, Bizzarri B, Giampietro S, De Curtis M. Feeding intolerance in preterm infants. How to understand the warning signs. *J Matern Fetal Neonatal Med* 2011; **24 Suppl 1**:72-74.
6. Su B. Optimizing Nutrition in Preterm Infants. *Pediatr Neonatol* 2013; **55**: 5-13.
7. Shah V, Chirinian N, Lee S. Does the use of glycerin laxatives decrease feeding intolerance in preterm infants? *Paediatr Child Health* 2011; **16**: e68-70.
8. Livingston MH, Shawyer AC, Rosenbaum PL, Williams C, Jones SA, Walton JM. (2015) Glycerin Enemas and Suppositories in Premature Infants: A Meta-analysis. *Pediatrics* 135:1093-1106.

9. Haiden N, Jilma B, Gerhold B et al. Small volume enemas do not accelerate meconium evacuation in very low birth weight infants. *J Pediatr Gastroenterol Nutr* 2007; **44**: 270-273.
10. Khadr SN, Ibhanesebhor SE, Rennix C et al. Randomized controlled trial: impact of glycerin suppositories on time to full feeds in preterm infants. *Neonatology* 2011; **100**: 169-176.
11. Shinde S, Kabra NS, Sharma SR, Avasthi BS, Ahmed J. Glycerin suppository for promoting feeding tolerance in preterm very low birthweight neonates: a randomized controlled trial. *Indian Pediatr* 2014; **51**: 367-370.
12. Walton JM. Glycerin Suppositories Used Prophylactically in Premature Infants (SUPP): a Pilot Randomized Placebo-controlled Trial. NCT02153606. https://clinicaltrials.gov/ct2/show/NCT02153606.
13. Livingston MH, Zequiera J, Blinder H, Pemberton J, Williams C, Walton JM. Glycerin Suppositories Used Prophylactically in Premature infants (SUPP) Trial: a study protocol for a pilot randomized controlled trial. *Pilot and Feasibility Studies* 2015; (in press).
14. Shim SY, Kim HS, Kim DH, Kim EK, Son DW, Kim BI et al. Induction of early meconium evacuation promotes feeding tolerance in very low birth weight infants. *Neonatology* 2007; **92**: 67-72.

15. Karagol BS, Zenciroglu A, Okumus N, Polin RA. Randomized controlled trial of slow vs rapid enteral feeding advancements on the clinical outcomes of preterm infants with birth weight 750-1250 g. *JPEN J Parenter Enteral Nutr* 2013; **37**: 223-228.
16. Haiden N, Norooz F, Klebermass-Schrehof K, Horak AS, Jilma B, Berger A et al. The effect of an osmotic contrast agent on complete meconium evacuation in preterm infants. *Pediatrics* 2012; **130**: e1600-1606.
17. Gregory KE, Connolly TC. Enteral feeding practices in the NICU: results from a 2009 Neonatal Enteral Feeding Survey. *Adv Neonatal Care* 2012; **12**: 46-55.
18. McMaster Children's Hospital. *NICU Nutrition Summary Guidelines*. Revised July 2012.
19. Premji SS, McNeil DA, Scotland J. Regional neonatal oral feeding protocol: changing the ethos of feeding preterm infants. *J Perinat Neonatal Nurs* 2004; **18**: 371-384.
20. Julious SA. Sample size of 12 per group rule of thumb for a pilot study. *Pharm Stat* 2005; **4**: 287-291.
21. Browne RH. On the use of a pilot sample for sample size determination. *Stat Med* 1995; **14**: 1933-1940.
22. Thabane L, Ma J, Chu R, Cheng J, Ismaila A, Rios LP et al. A tutorial on pilot studies: the what, why and how. *BMC Med Res Methodol* 2010; **10**: 1.

23. Billingham SA, Whitehead AL, Julious SA. An audit of sample sizes for pilot and feasibility trials being undertaken in the United Kingdom registered in the United Kingdom Clinical Research Network database. *BMC Med Res Methodol* 2013; **13**: 104.

24. Dupont WD, Plummer WD. *PS Power and Sample Size Calculations Version 3.0*. January 2009.

25. Hróbjartsson A, Gøtzsche PC. Placebo interventions for all clinical conditions. *Cochrane Database Syst Rev* 2010:CD003974.

26. Niemansburg SL, van Delden JJ, Dhert WJ, Bredenoord AL. Reconsidering the ethics of sham interventions in an era of emerging technologies. *Surgery* 2015; **157**: 801-810.

27. Altman DG, Bland JM. Absence of evidence is not evidence of absence. *BMJ* 1995; **311**: 485.

28. Schulz KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 2010; **340**: c332.

Table 2.1: Inclusion and exclusion criteria

Criteria	Definition
Inclusion (any of the following)	
Gestational age	24 – 32 weeks gestation
Birth weight	500 – 1 500 grams
Exclusion (any of the following)	
Congenital gastrointestinal anomalies	Any congenital gastrointestinal anomalies
Clinically unwell	Major surgery within 48 hours of birth
	Culture-proven sepsis
	Vasopressors
	Nitric oxide
	Prostaglandins
Suspected coagulopathy	Mucosal bleeding from any orifice
Confirmed coagulopathy	International Normalized Ratio>1.4
	Partial Thromboplastin Time>39 seconds
	Fibrinogen<1.00 grams/liter
	Platelet count$<100\times10^9$/liter
Neutropenia	Absolute neutrophil count$<0.5\times10^9$/L
Complete meconium evacuation	Two bowel movements with no meconium

Table 2.2: Baseline characteristics of premature infants randomized to glycerin suppositories or placebo procedure

Characteristic	Intervention (n=11) (suppositories in rectum)	Control (n=11) (suppositories in diaper)
Gestational age, mean (SD)	30^{1} (1^{0}) weeks	30^{3} (1^{1}) weeks
Birth weight, mean (SD)	1364 (258) grams	1334 (205) grams
Sex, n (%)		
Male	8 (73%)	5 (46%)
Female	3 (27%)	6 (55%)
Delivery, n (%)		
Vaginal	2 (18%)	5 (46%)
Caesarean section	9 (82%)	6 (55%)
Location of birth, n (%)		
Same hospital	10 (91%)	10 (91%)
Other hospital	1 (9%)	1 (9%)

