Unintentional Overfeeding of Formula Fed Infants

"Whoa Baby"

NCT02701868
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Version 3

PROTOCOL

Anne Gilmore, Ph.D. Principal Investigator
Leanne M. Redman, Ph.D. Co- Principal Investigator
Abby Altazan, M.S. Co-Investigator
Jeffrey Burton, Ph.D. Co-Investigator

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1. SUMMARY

Although breast milk is recommended exclusively until 6 months of age, two-thirds of infants in the U.S. are fed infant formula. Despite an almost identical energy density between infant formula and breast milk, formula fed infants experience greater weight gain in the first year of life. We propose that unintentional overfeeding, of nearly one additional day of calories per week, due to the "over-scooping" of powdered formula contributes significantly to this phenomenon and potentially to the early development of childhood obesity, a significant public health problem. Research of US infants indicates that infant formula use is most prevalent in families with lower levels of education and poverty and as such infant formula is provided to families within the National Women, Infants and Children program. Given the higher prevalence of formula feeding in lower socioeconomic populations, there is an increased need for easy to follow instructions for formula preparation.

We recently completed the Baby Bottle Study at Pennington Biomedical (NCT01762631) which adapted the Remote Food Photography Method to estimate infant formula consumption in infants. An unexpected finding, despite following manufacturer infant formula preparation instructions, was that the weight of a single scoop of formula varied significantly. Ninety-five percent of the prepared scoops weighed more than the intended gram weight stated on the package. "Over-scooping" can result in increased caloric density and unintentional overfeeding of the infant. While the error for one scoop may have little impact on the overall nutrition of the infant, the error is compounded with every scoop and bottle fed over the course of a day.

In the proposed study we will assess the literacy and understanding of individuals to follow commercially available infant formula instructions. We will collaborate with the LA CaTS Health Literacy Core to improve the reading and comprehension of the manufacturer package instruction, and we will solicit the input of caregivers and key stakeholders to inform the development of educational intervention around infant feeding. Following modification of infant formula preparation instructions, we will conduct a randomized controlled trial that will test the efficacy of the modified instructions on infant overfeeding in comparison with the manufacturer package instructions. One hundred and fifty individuals will be asked to complete one study visit during which they will be asked to measure various serving sizes of infant formula following either the manufacturer package instructions or the modified instructions. The primary outcome variable will be powdered formula weight. We have designed qualitative and quantitative research studies to test the following specific aims.

1. **Aim 1 is to evaluate parental and caregiver literacy related to the use of commercially-available instructions for the preparation of infant formula.** *Hypothesis A: the health literacy of parents and caregivers to the instructions of commercially-available infant formula is variable. Hypothesis B: More than half of the parents and caregivers studied will not demonstrate adequate understanding of the infant formula instructions of the most commonly used infant formula in the US.*

2. **Aim 2 is to improve the accuracy and precision by which individuals' measure powdered infant formula by increasing reading ease and clarity of preparation instructions.** *Hypothesis: Improving reading ease and adding explicit pictures that convey preparation instructions will increase the accuracy and precision of scoop weight when compared to the standard instructions given on the food label. We hypothesize that preparation instructions developed with a health literacy focus (Aim 1) will reduce the proportion of over-scooped bottles from 89% to 50%.*

2. BACKGROUND AND SIGNIFICANCE

2.1. Significance

The 1,000 days between a woman's pregnancy and the child's second birthday is a critical period for growth and development. During this time the child is particularly vulnerable to the environment and permanent metabolic and developmental changes that may occur. Inadequate nutrition and improper feeding practices early in life leads to childhood obesity and chronic diseases in adulthood such as obesity, diabetes, and cardiovascular disease [1] and therefore, the National Institutes of Health is currently soliciting proposals to understand the development of obesity in children within this critical window for metabolic programming (PAR-14-323). From the outset, the introduction of synthetic infant formula as an alternative to breast milk was met with tremendous scrutiny [2]. While improvements to the composition of synthetic infant formula have been made since its development in 1865 including fortification with iron and omega-3 fatty acids 2 and despite an almost identical energy density between formula and breast milk, formula fed infants experience greater weight gain in the first year of life than exclusively breastfed infants [3]. Potential mechanisms linking infant formula feeding and childhood adiposity include lower levels of feeding self-regulation and increased appetite due to differences in sucking biomechanics between the bottle and breast [4-6], differences in established feeding practices [7], and maternal attitudes towards infant feeding.