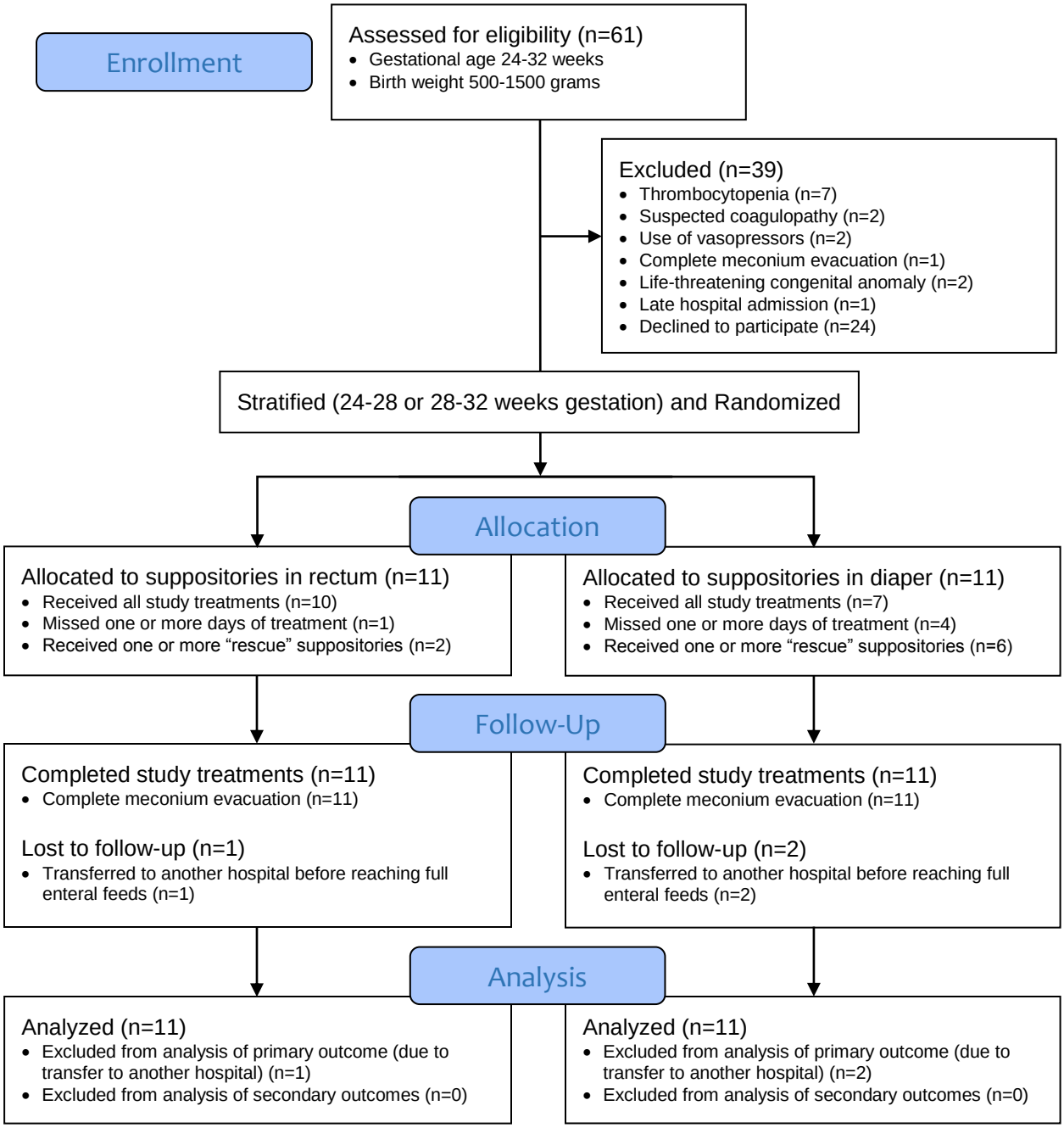
SD = standard deviation

Table 2.3: Safety and feasibility outcomes of premature infants randomized to glycerin suppositories or placebo procedure

Outcome	Intervention (n=11) (suppositories in rectum)	Control (n=11) (suppositories in diaper)
Adverse events, n (%)		
Rectal perforation	0 (0%)	0 (0%)
Rectal bleeding	2 (18%)	1 (9%)
Anal fissure	1 (9%)	1 (9%)
Necrotizing enterocolitis, n (%)	0 (0%)	0 (0%)
Days of treatment, mean (SD)	5.2 (1.7) days	6.6 (3.6) days
Range	4-9 days	1-14 days
Days to meconium evacuation, mean (SD)	7.4 (1.2) days	8.9 (3.3) days
Range	6-10 days	3-15 days
Days to full enteral feeds, mean (SD)	6.5 (3.1) days	8.0 (2.9) days
Range	3.1-13.5 days	2.6-13.1 days
Reached full enteral feeds, n (%)	10/11 (91%)	9/11 (82%)

SD = standard deviation

Figure 2.1: Recruitment of participants using the Consolidated Standards of Reporting Trials (CONSORT) flow diagram [28]



APPENDIX 1. GRANT APPLICATION FOR PILOT STUDY

Submitted to *Health Research Methodology 730*

as final course paper on December 11, 2013

Submitted to *McMaster Surgical Associates Innovation Grant*

competition on January 31, 2014

Grant awarded for *$29,641* on April 17, 2014

McMaster Surgical Associates

Guidelines for INNOVATION Research Proposals

Please forward your application by January 31st 2014 at 12 noon
To Catherine Gill Pottruff, gillc@mcmaster.ca
Office of Surgical Research Services
Department of Surgery

Please note that paper copies will not be accepted and you will receive a confirmation email within 2 working days of receipt of your electronic application.

Grant Title: "Do glycerin suppositories decrease the time to full enteral feeds in premature infants? A pilot study for a multicenter randomized placebo-controlled trial"

Principal Investigator: Dr. Mark Walton

Co-Investigators: Dr. Michael Livingston, Dr. Jorge Zequiera, Julia Pemberton, Dr. Connie Williams

Lay Summary:

All premature babies have problems with feeding and nutrition. Some can develop a life-threatening bowel infection called necrotizing enterocolitis and need emergency surgery. This can result in the loss of bowel, lifelong feeding problems, and death. Giving premature babies glycerin suppositories may be one way to stimulate the digestive tract and help prevent these problems. To see if this treatment works, we need to study hundreds of premature babies in a large trial involving multiple hospitals. The purpose of this project is to carry out a small study first and make sure that the larger trial is feasible. We will invite approximately 30 premature babies from McMaster University Medical Centre to participate over a 6-month period. We will focus on feasibility issues, including cost, safety, and rate of participation. This will allow us to rigorously test our study protocol and lay the groundwork for the larger study involving multiple hospitals.

Total Amount Requested: $29,641

1. THE NEED FOR A TRIAL

1.1. *What is the problem to be addressed?*

Adequate feeding and nutrition is a significant challenge for premature infants in the neonatal intensive care unit (NICU) [1,2]. These babies have immature digestive tracts and can develop a life-threatening bowel infection called necrotizing enterocolitis (NEC) [3,4]. Treatment of this condition may require surgery and is associated with significant morbidity and mortality. This includes short bowel syndrome, lifelong dependence on intravenous nutrition, and the need for additional surgery [5,6]. Infants who do not develop NEC still have significant feeding issues. Many are supported initially with intravenous nutrition and are then gradually transitioned to enteral feeding over a period of 2 to 3 weeks. This process can be delayed if infants develop feeding intolerance, characterized by abdominal distension, undigested feeds in the stomach, and decreased bowel movements [7]. This can lead to increased reliance on intravenous nutrition, which is associated with sepsis, extrauterine growth restriction, and poor neurodevelopmental outcomes [1,2,8].

Glycerin suppositories are commonly used in premature infants to stimulate the passage of meconium and improve feeding tolerance [9]. This practice is based on the observation that preterm infants experience significant delays in the passage of meconium, which is more viscous than normal stool [10,11]. Delays in meconium evacuation appear to be associated with a delay in the transition to enteral feeding [12]. Thus, if meconium evacuation could be expedited through the use of glycerin suppositories, this may lead to faster transition to enteral feeding, decreased reliance on intravenous nutrition, and better outcomes. Using this treatment regimen may even reduce the incidence of NEC. Unfortunately, there is little evidence to guide clinicians regarding treatment decisions.

1.2. What is the principal research question to be addressed?

What is the feasibility of a multicenter randomized controlled trial that assesses whether using glycerin suppositories decreases the time to full feeding in premature infants?

1.3. Give references to relevant systematic reviews and discuss the need for your trial

A recent systematic review assessed whether glycerin enemas and suppositories decrease feeding intolerance in premature infants [9]. This study considered the results from one cohort study and one randomized controlled trial. The cohort study included 83 very low birth weight infants from a single hospital in South Korea [13]. In this study, outcomes for the control period (where glycerin enemas were given as a rescue treatment) were compared to the treatment period (where glycerin enemas were given prophylactically to facilitate meconium evacuation). Full enteral feeding was achieved within 16 days during the treatment period and 22.9 days for the control period ($p<0.001$). Significant improvements were also reported for days to passage of first meconium, duration of central catheter usage, sepsis after 7 days of life, and positive catheter culture.

The randomized controlled trial included in the systematic review reported on 81 very low birth weight infants from a single hospital in Austria [14]. All participants were enrolled in the study shortly after birth and stratified by gestational age. Infants in the intervention group received daily glycerin enemas until complete evacuation was achieved, while those in the control group did not receive any intervention. This study appeared to be an open trial since method of randomization, allocation concealment, and blinding were not described or discussed. The primary outcome was the number of days to complete evacuation of meconium

and the study was powered to detect a 30% difference. There was a trend towards a treatment effect with complete evacuation of meconium occurring at a median of 6.5 days in the intervention group and 9 days in the control group, but this difference was not statistically significant (p=0.11). Approximately 28% of the participants experienced at least one study violation with almost twice as many events in the intervention group. This may have diluted the treatment effect and was likely a source of bias.

The authors of the systematic review indicated that the cohort study from South Korea was of fair quality and reported positive results, while the randomized controlled trial from Austria was of good quality but reported negative results [9]. As a result, they concluded that: "The evidence regarding the effectiveness of glycerin [enemas or suppositories] for improving feeding intolerance in very low birth weight infants is inconclusive" [9].

A more recent randomized controlled trial examined the use of glycerin suppositories to decrease feeding intolerance [15]. This study included 54 preterm infants born less than 32 weeks gestational age. Participants were enrolled in the study shortly after birth and randomized from a stack of opaque envelopes. This study was an open trial and there were no attempts to maintain allocation concealment or blinding. There was a trend towards a decrease in time to full feeds of 1.6 days, but the study was only powered to detect a difference of 3.6 days. Despite this, infants in the intervention group tended to pass their first stool earlier (day 2) than controls (day 4).