Despite the benefits of breastfeeding and the recommendation of the World Health Organization and the American Academy of Pediatrics for exclusive breastfeeding until six months of life [8], over 80% of infants in the United States receive infant formula prior to their six month birthday [9, 10]. Given that the majority of infants in the U.S. receive infant formula in the first year of life, it is perplexing that there is little to no current NIH funding dedicated to better understanding the link between infant formula and obesity development in young children. On the other hand, there is an abundance of research that focuses on the promotion of breastfeeding or mechanistic studies to understand why breast milk may be protective against childhood obesity which seems counterintuitive. We agree that research on breastfeeding and breast milk is important however the scientific community is neglecting the equally or arguably more important research of infant feeding in general and in particular feeding with infant formula. If this trend research continues, the childhood obesity epidemic will likely only be worsened because many of barriers to breastfeeding in the US, as pointed out in the US Surgeon General Report in 2011 [11], including maternal obesity, lack of social and family support and unpaid maternity leave are not likely to be overcome in the near future. Furthermore, evaluating the health literacy and comprehension of infant formula instructions is critical because the USDA's Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) is the largest purchaser of infant formula making WIC recipients the largest consumer of infant formula in the United States [12]. Individuals who receive WIC benefits are generally from racial and ethnic minority groups, GED certificate recipients or individuals below the 12th grade education level [13], and these demographics are consistent with a population with low health literacy [14]. Infant formula preparation instructions must therefore be written and designed to accommodate a low level of health literacy to ease readability, improve caregiver competency, and increase preparation accuracy to ensure proper feeding of the nearly 662,000 formula fed infants receiving WIC services per year. Thereby, research examining the health literacy of individuals in the understanding of infant feeding and in particular in the preparation of infant formula are critical to evaluate. Moreover interventions for caregivers that focus on infant feeding in general and infant formula in addition to breastfeeding in both the pre- and post-natal periods need to be developed and tested to address this major public health issue.

2.2. Innovation

Through this highly innovative project we will, for the first time, conduct qualitative research to evaluate the health literacy of infant formula instructions. In collaboration with the LA CaTS Health Literacy Core, we aim to improve the reading and comprehension of package preparation instructions. The revised infant formula

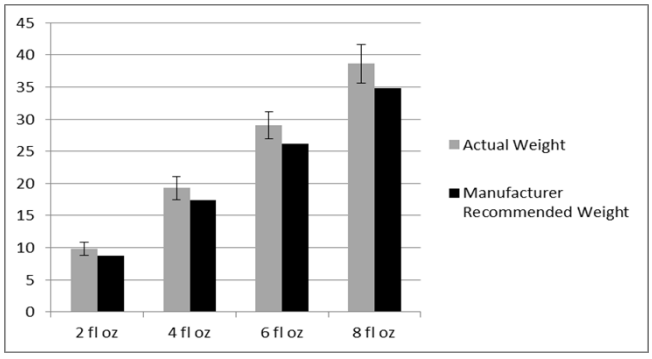
preparation instructions will be further modified through the recommendations of repeated focus groups. Lastly, we test the efficacy of the new instructions on infant overfeeding by asking volunteers to measure infant formula using the standard preparation instruction or the revised instructions in a randomized controlled trial. This is a novel translational study which will demonstrate that toolkits aimed to educate caregivers in the proper preparation of infant formula along with improved preparation instructions need to be developed. With easier to follow preparation instructions, caregivers will less likely overfeed infants resulting in decrease infant adiposity and risk for childhood obesity.

Our study is highly novel because: 1) it addresses infant formula feeding which is postulated to be a major factor in the development of childhood obesity which is a major public health concern, 2) we have highly provocative preliminary data from a recently completed study at Pennington Biomedical Research Center (PBRC) that shows adults including caregivers of infants grossly overestimate infant formula which would lead to a significant overfeeding, 3) we plan for the first time to carryout qualitative research that assesses the literacy and understanding of individuals to follow commercially available infant formula instructions and 4) we will solicit the input of caregivers and key stakeholders to inform the development of educational intervention around infant feeding. The results of our study therefore translate our previous research findings to improve the understanding of infant feeding and in particular the accuracy of infant formula preparation to prevent unintentional overfeeding and to inform public health strategies related to the prevention of obesity from birth.

The majority of the commercially available infant formula exists in powdered form. Caregivers are required to correctly interpret the package label to accurately estimate the serving size and prepare a bottle of formula for the infant. This is particularly problematic because adults are unable to accurately measure estimate portion sizes [8, 15] and the preparation of infant formula requires an accurate estimate of not one but two parts that can impact the energy density of the meal; the powdered infant formula and the added water. Despite strict governance by the FDA on the precise formulation of infant formula [9], it is surprising that there is no consistency between products in the preparation instructions provided to consumers on the label and the measuring scoop used to prepare the required serving size. Difficulty in understanding preparation instructions together with an inaccurate estimation of portion sizes would lead to an incorrect preparation of infant formula bottle.