These trials are small, underpowered, and fraught with methodological issues. As such, the evidence regarding the role of glycerin suppositories among premature infants remains unclear. A simple randomized controlled trial powered for a modest effect would help clarify whether this treatment facilitates transition to enteral feeding in preterm infants. This study would require appropriate methods of randomization, allocation concealment, and blinding. A trial with this level of methodological rigor has significant logistical challenges and requires an assessment of feasibility on a small scale prior to a multicenter study. This two-stage approach has gained increasing acceptance among trial experts [16,17]. Performing a pilot study first will allow us to ensure that the methodological issues encountered in previous trials are addressed prior to investigating significant resources in a multicenter study.

1.4. How will the results of this trial be used?

The results of this study will be used to assess the feasibility of a multicenter randomized controlled trial. If our protocol does not require significant modifications, then participants assessed in the pilot study will be folded in to the multicenter trial. Completing the pilot study will also allow Dr. Michael Livingston to fulfill his requirements as a general surgery resident in the Clinical Investigator Program at McMaster University.

The results from the multicenter trial will allow us to adequately assess the effectiveness of glycerin suppositories in preterm infants. This intervention is low cost, readily available, and easily administered. If proven to be effective in the larger trial, this regimen could easily be implemented in any NICU in the world. If the treatment is not shown to be more effective than placebo, then these findings will encourage clinicians to reflect on their practice and consider not using glycerin suppositories routinely in the NICU.

2. **THE PROPOSED TRIAL**

2.1. What is the proposed trial design?

This pilot study will assess the feasibility of a protocol for a parallel-design, multicenter, randomized controlled trial that assesses whether glycerin suppositories facilitate the transition to full enteral feeding in premature infants.

2.2. What are the planned trial interventions?

The treatment intervention will be a 250 mg glycerin suppository placed in the rectum once daily starting 12-36 hours after birth. Glycerin suppositories will be used as a prophylactic therapy to facilitate meconium evacuation and expedite the transition to enteral feeds. This treatment will continue daily until infants pass two normal bowel movements free of meconium staining. A similar approach was used for the intervention in the randomized controlled trial of glycerin enemas from Austria [14]. In our trial, the intervention will be administered by a part-time research nurse.

Participants in the control group will be subjected to sham therapy. Sham suppositories will be created by partially dissolving a 250 mg glycerin suppository in a cup of water. In usual clinical practice, partially dissolved suppositories are often ejected from the rectum either with or without stool. In our trial, leaving a partially dissolved suppository in the diaper (but not in the rectum) makes it ambiguous as to whether it was placed in the rectum and ejected, or simply placed in the diaper. Thus, this intervention works well as a non-invasive placebo and will help maintain blinding.

2.3. What are the proposed practical arrangements for allocating participants to trial groups?

Infants will be allocated to treatment groups via web-based stratified blocked randomization. Previous studies have shown that the size of the infant is highly predictive of the time to full enteral feeds [13]. In order to maintain prognostic balance, participants will be stratified by gestational age: (1) 24 weeks – 27 weeks 6 days; or (2) 28 weeks – 31 weeks 6 days. This strategy has been used in other randomized controlled trials examining feeding intolerance in preterm infants [13,18,19].

Block size will range from 4 to 6 to guarantee an equal number of participants in the treatment and control groups. The sequence and size will be randomly generated so that the allocation sequence cannot be predicted. Randomization of study participants will be completed online by the research nurse immediately prior to the administration of the first study intervention. This system will be developed using Research Electronic Data Capture (REDCap) software [20].

2.4. What are the proposed methods for protecting against other sources of bias?

Adequate blinding of nursing and medical staff is essential to the study design since these clinicians typically make decisions about advancing or holding feeds during daily patient rounds. If these individuals know which intervention the participant is receiving there is a chance that this will affect their decision-making and bias the results. We will employ several strategies to maintain blinding. First, participants in the control group will receive a sham intervention rather than no intervention at all. Second, all study interventions will be administered when the participant's crib is covered. This will ensure that only the clinician administering the treatment is able to observe which intervention is given. Third, we will hold multiple meetings with the NICU nursing staff prior to the start of the trial to discuss the purpose of blinding and ensure that we have adequate buy-in from these individuals.

Another issue to consider is post randomization withdrawal or exclusion. While this is an issue in any randomized controlled trial, this will present a unique challenge in our study since the participant will have been alive for less than 24 hours when they are enrolled. Some patients may not have had prenatal screening and serious congenital anomalies that would have excluded them from the study may not be diagnosed until days or weeks post randomization. Even in cases of excellent prenatal care, some conditions (e.g., Hirschsprung's disease) cannot diagnosed until the postnatal period [21,22]. The best way to handle this will be to follow all randomized participants to the primary endpoint of full enteral feeds and analyze the data on the basis of Intention-to-treat.

2.5. What are the planned inclusion/exclusion criteria?

Inclusion criteria include gestational age less than 32 weeks or birth weight less than 1500 grams. Exclusion criteria include: severe congenital anomalies; primary gastrointestinal abnormalities (e.g., meconium ileus); surgery within 24 hours of birth; vasopressor support; and parent or legal guardian unable to understand English

2.6. What is the proposed duration of treatment period?

Infants enrolled in the trial will receive glycerin suppositories or the sham procedure daily until meconium evacuation is complete. Previous studies have demonstrated that this process takes approximately 6 to 9 days [14]. The study intervention will be withheld and reassessed daily in cases of thrombocytopenia (defined as platelet count<100) and confirmed or suspected coagulopathy. The study intervention will be stopped in cases of clinical deterioration (i.e., vasopressor support, respiratory failure, sepsis, NEC, or death) or treatment-related adverse events (i.e., rectal bleeding or perforation). Previous trials of glycerin suppositories and enemas did not report single case of rectal bleeding or perforation [14,15]. As such, the risk of these events occurring in our study is low.

2.7. What is the proposed frequency and duration of follow-up?

Outcome data will be extracted from the medical record after participants are discharged from hospital, reach a corrected gestational age of 40 weeks, or die (whichever happens first). The research coordinator will also assess participants on a weekly basis to maintain contact with nursing staff, discuss any protocol violations, monitor for adverse events, and address any other issues that arise. Nursing staff will also be instructed to submit possible adverse events to the research coordinator as soon as they occur. These include any cases of overt rectal bleeding or rectal perforation.

2.8. What are the proposed primary and secondary outcome measures?

Outcomes for the pilot study will be cost, recruitment rate (percentage of eligible infants enrolled), randomization rate (percentage of participants randomized), and percentage of infants reaching the primary endpoint of full enteral feeds. We will also assess the frequency of protocol violations, post-randomization exclusions, and adverse events related to the study intervention, including rectal bleeding and rectal perforation.

The primary outcome for the multicenter trial will be time in days to full enteral feeding (defined as 150 mL/kg/day). Advancing the rate of enteral feeds is typically based on a standardized NICU feeding protocol [23-25]. Deviations from this protocol occur when infants become unwell, develop signs of feeding intolerance, or if there are other clinical concerns.

Secondary outcomes will include feeding volume on day 14 of life (in mL/kg), days to complete meconium evacuation, days of parenteral nutrition, incidence of necrotizing colitis, incidence of line sepsis, and mortality.

2.9. What is the proposed sample size?

Previous studies have demonstrated that time to full enteral feeding ranges from 16 to 27 days with a standard deviation from 6 to 9 days [13,14]. Treatment differences were 1 to 1.6 days but these were not statistically significant [14,15]. Our trial will be powered to detect a treatment effect of 2 days since: (1) this is the approximate size of the true effect (if one actually exists); and (2) differences in time to full feeds less than 2 days are probably not clinically significant. This results in a 'small' to 'moderate' effect size of 0.33 [26]. Setting α at 0.05 and power at 0.8 will require at least 142 participants in each group with a total sample size of 284 [27].

For our pilot study, we hope to recruit 30 participants (15 per group) over a 6-month period. Previous reviews recommend using at least 12 participants per group and some indicated that a total sample size of 30 may be more appropriate [28,29,30].

2.10. What is the planned recruitment rate?

Our multicenter trial will be preceded by a 6-month pilot study in the NICU at McMaster University Medical Centre. This is a busy Level 3 unit and we would expect approximately 80 eligible infants during this time period.