2.3. Preliminary Studies

Figure 1. Actual vs. recommended scoop weights



Preliminary data obtained in the Baby Bottle study points to an unintentional overfeeding of formula fed infants. From 2012-2013 we conducted the Baby Bottle study at PBRC (NCT01762631). The purpose of the baby bottle study was to validate the remote food photography method (RFPM) for estimating energy intake for formula-fed infants. To this end, we recruited 53 men and women aged 18-80 to prepare bottles of infant formula and take photographs of the bottles simultaneously for the RFPM. The subjects were self-identified as caregivers, that is an individual who had prepared a bottle of infant formula in the past year or non-caregivers. Subjects completed two visits approximately one-week apart and prepared three bottles measuring 2fl oz, 4 fl oz, 6 fl oz and 8 fl oz, such that two trials were conducted at Visit 1 and the final trial was conducted at visit 2. The gram weights recommended for each serving of infant formula is shown in **Figure 1**, alongside the mean±se of actual gram weights of servings prepared by the study subjects. Of the 636 bottles prepared, 607 or 95% contained significantly more infant formula powder (by weight) than what was recommended on the package label ($p<.0001$). This finding was

evident for each bottle size which is suggested for infant feeding from birth until 12 months of age and averaged 11± 0.6% (range: -24 to +140%) over-scooping across all bottles. The caloric density of a standard scoop of infant formula is 20 kcal/fl oz and therefore with this unintentional over-scooping the prepared bottles of formula had a caloric density of 22 kcal/fl oz, the caloric density often prescribed to premature infants to accelerate infant growth rate [16]. While this error may appear to be marginal for a single bottle, the error was consistent across trials. We can therefore assume that the unintentional over-scooping is consistent within an individual and the overfeeding of the infant would then compound throughout the day as multiple bottles are consumed. These data are summarized in **Table 1**. For each bottle size the additional formula added to each bottle, would result in an almost entire day of additional energy intake per week. Furthermore, there was no difference between caregivers and non-caregivers which points to a fundamental error in understanding the package instructions.

Table 1. Unintentional overfeeding of formula fed infants

Infant Age	% Overfed/ meal	Energy Intake Kcal/day	Overfed (kcal/d)	Overfed (kcal/wk)
Newborn	11	350	39	273
2 months	11	480	53	371
4 months	11	500	55	385
6 months	11	590	65	455

3. RESEARCH PLAN: PHASE 1

The proposed study is divided into two phases. Phase I will utilize qualitative data gathered from focus groups to direct the modification of infant formula manufacturer preparation instructions to enhance user understanding and accuracy.

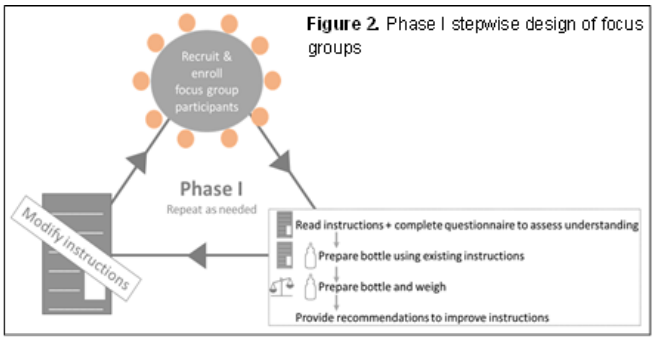


Figure 2. Phase I stepwise design of focus groups

In collaboration with the LA CaTS Health Literacy Core, the health literacy elements of infant formula preparation instructions will be assessed (**Figure 2**). Initial modifications to the manufacturer instructions will be made to increase reading ease and ability to understand and act on instructions. Tools used to assess the health literacy elements and to inform necessary modifications to the standard instructions include the Patient Education Materials Assessment Tool (PEMAT) [17], Ensuring Quality Information for Patients (EQUIP) [18], and the Health Literacy INDEX [19]. These literacy tools encourage the use of plain language, statement of a clear purpose, specific concrete instructions, use of short sentences, use of pictures to clarify message, clear layout, use of headings, chunking of related information, and audience appropriateness. Directed by the health literacy assessment, we will modify the manufacturer infant formula preparation instructions to decrease the level of health literacy required to properly prepare a bottle of formula. The instructions will be modified to be easier to read and navigate. The aim is to increase the participant's ability to understand the words and measurements and increase their ability to properly act on the instructions. The modified instructions will then be presented to focus groups in a stepwise fashion to continually improve consumer comprehension of the instructions and until feedback from the focus groups is completely addressed.

3.1. Study Population

Up to 40 individuals will be recruited to participate in focus groups at Pennington Biomedical Research Center. Approximately six focus groups (each with 5-10 participants) will be conducted at the PBRC Demonstration Kitchen over the course of approximately 3 months. Focus group participants will be matched based on demographic representation (i.e. level of education, household income, gender, and relation to infants) to encourage equal participation of focus group attendees. A primary caregiver is defined as an individual who has routinely cared for an infant in the last year for more than 12 hours per day. Secondary caregiver is defined as

an individual who has routinely cared for an infant in the last year for less than 12 hours per day. A non-caregiver is an individual who has not routinely cared for an infant in the last year.

3.1.1. Eligibility Criteria

Participants are eligible to participate in this study if they are:

- ≥18 years of age
- Willing to participate in 1 focus group visit at Pennington Biomedical Research Center
- Willing to disclose level of education, household income, gender, and relation to infants (i.e. primary caregiver, secondary caregiver, or non-caregiver)
- English speaking

3.1.2. Exclusion Criteria

Participants are ineligible to participate in this study if they are:

- <18 years of age
- Not willing to participate in 1 focus group visit at Pennington Biomedical Research Center
- Not willing to disclose level of education, household income, gender, and relation to infants (i.e. primary caregiver, secondary caregiver, or non-caregiver)
- Non-English speaking

3.2. Recruitment

Potential participants will be recruited from within PBRC and the Greater Baton Rouge area via Institutional Review Board (IRB) approved posters, flyers and advertisements posted on the PBRC website (http://www.pbrc.edu/clinicaltrials) and through PBRC approved social media outlets including but not limited to Facebook, blogs, and Craigslist. In addition to recruitment in the community, the Reproductive Endocrinology and Women's Health Laboratory has successfully recruited participants for pregnancy, breastfeeding, and women's health studies through partnerships with Baton Rouge General and Ochsner OB/GYNs and local Women, Infants, and Children (WIC) clinics. Referrals from these collaborators will also be used in recruitment efforts.

3.3. Study Screening

Interested participants will complete initial screening via phone or the online webscreener survey. Interested participants may contact study staff for study description and basic eligibility details via phone or email correspondence. Participants not meeting these basic criteria from the webscreener will be excluded. All eligible volunteers will be assigned a unique study ID number and referred to study staff for study specific screening and focus group scheduling.

3.4. Assessment Schedule and Procedures

Potential participants will complete screening to determine eligibility prior to enrollment. Interested and eligible participants will be scheduled for a focus group visit. At the beginning of the focus group visit, participants will be provided the Whoa Baby Phase 1 informed consent and HIPAA authorization for review and completion. After signing the Whoa Baby Phase 1 informed consent and HIPAA authorization, participants will complete several procedures and participate in focus group discussion at the focus group visit. See **Table 2**, Schedule of Procedures.

TABLE 2. Schedule of Procedures

Visit Assessment	Focus Group Visit
Informed Consent (Adult)	X
Height	X
Weight	X
Individual Interview	X
Health Literacy Assessment via Interview	X
Infant Formula Preparation	X
Focus Group Discussion	X

3.4.1. Focus Group Visit

When an individual arrives to the assigned focus group visit at the PBRC Demonstration Kitchen, the Whoa Baby Phase 1 informed consent will be provided for reading and review. If the participant agrees, the participant will sign the consent prior to the initiation of study procedures. After signing the informed consent, study procedures will be completed as part of the focus group visit. Height and non-fasting body weight will be measured by trained study staff. Before focus group discussion, individuals will be asked (one participant at a time) to go to a different room for the individual structured interview for health literacy assessment and the infant formula preparation procedure. After all participants have completed the individual, structured interviews, infant formula preparation, and anthropometric assessments, the focus group discussion will be completed where the focus group will elicit barriers and facilitators to infant formula using standard package instructions and provide recommendations for improvement of standard infant formula preparation instructions to enhance readability and improve understanding.

3.5. Measures and Outcome Assessments

3.5.1. Anthropometrics

Height and body weight will be measured using standard procedures of PBRC. Non-fasting body weight will be recorded. Body mass index (BMI) will then be calculated from the recorded height and body weight. BMI will be computed as a cohort demographic characteristic to ensure that focus group population is representative of the general population in the Baton Rouge area.

3.5.2. Individual Interview

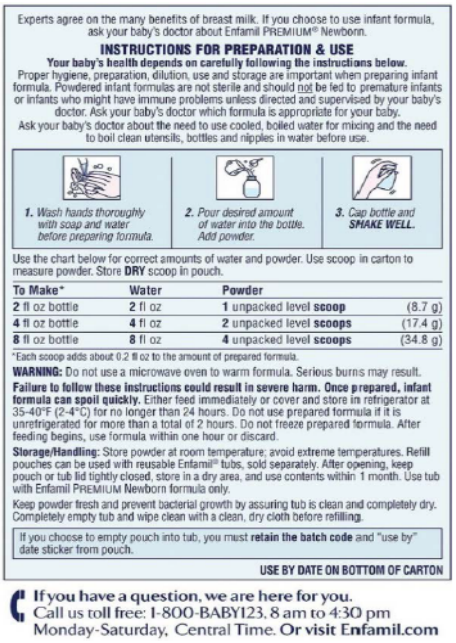
1. **General information:** A research assistant will conduct a structured interview with participants to collect general information (e.g. age, race, income, education, experience with infant formula preparation, number of children, caregiver status, etc.), experience with infant formula preparation, and relation to infants.