2.11. Are there likely to be any problems with compliance or rate of loss to follow-up?

Compliance was a serious issue in the randomized controlled trial of glycerin enemas from Austria [14]. This may have occurred because some clinicians did not believe in the efficacy of glycerin enemas and tended to withhold this intervention among participants in the therapeutic arm. This will be less of an issue in our trial since glycerin suppositories are commonly used and much less invasive, but we will still have to monitor for these events.

Losing participants to follow-up is unlikely to occur since all premature infants are monitored in hospital until they are tolerating full enteral feeds and reach 37 weeks corrected gestational age. Some participants who are doing well clinically may be transferred to a NICU in a community hospital that is not part of this study, but this is unlikely to occur until after these infants have reached full enteral feeds. Finally, the mortality rate in this population is approximately 20% [31,32]. As such, some participants who are enrolled may die before they are randomized, complete the treatment regimen, or reach full enteral feeds.

2.12. Give details of the planned statistical analyses.

Frequencies and 95% confidence intervals will be used to estimate recruitment rate, randomization rate, percentage infants reaching the primary endpoint, rate of protocol violations, rate of post-randomization exclusions, and incidence of adverse events related to the study intervention. We will report cost per participant in terms of mean and standard deviation. All data will be analyzed in IBM® SPSS® Statistics Version 21 [33].

2.13. What is the estimated cost and duration of the trial?

The 6-month pilot study can be completed as a graduate Thesis project that will require only a moderate amount of additional funding. The majority of this support will be needed to

compensate the research nurses for the time required to administer the study intervention. We will also to hire a part-time research assistant to help with database development, quality testing, study communication, patient consent, and general research administration. The multicenter trial will require more substantial funding and would be approximately 3 years in length.

3. DETAILS OF TRIAL TEAM

3.1. Briefly describe the role of each applicant proposed.

The principal investigator will be Dr. Mark Walton, who is a pediatric general surgeon with an interest in gastrointestinal issues among premature infants. He will be responsible for identifying funding for the full-scale trial and act as a clinical liaison on the steering committee. Dr. Michael Livingston is a general surgery resident in the Clinical Investigator Program at McMaster University. His main responsibility will be to act as the research coordinator for the 6-month pilot study. Dr. Jorge Zequiera is a pediatric general surgery fellow at McMaster University. He will assist with the ethics application for the pilot study. Julia Pemberton is a clinical epidemiologist and experienced research coordinator. She will assist with trial registration for the pilot study and full-scale trial, ethics applications, and manuscript writing. If we obtain funding, she will serve as the research coordinator for the full-scale trial. Dr. Connie Williams is a neonatologist with an interest in health outcomes research. Like Dr. Walton, she will serve as a clinical liaison and help identify funding for the multicenter trial.

3.2. Please discuss the nature of and need for any multicenter collaboration.

This project will be completed as a pilot study in preparation for a multicenter trial. This approach will ensure that we have an accurate assessment of the feasibility issues and that any problems with the protocol are addressed prior to starting the larger study.

3.3. Please list the proposed participating centers.

Participants in the pilot study will be recruited from the NICU at McMaster University Medical Centre. Proposed participating centers for the full-scale study include Children's Hospital of Western Ontario in London, Children's Hospital of Eastern Ontario in Ottawa, and BC Children's Hospital in Vancouver.

REFERENCES

1. Su B. Optimizing Nutrition in Preterm Infants. *Pediatr Neonatol* 2013 Sept 16 [Epub ahead of print].
2. Belfort MB, Rifas-Shiman SL, Sullivan T, Collins CT, McPhee AJ, Ryan P, Kleinman KP, Gillman MW, Gibson RA, Makrides M. Infant growth before and after term: effects on neurodevelopment in preterm infants. *Pediatrics*. 2011;128:e899.
3. Sharma R, Hudak ML. A clinical perspective of necrotizing enterocolitis: past, present, and future. *Clin Perinatol*. 2013;40:27-51.
4. Raval MV, Hall NJ, Pierro A, Moss RL. Evidence-based prevention and surgical treatment of necrotizing enterocolitis-a review of randomized controlled trials. *Semin Pediatr Surg*. 2013;22:117-21.
5. Gardener VA, Walton JM, Chessel L. Refeeding technique in a Premature infant with Short Bowel Syndrome. *Adv Neonatal Care.* 2003;6:258-71.
6. Al-Harbi K, Walton JM, Gardner V, Chessel L, Fitzgerald P. Mucous fistula Refeeding in Neonates with Short Bowel Syndrome. *J Pediatr Surg*. 1999;34:1100-3.
7. Lucchini R, Bizzarri B, Giampietro S, De Curtis M. Feeding intolerance in preterm infants. How to understand the warning signs. *J Matern Fetal Neonatal Med* 2011;24 Suppl 1:72-4.
8. Morris BH, Miller-Loncar CL, Landry SH, Smith KE, Swank PR, Denson SE. Feeding, medical factors, and developmental outcome in premature infants. *Clin Pediatr (Phila)*. 1999;38:451.
9. Shah V, Chirinian N, Lee S. Does the use of glycerin laxatives decrease feeding intolerance in preterm infants? *Paediatr Child Health.* 2011;16:e68-70.
10. Loening-Baucke V, Kimura K. Failure to pass meconium: Diagnosing intestinal obstruction. *Am Fam Physician*. 1990;60:2040-50.
11. Meetze WH, Franz AR, Linder W, Pohlandt F. Meconium passage in very-low birthweight infants. *J Parenter Enteral Nutr*. 1993;17:1537-40.
12. Mihatsch WA, Franz AR, Lindner W, Pohlandt F. Meconium passage in extremely low birthweight infants and its relation to very early enteral nutrition. *Acta Paediatr*. 2001;90:409-11.
13. Shim SY, Kim HS, Kim DH et al. Induction of early meconium evacuation promotes feeding tolerance in very low birth weight infants. *Neonatology*. 2007;92:67-72.
14. Haiden N, Jilma B, Gerhold B et al. Small volume enemas do not accelerate meconium evacuation in very low birth weight infants. *J Pediatr Gastroenterol Nutr*. 2007;44:270-273.
15. Khadr SN, Ibhanesebhor SE, Rennix C et al. Randomized controlled trial: impact of glycerin suppositories on time to full feeds in preterm infants. *Neonatology*. 2011;100:169-176.
16. Thabane L, Ma J, Chu R, Cheng J, Ismaila A, Rios LP, Robson R, Thabane M, Giangregorio L, Goldsmith CH. A tutorial on pilot studies: the what, why and how. *BMC Med Res Methodol*. 2010;10:1.

17. Braga LH, Pemberton J, Heaman J, DeMaria J, Lorenzo AJ. Pilot randomized placebo-controlled trial to investigate the effect of antibiotic prophylaxis on the rate of urinary tract infection in infants with antenatal hydronephrosis. (in preparation)
18. Karagol BS, Zenciroglu A, Okumus N, Polin RA. Randomized controlled trial of slow vs rapid enteral feeding advancements on the clinical outcomes of preterm infants with birth weight 750-1250 g. *JPEN J Parenter Enteral Nutr*. 2013;37:223-8.
19. Haiden N, Norooz F, Klebermass-Schrehof K, Horak AS, Jilma B, Berger A, Repa A. The effect of an osmotic contrast agent on complete meconium evacuation in preterm infants. *Pediatrics*. 2012;130:e1600-6.
20. Research Electronic Data Capture (REDCap) Consortium. http://project-redcap.org/
21. Kenny SE, Tam PK, Garcia-Barcelo M. Hirschsprung's disease. *Semin Pediatr Surg*. 2010;19:194-200.
22. Schäppi MG, Staiano A, Milla PJ, Smith VV, Dias JA, Heuschkel R, Husby S, Mearin ML, Papadopoulou A, Ruemmele FM, Vandenplas Y, Koletzko S. A practical guide for the diagnosis of primary enteric nervous system disorders. *J Pediatr Gastroenterol Nutr*. 2013;57:677-86.
23. Gregory KE, Connolly TC. Enteral feeding practices in the NICU: results from a 2009 Neonatal Enteral Feeding Survey. *Adv Neonatal Care*. 2012 Feb;12(1):46-55.
24. McMaster Children's Hospital. *NICU Nutrition Summary Guidelines*. Revised July 2012.
25. Premji SS, McNeil DA, Scotland J. Regional neonatal oral feeding protocol: changing the ethos of feeding preterm infants. *J Perinat Neonatal Nurs*. 2004;18:371-84.
26. Cohen, J. (1988). *Statistical power analysis for the behavioral sciences (2nd edition).* Hillsdale, NJ: Lawrence Earlbaum Associates.
27. Dupont WD, Plummer WD. *PS Power and Sample Size Calculations Version 3.0*. January 2009.
28. Julious SA. Sample size of 12 per group rule of thumb for a pilot study. *Pharm Stat*. 2005;4:287-91.
29. Billingham SA, Whitehead AL, Julious SA. An audit of sample sizes for pilot and feasibility trials being undertaken in the United Kingdom registered in the United Kingdom Clinical Research Network database. *BMC Med Res Methodol*. 2013;13:104.
30. Browne RH. On the use of a pilot sample for sample size determination. *Stat Med*. 1995;14:1933-40.
31. Matthews TJ, MacDorman MF. Infant mortality statistics from the 2005 period linked birth/infant data set. *Natl Vital Stat Rep* 2008;57:1.
32. Mandy GT. Incidence and mortality of the premature infant. *UpToDate* last updated: July 24, 2013.
33. *IBM® SPSS® Statistics Version 21*. 2012.

BUDGET

Budget Items	Total ($)
Personnel	
Research Assistant: $20.00/hour + $2.60/hour fringe benefits = $22.60/hour x 7 hours/week x 52 weeks (~1 day/week)	$8,227
Research Nurse: $30.00/hour + $9.60/hour fringe benefits = $39.60/hour x 7 hours/week x 52 weeks (7 hours/week)	$14,414
Total Personnel	**$22,641**
Materials and Supplies	
Recruitment materials, posters, brochures, cards, patient study binders	$1,000
Administrative	$1,000
Research Electronic Data Capture (REDCap) setup and hosting	$3,000
Travel to present findings at an international meeting	$2,000
Total Materials and Supplies	**$7,000**
TOTAL PROJECT BUDGET REQUESTED	**$29,641**

Justification

1. **Personnel:** This study requires a Research Assistant (RA) who has experience with clinical health research to ensure high quality data management. The RA will be responsible for database development, quality testing, study communication, patient consent, and general research administration. The RA will be budgeted for approximately 1 day per week for 1 year. This study also requires a research nurse to administer the study intervention to maintain blinding among the clinical care team. We have estimated that his person will need approximately 7 hours per week to accomplish this task.

2. **Materials and Supplies:** A total of $7,000 is requested for materials and supplies. This includes recruitment materials designed by a graphic designer, administrative supplies, and REDCap training and server space costs.

APPENDIX 2. STUDY PROTOCOL FOR PILOT STUDY

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Glycerin Suppositories Used Prophylactically in Premature infants (SUPP) Trial: a study protocol for a pilot randomized controlled trial

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Abstract

Background

Feeding is a significant challenge for premature infants in the neonatal intensive care unit (NICU). These patients are often treated with glycerin suppositories to stimulate the passage of meconium and prevent feeding intolerance. Unfortunately, the evidence for this practice is inconclusive.

Methods/Design

This protocol is for an external pilot study that will assess the feasibility of a superiority, placebo-controlled, parallel-design, multicenter randomized controlled trial. Participants are premature infants treated in a Level 3 NICU with a gestational age 24 to 32 weeks and/or birth weight of 500 to 1500 grams. Thirty participants will be recruited as part of this external pilot study. Participants will be randomized to glycerin suppository (250 mg) or placebo starting 48 to 72 hours after birth, and continuing once daily until meconium evacuation is complete or for a maximum of 12 days. The placebo consists of a 250 mg glycerin suppository placed in the diaper rather than the rectum. Study treatments are administered by the charge nurse on duty who is not otherwise involved in patient care. All other clinicians and research personnel will remain blinded. Outcomes for the pilot study are percentage of eligible participants randomized, percentage of infants reaching full enteral feeds, cost, and treatment-related adverse events (rectal bleeding, rectal perforation, and anal fissure).

Discussion

This external pilot study will assess the feasibility of a multicenter randomized controlled trial of glycerin suppositories in premature infants. The subsequent multicenter trial will have sufficient power to determine whether this treatment strategy is associated with decreased time to full enteral feeds.

Trial registration

ClinicalTrials.gov: NCT02153606

Keywords

prematurity, newborn, feeding intolerance, suppository, necrotizing enterocolitis

Background

Feeding is a significant challenge for premature infants in the neonatal intensive care unit (NICU) [1,2]. These babies have immature digestive tracts and can develop a life-threatening bowel infection called necrotizing enterocolitis (NEC) [3,4]. Treatment of this condition may require surgery and is associated with substantial morbidity and mortality. This includes short bowel syndrome, dependence on parenteral nutrition, and/or need for additional surgery [5,6].

Infants who do not develop NEC can still have issues with feeding and growth. Most are supported with intravenous nutrition while enteral feeds are advanced over a period of 1 to 3 weeks. This process can be delayed if infants develop feeding intolerance, characterized by abdominal distension, undigested feeds in the stomach, and decreased bowel movements [7]. This can lead to increased reliance on intravenous nutrition, which is associated with sepsis, extrauterine growth restriction, and poor neurodevelopmental outcomes [1,2,8].

Glycerin suppositories are commonly used in premature infants to stimulate the passage of meconium and improve feeding tolerance [9]. This practice is based on the observation that preterm infants experience significant delays in the passage of meconium, which is more viscous than normal stool [10,11]. Delays in meconium evacuation appear to be associated with a delay in the transition to enteral feeding [12]. Thus, if meconium evacuation could be expedited through the use of glycerin suppositories, this may lead to

faster transition to enteral feeding, decreased reliance on intravenous nutrition, and better outcomes. Unfortunately, there is little evidence to support this practice [9-17].

We recently conducted a systematic review on the use of glycerin suppositories and enemas in premature infants [17]. We identified a total of 185 infants from three single-center, randomized controlled trials [14-6]. These studies focused on the prophylactic use of glycerin suppositories (two trials) or enemas (one trial). Across all three trials, there were no differences in terms of meconium evacuation, transition to full enteral feeding, or mortality. There were no reports of rectal bleeding or perforation, but meta-analyzed data revealed a non-significant trend towards increased risk of NEC with active treatment. We concluded that going trials should be carefully monitored and stopped if it becomes clear that this trend is a real effect and not just a spurious correlation.

The results of our systematic review were complicated by the fact that all three trials were underpowered and affected by one or more major methodological issues. As a result, the quality of evidence was low to very low. We concluded that the evidence for the use of glycerin suppositories or enemas in premature infants is inconclusive and that further research is required. As a result, we designed an external pilot study to assess the feasibility of a multicenter randomized controlled trial of prophylactic glycerin suppositories in premature infants.

Methods/Design

Study design and objective

The Glycerin Suppositories Used Prophylactically in Premature infants (SUPP) Trial is an external pilot study for a superiority, placebo-controlled, parallel-design, multicenter randomized controlled trial [18]. The purpose of the multicenter trial is to determine whether glycerin suppositories decrease the time to full enteral feeding in premature infants. We hypothesize that the multicenter trial will demonstrate that using glycerin suppositories in premature infants results in earlier completion of meconium evacuation. Whether this treatment strategy results in earlier full enteral feeding or improvements in other outcomes remains unclear [17]. The protocol described here is for an external pilot study to assess the feasibility of a multicenter randomized controlled trial [19,20].

Setting

Premature infants will be recruited from the Level 3 NICU at McMaster Children's Hospital in Hamilton, Ontario, Canada. This unit treats almost 1000 infants per year of which approximately 150 would be eligible for our trial.

Participants

The participants will be premature infants 24 to 32 weeks and/or birth weight 500 to 1500 grams (Table 1). Exclusion criteria include: congenital gastrointestinal anomalies; surgery within 48 hours of birth; culture-proven sepsis; vasopressors; nitric oxide; prostaglandins; suspected coagulopathy (bleeding from any orifice); confirmed

coagulopathy (International Normalized Ratio>1.4, Partial Thromboplastin Time>39 seconds, Fibrinogen<1.00 grams/liter, platelet count<100×109/liter); neutropenia (absolute neutrophil count<0.5×10^9/L); and complete meconium evacuation within 48 hours after birth.

Interventions

Participants randomized to active treatment will receive a 250 mg glycerin suppository once daily starting 48 to 72 hours after birth (i.e., on day-3 of life). This smaller suppository will be created by cutting the tip off of a 1440 mg glycerin suppository. In order to maintain consistent dosing, we created a plastic measurement guide that results in a 250 mg suppository (Figure 1). This "tip" will be covered with a water-based lubricant and placed in the infant's rectum.