2. **Health Literacy Assessment:** Health literacy and numeracy skills will be assessed through structured interview. The following assessments will be completed as part of the health literacy assessment.
    a. *Rapid Estimate of Adult Literacy in Medicine (REALM-SF):* The REALM-SF [20] asks participants to read medical terms aloud.
    b. *Newest Vital Sign:* The Newest Vital Sign [21] is based on a nutrition label from an ice cream container. Participants are given the label and then asked 6 questions about it. Participants can refer to the label while answering questions. The questions are asked orally and the responses recorded by study staff on a special score sheet, which contains the correct answers. Based on the number of correct responses, the study staff can assess the patient's health literacy level.
    c. *Modified Parental Health Literacy Activities Test (PHLAT):* The PHLAT [22] assesses parental health literacy and numeracy skills in understanding instructions for caring for young children.

3.5.3. Infant Formula Preparation

Participants will be asked to prepare a single 2 fl oz bottle of infant formula according to the manufacturer package instructions without assistance or interruption (**Figure 3**). Participants will be asked to prepare a 2 fl oz bottle again, but this time the weight of the dry powder scooped and the weight of the prepared formula will be recorded. Weights will be obtained (to the nearest tenth of a gram) using a METTLER TOLEDO PB3001 scale. Discrepancies between actual weight and the weight recommend by the manufacturer will be a point of discussion during the focus group. Both trials will be video recorded for future review by study staff to identify common points of error or confusion.

Figure 3: Commercially available instructions for preparation of infant formula


Experts agree on the many benefits of breast milk. If you choose to use infant formula, ask your baby's doctor about Enfamil PREMIUM® Newborn.

INSTRUCTIONS FOR PREPARATION & USE

Your baby's health depends on carefully following the instructions below.

Proper hygiene, preparation, dilution, use and storage are important when preparing infant formula. Powdered infant formulas are not sterile and should not be fed to premature infants or infants who might have immune problems unless directed and supervised by your baby's doctor. Ask your baby's doctor which formula is appropriate for your baby.

Ask your baby's doctor about the need to use cooled, boiled water for mixing and the need to boil clean utensils, bottles and nipples in water before use.

1. *Wash hands thoroughly with soap and water before preparing formula.*
2. *Pour desired amount of water into the bottle. Add powder.*
3. *Cap bottle and* ***SHAKE WELL.***

Use the chart below for correct amounts of water and powder. Use scoop in carton to measure powder. Store **DRY** scoop in pouch.

To Make*	Water	Powder	
2 fl oz bottle	2 fl oz	1 unpacked level **scoop**	(8.7 g)
4 fl oz bottle	4 fl oz	2 unpacked level **scoops**	(17.4 g)
8 fl oz bottle	8 fl oz	4 unpacked level **scoops**	(34.8 g)

*Each scoop adds about 0.2 fl oz to the amount of prepared formula.

WARNING: Do not use a microwave oven to warm formula. Serious burns may result.

Failure to follow these instructions could result in severe harm. Once prepared, infant formula can spoil quickly. Either feed immediately or cover and store in refrigerator at 35-40°F (2-4°C) for no longer than 24 hours. Do not use prepared formula if it is unrefrigerated for more than a total of 2 hours. Do not freeze prepared formula. After feeding begins, use formula within one hour or discard.

Storage/Handling: Store powder at room temperature; avoid extreme temperatures. Refill pouches can be used with reusable Enfamil® tubs, sold separately. After opening, keep pouch or tub lid tightly closed, store in a dry area, and use contents within 1 month. Use tub with Enfamil PREMIUM Newborn formula only.

Keep powder fresh and prevent bacterial growth by assuring tub is clean and completely dry. Completely empty tub and wipe clean with a clean, dry cloth before refilling.

If you choose to empty pouch into tub, you must **retain the batch code** and "use by" date sticker from pouch.

USE BY DATE ON BOTTOM OF CARTON

If you have a question, we are here for you.
Call us toll free: 1-800-BABY123, 8 am to 4:30 pm
Monday-Saturday, Central Time. Or visit Enfamil.com