Participants in the control group will receive placebo suppositories. In usual practice, partially dissolved suppositories are often ejected from the rectum either with or without stool. In our trial, leaving a suppository in the diaper (but not in the rectum) makes it ambiguous as to whether it was placed in the rectum and ejected, or simply placed in the diaper. This approach also ensures that treatment will appear to have been administered to all infants, even if they happen to be in the control group.

Following administration of either treatment, the gluteal buttocks will be held together for 30 seconds to minimize the likelihood of the suppository being ejected from the

rectum. Participants in the each treatment group will receive study treatments once daily until they pass two normal bowel movements free of meconium staining. A similar duration of treatment was used in the randomized controlled trial of glycerin enemas from Austria [14]. Maximum treatment duration will be 12 days (i.e., all treatments will stop on day-14 of life).

All study interventions will be administered by one of the NICU charge nurses on duty. These individuals have years of experience working in the NICU but are not involved in the care of individual patients. All participants will receive a medical order of "nil per rectum" during the period of study treatments. This will be removed once study treatments stop. Study participants will be eligible for rescue glycerin suppository therapy if they are judged by the medical team to have feeding intolerance.

Randomization

Infants will be allocated to treatment groups via web-based stratified blocked randomization. Previous studies have shown that the size of the infant is highly predictive of the time to full enteral feeds [13]. In order to maintain prognostic balance, participants will be stratified by gestational age: (1) 24 weeks – 27 weeks 6 days; or (2) 28 weeks – 31 weeks 6 days. This strategy has been used in other randomized controlled trials of feeding intolerance in premature infants [14,15,21,22]. See Figure 2 for an overview of the external pilot study.

Sequence generation will be created using random number software by an unblinded research assistant not otherwise involved in the SUPP Trial. Block size will range from 4 to 6 to ensure that there are an equal number of participants in the treatment and control groups. Randomization of study participants will be completed online by a blinded research assistant immediately prior to the administration of the first study treatment. This web-based system will be created using Research Electronic Data Capture (REDCap) software [23]. For each participant, REDCap will assign a 3-digit treatment code that will be recorded by the blinded research assistant on the infant's bedside chart. The charge nurse will use a coding sheet to link the 3-digit number for each participant with either active treatment or placebo.

Blinding

The principal investigator, co-investigators, parents or guardians, physicians, bedside nurses, allied health professionals (e.g., dieticians), research assistants, outcome assessors, and statistical analysts will be blinded to treatment allocation. The only groups who will be unblinded are the participants (i.e., the premature infants who cannot communicate) and the charge nurses responsible for administering study treatments. Adequate blinding of medical staff, bedside nurses, and allied health professionals is essential since these clinicians typically make decisions about advancing or holding feeds during daily patient rounds. If these individuals are aware which treatment the participant is receiving, there is a chance that this knowledge will affect their decision-making and bias the results.

We will employ several strategies to maintain blinding. First, participants in the control group will receive suppositories placed in the diaper rather than no intervention at all. Second, all study treatments will be administered when the participant's crib is covered. This will ensure that only the charge nurse administering the treatment knows which intervention is given. Third, we have held multiple meetings with the NICU nursing staff prior to the start of the trial to discuss the purpose of blinding and ensure that we have adequate buy-in from these individuals.

Duration of treatment

Infants enrolled in this trial will receive active treatment or placebo once daily until meconium evacuation is complete. This will be defined as two bowel movements free of meconium staining. Previous studies have demonstrated that this process takes approximately 6 to 9 days [14]. The study intervention will be withheld and reassessed daily in cases of thrombocytopenia (platelet count$<100\times10^9$/L), suspected coagulopathy (bleeding from any orifice, confirmed coagulopathy (International Normalized Ratio>1.4, Partial Thromboplastin Time>39 seconds, Fibrinogen<1.00 grams/liter, Thrombocytopenia (platelet count$<100\times10^9$/liter), or neutropenia (absolute neutrophil count$<0.5\times10^9$/L).

The study intervention will be stopped early in cases of clinical deterioration (vasopressors, prostaglandins, culture-proven sepsis, NEC, or death) or treatment-related

adverse events (rectal bleeding, rectal perforation, or anal fissure). Previous trials of glycerin suppositories and enemas did not report single case of rectal bleeding, rectal perforation, or anal fissure [14-16]. As such, the risk of these events occurring in the current study is low.

Follow-up

Outcome data will be extracted from the medical record after participants are discharged from hospital, reach a corrected gestational age of 40 weeks, or die (whichever happens first). The research assistant will also assess participants on at least a weekly basis to maintain contact with nursing staff, discuss any protocol violations, monitor for adverse events, and address any other issues that arise. Nursing staff will also be instructed to submit possible adverse events to the research assistant as soon as they occur. These include any cases of rectal bleeding, rectal perforation, and anal fissure.

Outcomes

Outcomes for the external pilot study will be recruitment rate (i.e., percentage of eligible infants randomized), completion rate (i.e., percentage of infants reaching the primary endpoint of full enteral feeds), and treatment-related adverse events (i.e., safety outcomes). The pilot study will also allow us to better estimate the explicit cost per infant of conducting a randomized trial on this topic (i.e., considering the total costs of database design, data storage, printed materials, and salary for research assistants). We will also assess the frequency and type of protocol violations and post-randomization exclusions.

The primary outcome for the multicenter trial will be time in days to full enteral feeding (defined as 150 mL/kg/day). Advancing the rate of enteral feeds is typically based on a standardized NICU feeding protocol [24-26]. Deviations from this protocol occur when infants become unwell, develop signs of feeding intolerance, or if there are other clinical concerns. Secondary outcomes will include feeding volume on day 14 of life (in mL/kg), days to complete meconium evacuation, days of parenteral nutrition, incidence of NEC, incidence of line sepsis, compliance with treatment regimen, and mortality.

Sample size

For the external pilot study, we hope to recruit 30 participants (15 per group) over a 6-month period. Previous reviews recommend using at least 12 participants per group and some indicated that a total sample size of 30 may be more appropriate [27-29].

Potential pitfalls

Adherence to the study protocol was a serious issue in a randomized controlled trial of glycerin enemas from Austria [14]. This may have occurred because some clinicians did not believe in the efficacy of glycerin enemas and tended to withhold this intervention among participants in the therapeutic arm. This will be less of an issue in our trial since glycerin suppositories are commonly used in the NICU at McMaster Children's Hospital and are much less invasive.

Another issue to consider is post-randomization withdrawals or exclusions. While this is an issue in any randomized controlled trial, this will present a unique challenge in our study since participants will have been alive for less than 48 hours when they are enrolled. Some infants may not have had prenatal screening and serious congenital anomalies that would have excluded them from the study may not be diagnosed until days or weeks after randomization. Even in cases of excellent prenatal care, some conditions (e.g., Hirschsprung's disease) cannot be diagnosed until the postnatal period [30,31]. The best way to handle this will be to follow all randomized participants to the primary endpoint of full enteral feeds and analyze the data on the basis of intention-to-treat.

Losing participants to follow-up is unlikely to occur since all premature infants are monitored in hospital until they are tolerating full enteral feeds and reach 37 weeks corrected gestational age. Some participants who are doing well clinically may be transferred to a NICU in a community hospital that is not part of this study, but this is unlikely to occur until after these infants have reached full enteral feeds. Finally, the mortality rate in this population is approximately 10% [32,33]. While our exclusion criteria will exclude most of the infants at risk for postnatal mortality, some participants may die before they are randomized, complete the treatment regimen, or reach full enteral feeds.

Statistical analysis

Frequencies and 95% confidence intervals using normal approximation will be used to estimate recruitment rate and percentage infants reaching the primary endpoint. We will also report the rate of protocol violations, post-randomization exclusions, and incidence of adverse events related to the study intervention. We will also report the mean cost per infant randomized. All data will be analyzed in the Statistics Package for the Social Sciences (SPSS) [34].

Ethical and safety considerations

This study was approved by the Neonatal Research Committee at McMaster Children's Hospital, Hamilton Integrated Research Ethics Board (14-575), and Health Canada (9427-M1133-53C). All parents or guardians will provide written and informed consent prior to enrollment.

We have established a Data Safety and Monitoring Board (DSMB) for the pilot study, which consists of two neonatologists and one pediatric surgeon. This group will meet after the first 5 participants are randomized and then once every three months until the pilot study is complete. The DSMB will review safety outcomes (i.e., treatment-related adverse events) and can request unblinding should the need arise. Unblinding will be facilitated by the unblinded research assistant who performed sequence generation and is

not otherwise involved in the administration of this study. The principal investigator, co-investigators, and research assistant will remain blinded.