3.5.4. Focus Group Discussion

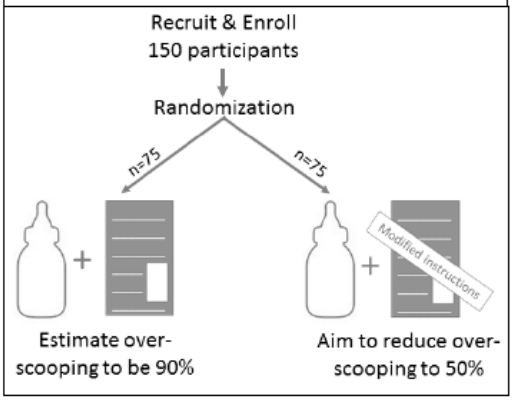
Focus group discussions will be led by a moderator who will follow a script of group discussion probes. During the focus group, participants will be asked to comment on discussion themes (e.g. attitudes towards infant feeding, current feeding practices, desired education regarding infant feeding, and recommendations to improve preparation instructions), and all responses will be audio and/or video tape-recorded and will be transcribed verbatim later. Responses will be de-identified in the transcript. Modified preparation instructions will be presented to the first focus group for discussion. The preparation instructions will be further modified based on participant feedback. Iterative focus groups will view and provide feedback on the preparation instructions modified by the previous focus group. Focus groups will continue until a satisfactory set of instructions are developed. Conducting focus groups in a stepwise fashion allow one focus group to build on the improvements recommended by another.

4. RESEARCH DESIGN: PHASE 2

Phase 2 is a randomized, controlled study to test the efficacy of the revised instructions on infant overfeeding in comparison with the instructions currently provided on the packaging (**Figure 4**). Individuals will be randomized in a 1:1 ratio to one of two groups:

1. **Group 1**: Group 1 participants will prepare infant formula following the standard infant formula preparation instructions provided by the manufacturer.
2. **Group 2**: Group 2 participants will prepare infant formula following the modified infant formula preparation instructions.

Figure 4. Phase II instruction efficacy



4.1. Study Population

Up to 150 individuals who did not participate in Whoa Baby Phase 1 will be recruited to participate in Whoa Baby Phase 2 at Pennington Biomedical Research Center.

4.1.1. Eligibility Criteria

Participants are eligible to participate in this study if they are:

- ≥18 years of age
- Did not participate in Whoa Baby Phase 1
- Willing to participate in 1 assessment visit at Pennington Biomedical Research Center
- English speaking

4.1.2. Exclusion Criteria

Participants are ineligible to participate in this study if they are:

- <18 years of age
- Did participate in Whoa Baby Phase 1
- Not willing to participate in 1 assessment visit at Pennington Biomedical Research Center
- Non-English speaking

4.2. Recruitment

Potential participants will be recruited from within PBRC and the Greater Baton Rouge area via Institutional Review Board (IRB) approved posters, flyers and advertisements posted on the PBRC website (http://www.pbrc.edu/clinicaltrials) and through PBRC approved social media outlets including but not limited to Facebook, blogs, and Craigslist. In addition to recruitment in the community, the Reproductive Endocrinology and Women's Health Laboratory has successfully recruited participants for pregnancy, breastfeeding, and women's health studies through partnerships with Baton Rouge General and Ochsner OB/GYNs and local Women, Infants, and Children (WIC) clinics. Referrals from these collaborators will also be used in recruitment efforts.

4.3. Study Screening

Interested participants will complete initial screening via phone or the online webscreener survey. Interested participants may contact study staff for study description and basic eligibility details via phone or email correspondence. Participants not meeting these basic criteria from the webscreener survey will be excluded. All eligible volunteers will be assigned a unique study ID number and referred to study staff for study specific screening and scheduling.

4.4. Assessment Schedule and Procedures

Potential participants will complete screening to determine eligibility prior to enrollment. Interested and eligible participants will be scheduled for an assessment visit. At the beginning of Visit 1, participants will be provided the Whoa Baby Phase 2 informed consent and HIPAA authorization for review and completion. After signing the Whoa Baby Phase 2 informed consent and HIPAA authorization, participants will complete several procedures at the Visit 1. See **Table 3**, Phase 2 Schedule of Procedures.

TABLE 3. Phase 2 Schedule of Procedures

Visit Assessment	Visit 1
Informed Consent (Adult)	X
Height	X
Weight	X
Individual Interview	X
Infant Formula Preparation	X

4.4.1. Visit 1

When an individual arrives to Visit 1 at the PBRC Demonstration Kitchen, the Whoa Baby Phase 2 informed consent will be provided for reading and review. If the participant agrees, the participant will sign the consent prior to the initiation of study procedures. After signing the informed consent, participants will learn of their group assignment and study procedures will be completed as part of Visit 1. Height and non-fasting body weight will be measured by trained study staff. Individuals will be asked to participate in an individual interview and prepare servings of powdered infant formula following the instructions provided.

4.5. Measures and Outcome Assessments

4.5.1. Anthropometrics

Height and body weight will be measured using standard procedures of PBRC. Non-fasting body weight will be recorded. Body mass index (BMI) will then be calculated from the recorded height and body weight.

4.5.2. Individual Interview

Study staff will give participants a brief structured interview to collect general information, e.g. age, race, income, education, typical use of infant formula, number of children and caregiver status.

4.5.3. Infant Formula Preparation

Participants will be asked to prepare 2 trials of 2, 4, 6, and 8 fl oz bottles of infant formula in random order. A total of 8 formula bottles will be prepared at Visit 1. For each bottle, the participant will follow their randomly assigned (Group 1 or Group 2 randomization) preparation instructions to measure the required amount of powdered formula. The participant will dispense the respective number of scoops of powdered formula into the formula bottle depending on which size bottle is being prepared. After all powdered formula is dispensed into the feeding bottle, PBRC staff will weigh the formula bottle with powdered formula only. The participant will continue following the instructions and pour water into the bottle and mix the formula. PBRC staff will weigh the formula bottle. This procedure will be repeated to assess intra- individual variability. Bottles will be weighed (to the nearest tenth of a gram) using a METTLER TOLEDO PB3001 scale, and dry formula and prepared formula weights will be recorded. Study staff who record the formula weights will be blinded to the randomization assignment and intended bottle size.

5. PARTICIPANT SAFETY AND CONFIDENTIALITY

5.1. Risks to participants

This study involves humans, but does not meet the definition of a clinical trial because there is no prospective intervention that may impact health. This study does not involve major risk to screeners and trial participants. Efforts to minimize the potential risks of the assessment methods and outcome variables include monitoring by the investigators to assure there are no incidents that occur while at Pennington Biomedical Research Center as a result of participating in the research.

Potential Risks associated with the study procedures include (presented in alphabetical order):

- Body weight. There is no risk to participants who record their body weight.
- Focus groups. There is minimal risk associated with participation in a focus group. Participants will be told that the discussion during a group is confidential prior to the start of a discussion. Participants will be encouraged to participate in the discussion, but will not be required to do so if they feel uncomfortable.
- Infant formula preparation. There is no risk to participants who prepare infant formula. The formula will be poured out after preparation, and no formula will be consumed.

- Interview: There is no known or anticipated risk associated with the completion of an interview. Participants will be encouraged to answer all questions, but will be told that they can choose not to answer any questions if they feel uncomfortable answering them. Responses to the questions will be coded to protect confidentiality.

5.2. Adverse events

Pennington Biomedical Research Center is an AAHRPP accredited institution and, above all else, is committed to ensuring and maintaining the safety of its participants. Participants in the study will come to Pennington Biomedical Research for one visit and will not be followed longitudinally. Therefore, adverse events and medications will not be tracked. There are three physicians on site at Pennington Biomedical Research Center who will be available if an incident occurs during a study visit. Study staff will follow institutional procedures for reporting any incident.

Protection of subjects from risks related to the study is of paramount concern to investigators. Due diligence will be applied to ensure that study subjects are not exposed to undue or untoward risk. Remedial action, including additions and changes to the protocol, will be taken as appropriate. The specific reporting mechanism currently in place is also outlined in PBRC HRPP Policy 8.0 Unanticipated Problems Involving Risks to Subjects or Others. While Federal guidelines require that Unanticipated Problems Involving Risks to Subjects or Others [21 CFR 56.108(b)] be reported to the IRB [45 CFR 46.103(b)(5)], related internal and external events involving risk but not meeting the prompt reporting requirements should be reported to the IRB in summary form at the time of continuing review. Surveillance and reporting procedures are summarized in the following section.

5.2.1. Surveillance

All incidents will be recorded and reviewed by the study Principal Investigator and a Center physician. Proper action as directed by Center standard operating procedures or physician advice will be followed.

5.2.2. Reporting

The following events may represent unanticipated problems involving risks to subjects or others and should be promptly reported to the IRB:

Breach of confidentiality involving risks

- Event requiring prompt reporting (Report only when required by the protocol, sponsor, or funding agency.)
- New information (Report information indicating an unexpected change in risks or potential benefits, e.g., literature/scientific report or other published finding.)
- Subject complaint (complaints indicating unanticipated risks or those that cannot be resolved by the research staff.)
- Unapproved change made to the research to eliminate an apparent immediate hazard
- Other problem or finding (e.g., loss of study data, a subject becomes a prisoner while participating in research)
- New information that may affect adversely the safety of the participants or the conduct of the clinical trial.
- Any changes significantly affecting the conduct of the clinical trial or increasing the risk to participants.

Both internal events and external events that may represent unanticipated problems involving risks to subjects or others should be promptly reported. Unanticipated problems involved risks to subjects or others will be reported within 10 working days of the Principal Investigator or research staff becoming aware of the

unanticipated problem. Events resulting in temporary or permanent interruption of study activities by the investigator or sponsor to avoid potential harm to subjects should be reported within 48 hours when possible.