Discussion

The evidence for the use of glycerin suppositories in premature infants is inconclusive [9,17]. In our recent systematic review, we considered the results from three single-center randomized controlled trials of glycerin suppositories (two studies) or enemas (one study) [17]. The trial focused on glycerin enemas included 81 very low birth weight infants from a single hospital in Austria [14]. All participants were enrolled in the study shortly after birth and stratified by gestational age: either (1) 24 weeks – 27 weeks 6 days; or (2) 28 weeks – 31 weeks 6 days. Infants in the intervention group received daily glycerin enemas if they did not pass meconium spontaneously within 12 hours of birth. These enemas continued until complete evacuation was achieved. The control group did not receive any intervention.

This study was an open trial and the primary outcome was the number of days to complete evacuation of meconium and the study was powered to detect a 30% difference. There was a trend towards a treatment effect with complete evacuation of meconium occurring at a median of 6.5 days in the intervention group and 9 days in the control group, but this difference was not statistically significant ($p=0.11$). No differences were reported for any of the secondary outcomes, including duration of hospital stay, weight at discharge, days to introduction of oral feedings, feeding volume on day 14 of life, days to

passage of first meconium, or days to full enteral feeding. This trial was limited by the lack of blinding, possibility of selective reporting, small sample size, and frequent protocol violations.

The second trial explored whether glycerin suppositories decrease feeding intolerance in premature infants [15]. Participants were enrolled shortly after birth and were randomized from a stack of opaque envelopes. This study was an open trial and there were no attempts to maintain blinding. The primary outcome was days to full enteral feeding. There was a trend towards a decrease in time to full feeds of 1.6 days, but the study was only powered to detect a difference of 3.6 days. There were also no significant differences for any of the secondary outcomes, including incidence of NEC, episodes of culture-positive sepsis, feeding intolerance during the first 10 days, growth and nutrition, and ventilation. Despite this, infants in the intervention group passed their first stool earlier (day 2) than controls (day 4) ($p=0.016$), and were less likely to pass their first stool after 48 hours of life (24% versus 64%) ($p=0.003$).

The third randomized controlled trial was published in 2014 [16]. This study included 50 premature infants from a single hospital in India with a gestational age of 28 to 32 weeks and birth weight 1000 to 1500 grams. Infants less than 28 weeks gestation or 1000 grams were excluded. Participants randomized to active treatment received a 1000 mg glycerin suppository once daily starting on day-2 of life and continuing until day-14, regardless of stooling pattern. Infants in the control group underwent a placebo procedure, where the

diaper was opened and closed again, but no active treatment was administered. All study treatments were administered by a research nurse and blinding was maintained for all other clinical and research personnel.

This trial reported no differences between treatment groups for any of the outcomes, including time to full enteral feeds, time to regain birth weight, NEC, frequency of feeds being withheld, and length of hospital stay. The main limitations were small sample size, possibility of selective reporting, and number of participants lost to follow-up. In each group, 3/25 participants (greater than 10% of the total sample size) were transferred to another hospital before complete outcomes could be obtained [16].

As shown above, previous trials on the use of glycerin suppositories in premature infants are small, underpowered, and affected by a variety of methodological issues [17]. As such, the evidence for this treatment strategy is inconclusive and clinical equipoise remains. The SUPP Trial will start as an external pilot study to assess feasibility. If minimal changes are required, we will develop a similar protocol for a superiority, placebo-controlled, parallel-design, multicenter randomized controlled trial. Once completed, the multicenter trial will have sufficient power to determine whether glycerin suppositories facilitate meconium evacuation and transition to enteral feeding in premature infants.

Trial status

The SUPP Trial started recruiting participants in January 2015 and is on track to complete enrollment of 30 participants for the external pilot study by July 2015. An update with results from the external pilot study will be provided in 2016.

Abbreviations

NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit

Competing interests

The authors declare they have no financial conflict or competing interests.

Authors' contributions

MHL designed the study protocol, helped obtain funding, completed the ethics review, and assisted with trial management. JZ assisted with the literature review and study conception. HB revised the study protocol and helped complete the ethics review. JP assisted with ethics review, trial registration, and trial management. CW assisted with the funding application, ethics application, and trial management. JMW conceived this study, completed the funding application, and assisted with trial management. All authors read and approved the final manuscript.

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References

1. Su B. Optimizing Nutrition in Preterm Infants. *Pediatr Neonatol* 2014, 55:5-13.
2. Belfort MB, Rifas-Shiman SL, Sullivan T, Collins CT, McPhee AJ, Ryan P, Kleinman KP, Gillman MW, Gibson RA, Makrides M. Infant growth before and after term: effects on neurodevelopment in preterm infants. *Pediatrics*. 2011, 128:e899.
3. Sharma R, Hudak ML. A clinical perspective of necrotizing enterocolitis: past, present, and future. *Clin Perinatol*. 2013, 40:27-51.
4. Raval MV, Hall NJ, Pierro A, Moss RL. Evidence-based prevention and surgical treatment of necrotizing enterocolitis-a review of randomized controlled trials. *Semin Pediatr Surg*. 2013, 22:117-21.
5. Gardener VA, Walton JM, Chessel L. Refeeding technique in a Premature infant with Short Bowel Syndrome. *Adv Neonatal Care.* 2003, 6:258-71.
6. Al-Harbi K, Walton JM, Gardner V, Chessel L, Fitzgerald P. Mucous fistula Refeeding in Neonates with Short Bowel Syndrome. *J Pediatr Surg*. 1999, 34:1100-3.
7. Lucchini R, Bizzarri B, Giampietro S, De Curtis M. Feeding intolerance in preterm infants. How to understand the warning signs. *J Matern Fetal Neonatal Med* 2011, 24 Suppl 1:72-4.
8. Morris BH, Miller-Loncar CL, Landry SH, Smith KE, Swank PR, Denson SE. Feeding, medical factors, and developmental outcome in premature infants. *Clin Pediatr (Phila)*. 1999, 38:451-7.
9. Shah V, Chirinian N, Lee S. Does the use of glycerin laxatives decrease feeding intolerance in preterm infants? *Paediatr Child Health.* 2011, 16:e68-70.

10. Loening-Baucke V, Kimura K. Failure to pass meconium: Diagnosing intestinal obstruction. *Am Fam Physician*. 1990, 60:2040-50.
11. Meetze WH, Franz AR, Linder W, Pohlandt F. Meconium passage in very-low birthweight infants. *J Parenter Enteral Nutr*. 1993, 17:1537-40.
12. Mihatsch WA, Franz AR, Lindner W, Pohlandt F. Meconium passage in extremely low birthweight infants and its relation to very early enteral nutrition. *Acta Paediatr*. 2001, 90:409-11.
13. Shim SY, Kim HS, Kim DH et al. Induction of early meconium evacuation promotes feeding tolerance in very low birth weight infants. *Neonatology*. 2007, 92:67-72.
14. Haiden N, Jilma B, Gerhold B et al. Small volume enemas do not accelerate meconium evacuation in very low birth weight infants. *J Pediatr Gastroenterol Nutr*. 2007, 44:270-273.
15. Khadr SN, Ibhanesebhor SE, Rennix C et al. Randomized controlled trial: impact of glycerin suppositories on time to full feeds in preterm infants. *Neonatology*. 2011, 100:169-176.
16. Shinde S, Kabra NS, Sharma SR, Avasthi BS, Ahmed J. Glycerin suppository for promoting feeding tolerance in preterm very low birthweight neonates: a randomized controlled trial. *Indian Pediatr*. 2014, 51:367-370.
17. Walton JM. Glycerin Suppositories Used Prophylactically in Premature Infants (SUPP): a Pilot Randomized Placebo-controlled Trial. *NCT02153606*. https://clinicaltrials.gov/ct2/show/NCT02153606