5.3. Stopping rules

There is minimal risk for participating in this trial. The most likely scenario that would indicate a cessation of the study would be failure to recruit participants. Nevertheless, in addition to monitoring recruitment, we also will monitor the rates of injury in our participants. The study investigators will alert the IRB, if a larger than reasonably expected injury rate occurs in our participants. Other issues that are related to the stopping rules include:

- New information – It is unlikely that new information will become available during this study that would result in discontinuing the trial.
- Limits of assumption – It is possible that the value of data analysis will be limited by differences between the intervention groups at baseline or because of study dropouts or missing data. Baseline differences will be analyzed annually and effects on the power to detect differences in the outcome measures will be evaluated and discussed with the PI and other officials. Although an excessive number of dropouts could occur, this has not been our past experience. In CALERIE study (24 weeks), the dropout rate was only 2% (1 out of 36 enrolled). If the dropout rate for the proposed study exceeds 15%, the study coordinator will initiate a meeting with the PI to discuss strategies to increase retention. If the dropout rate exceeds 25%, the safety officer will meet with the study investigators to determine whether or not the study should continue.
- Limit of rules – We acknowledge that circumstances, other than what are listed, may justify stopping the study.

5.4. Confidentiality

All volunteers are assured of their anonymity and confidentiality both verbally and in the informed consent form. The clinical facilities are strictly limited to the staff of the research institution and to research volunteers. This is accomplished by a variety of stringent security measures. All medical records are stored in locked areas. Access to these areas is limited to the clinical support staff, director of the clinical facilities, and the PIs. Volunteers' medical records are filed according to ID numbers. All forms on the chart, with the exception of consent form, display only the ID number. Electronic data storage is similarly restricted with only the PIs and authorized persons having access to databases containing confidential clinical records, i.e. those containing name, Social Security Number or other identifying information.

Data will be collected from participants. Data are confidentially collected from study participants and are only used for research purposes. All records are kept in locked file cabinets, and participant data can be identified only by number. Data are used only in aggregate, and no identifying characteristics of individuals are published or presented.

6. DATA MANAGEMENT

6.1. Sample Size Consideration

In the Baby Bottle study, participants were asked to prepare a randomly ordered set of liquid baby formula (2, 4, 6, or 8 fl oz). Estimated proportions of participants over-scooping dry formula for bottles containing each amount were obtained from this preliminary data. A separate power analysis for the proposed study was carried out for each formula amount with the Baby Bottle estimates used to represent expected proportion of participants over-scooping dry formula when using the manufacturer preparation instructions. To ensure a well powered study, each estimate from the Baby Bottle data was reduced by an additional 15%. The most conservative results from the power analyses are presented in **Table 4** and provide a sufficient sample size to

achieve desired power when comparing the control and experimental group proportions of over-scooping for any individual formula amount as well as across all amounts. In the proposed study, we hope to see the proportion of participants over-scooping formula reduced to 50% following use of the new preparation instructions. 75 participants will be enrolled in each study group, ensuring 80% power to detect this decrease.

Table 4. Sample size calculations[1]

Power (%)	Proportion over-scooped: OLD	Proportion over-scooped: NEW	N Per Group
80	0.8	0.7	294
80	0.8	0.6	82
80	0.8	0.5	39
80	0.8	0.4	23

[1]*Old Proportion* represents the proportion of participants expected to over-scoop formula when using the existing instructions. *New Proportion* represents the reduced proportion of participants that are expected to over-scoop formula when using the new instructions.

6.2. Whoa Baby Phase 2 Randomization

A total of 150 participants will be randomized to one of two groups in Phase 2. A randomization schedule will be created and the study coordinator will ensure that each enrollee is put into the correct group. In addition to participant randomization, bottle size preparation order will also be randomized. At the visit, participants will receive a slip of paper revealing the order in which to prepare each bottle. A biostatistician will create the randomized sequences and each participant will be asked to prepare the bottles as dictated by the sequence provided to them.

6.3. Data Analysis Plan

To address the primary aim, dry formula weights will be compared to package recommendations (i.e. 8.7 g per scoop) and classified as under-scooped, accurate scoop, or over-scooped. The proportion of bottles that are over-scooped will be compared between the two groups. Both univariate and multivariate analyses will be employed. 2x2 contingency tables with counts of participants over-scooping in each treatment arm will be constructed and chi-square tests used to compare raw proportions. This will be carried out separately for each bottle size. In addition, estimated probabilities of over-scooping for each group will be obtained from logistic regression models allowing for adjustments for bottle size and any other chosen covariates. Group-level odds ratios constructed from the estimated probabilities will be compared using two-sample t-tests. For all tests, statistical significance is defined as $p<\alpha$. Adjustments for multiple comparisons will be made when appropriate.

7. SUBJECT PAYMENT

Participants enrolled in Whoa Baby Phase 1 or Whoa Baby Phase 2 will receive $25 for participation in the study to help offset transportation costs and their time. The compensation is in line with other studies conducted at PBRC, and checks will be requested from the Pennington Biomedical (LSU) payroll department.

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