18. Thabane L, Ma J, Chu R, Cheng J, Ismaila A, Rios LP, Robson R, Thabane M, Giangregorio L, Goldsmith CH. A tutorial on pilot studies: the what, why and how. *BMC Med Res Methodol*. 2010, 10:1.
19. Braga LH, Pemberton J, Heaman J, DeMaria J, Lorenzo AJ. Pilot randomized placebo-controlled trial to investigate the effect of antibiotic prophylaxis on the rate of urinary tract infection in infants with antenatal hydronephrosis. *J Urol* 2014, 191(5 Suppl): 1501-7.
20. Karagol BS, Zenciroglu A, Okumus N, Polin RA. Randomized controlled trial of slow vs rapid enteral feeding advancements on the clinical outcomes of preterm infants with birth weight 750-1250 g. *JPEN J Parenter Enteral Nutr*. 2013, 37:223-8.
21. Haiden N, Norooz F, Klebermass-Schrehof K, Horak AS, Jilma B, Berger A, Repa A. The effect of an osmotic contrast agent on complete meconium evacuation in preterm infants. *Pediatrics*. 2012, 130:e1600-6.
22. Research Electronic Data Capture (REDCap) Consortium. http://project-redcap.org/
23. Cohen, J. (1988). *Statistical power analysis for the behavioral sciences (2nd edition).* Hillsdale, NJ: Lawrence Earlbaum Associates.
24. Dupont WD, Plummer WD. *PS Power and Sample Size Calculations Version 3.0*. January 2009.
25. Julious SA. Sample size of 12 per group rule of thumb for a pilot study. *Pharm Stat*. 2005, 4:287-91.
26. Billingham SA, Whitehead AL, Julious SA. An audit of sample sizes for pilot and feasibility trials being undertaken in the United Kingdom registered in the United

Kingdom Clinical Research Network database. *BMC Med Res Methodol*. 2013, 13:104.

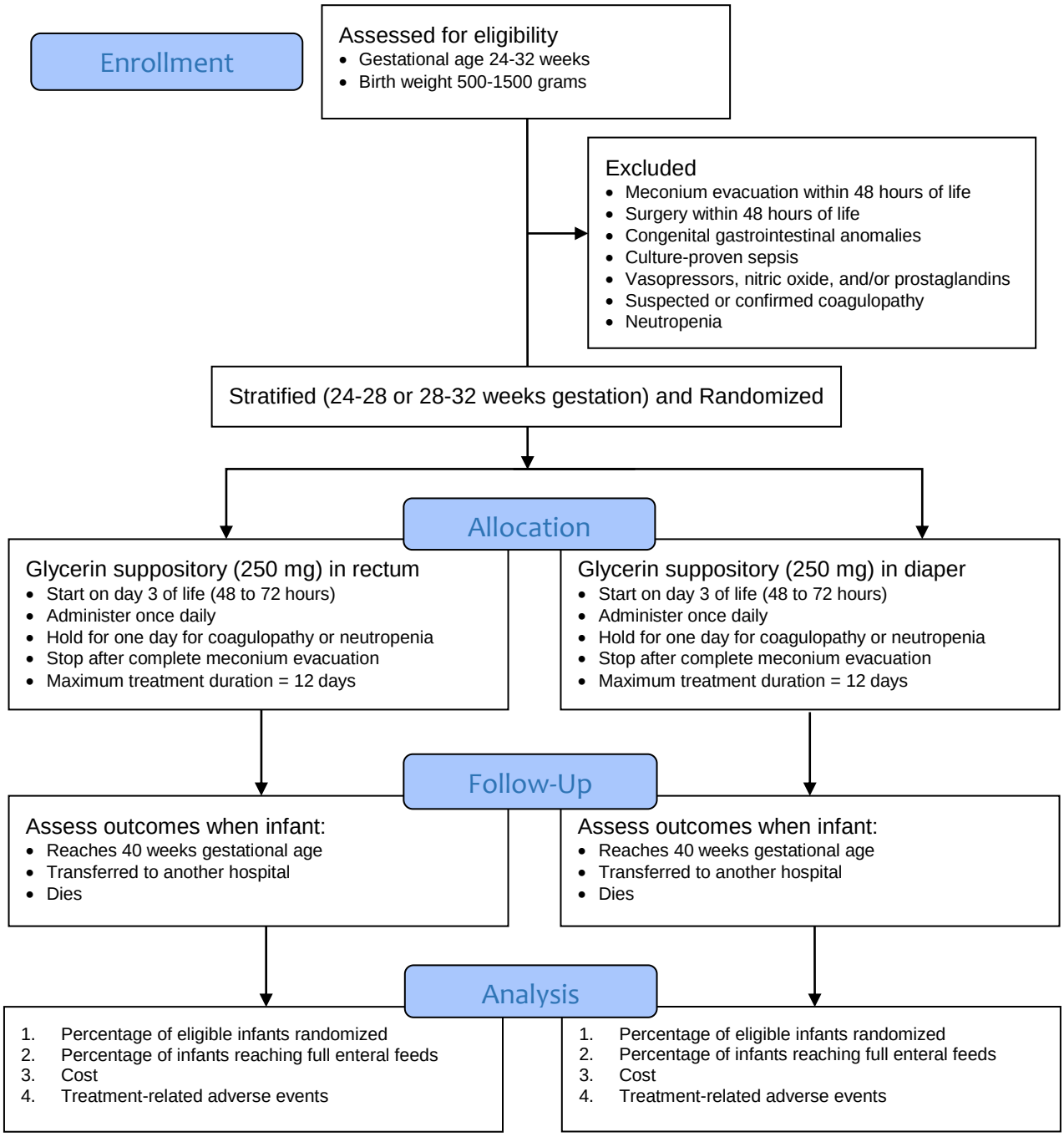
27. Browne RH. On the use of a pilot sample for sample size determination. *Stat Med*. 1995, 14:1933-40.
28. Kenny SE, Tam PK, Garcia-Barcelo M. Hirschsprung's disease. *Semin Pediatr Surg*. 2010, 19:194-200.
29. Schäppi MG, Staiano A, Milla PJ, Smith VV, Dias JA, Heuschkel R, Husby S, Mearin ML, Papadopoulou A, Ruemmele FM, Vandenplas Y, Koletzko S. A practical guide for the diagnosis of primary enteric nervous system disorders. *J Pediatr Gastroenterol Nutr*. 2013, 57:677-86.
30. Matthews TJ, MacDorman MF. Infant mortality statistics from the 2005 period linked birth/infant data set. *Natl Vital Stat Rep* 2008, 57:1.
31. Mandy GT. Incidence and mortality of the premature infant. *UpToDate* last updated: July 24, 2013.
32. *IBM® SPSS® Statistics Version 21*. 2012.

Table 1: Participant inclusion and exclusion criteria

Criteria	Definition
Inclusion (any of the following)	
Gestational age	24-32 weeks gestation
Birth weight	500-1500 grams
Exclusion (any of the following)	
Congenital gastrointestinal anomalies	Any congenital gastrointestinal anomalies
Clinically unwell	Major surgery within 48 hours of birth
	Culture-proven sepsis
	Vasopressors
	Nitric oxide
	Prostaglandins
Suspected coagulopathy	Mucosal bleeding from any orifice
Confirmed coagulopathy	International Normalized Ratio>1.4
	Partial Thromboplastin Time>39 seconds
	Fibrinogen<1.00 grams/liter
	Platelet count<100 $x10^9$/liter
Neutropenia	Absolute neutrophil count<0.5×10^9/L
Complete meconium evacuation	Two bowel movements with no meconium

Figure 1: Plastic measurement guide to ensure consistent dose of glycerin suppositories

Figure 2: Overview of Glycerin Suppositories Used Prophylactically in Premature infants (SUPP) Trial

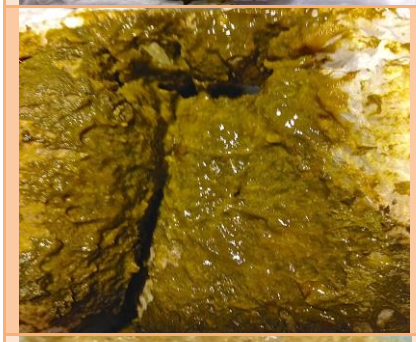


APPENDIX 3. STOOLING LEGEND

Developed by Stephanie Becker, Michael Livingston,

Henrietta Blinder, Connie Williams, and J Mark Walton

Stooling Documentation Legend

Example	Type	Description	Abbreviation
	Meconium	Viscous and sticky (tar like). Very dark green or brown. Almost odorless.	Mec
	Meconium-Transitional	Lighter green or brown with some sticky dark green/brown.	Mec+Tran
	Transitional	Light green, brown and/or yellow with looser texture. Some seeds present. NO sticky dark green/brown.	Tran
	Regular	Green changing to yellow or brown with a seedy-like texture.	Colour: Yellow = Y Green = G Brown = B Texture: Seedy = S Pasty = P Loose = L Watery = W

*Abbreviations consistent with the Neonatal Nursery's intake and output flow sheet.