THICKENING FLUIDS FOR PEDIATRIC PATIENTS WITH DYSPHAGIA: THE CHALLENGE OF ACHIEVING SAFE VISCOSITIES

A Thesis

presented to

the Faculty of the Graduate School

at the University of Missouri

In Partial Fulfillment

of the Requirements for the Degree

Master of Health Science

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by

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MAY 2018

The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled:

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ACKNOWLEDGEMENTS

Foremost, I would like to express my sincere gratitude to my thesis mentor and committee chair, Dr. Teresa Lever, for her continuous support. Her patience, motivation, and immense knowledge has added greatly to my graduate experience. Dr. Lever has provided me with extensive personal and professional guidance and taught me a great deal about scientific research and clinical application. She has taught me more than I can ever give her credit for here. Thank you, Dr. Lever, for pushing me further and providing me with skills to pursue my goals.

I would also like to extend my thanks to my thesis committee members, Dr. Judith Goodman and Dr. Eliav Gov-Ari, for offering their valuable time, support, and encouragement throughout the preparation and review of this thesis manuscript. Through this process, their ideas, questions, and feedback have been invaluable.

Special thanks to the speech-language pathology team at Women's and Children's Hospital - John Gianladis, Caitlin Vandell, and Chesney Willis. Without their efforts in recruiting donors, this project would not have been possible. I would also like to thank them for their personal time in providing suggestions and problem-solving throughout this project. I recognize that this entire experience would not have been possible without the financial assistance of SEARS grant funding through Women's and Children's Hospital in Columbia, Missouri.

Data collection for this project would not have been possible without the incredible team in the Lever Lab – Kate Osman, Ian Deninger, Marci Steinberg, Dani Lake, Erin White, and Allison Jarombek. I am grateful for the dedication and hard work you have put into this project. To everyone else in the Lever Lab, thank you for a great four years. You have all made this experience unforgettable and I wish you all the best!

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ABSTRACT

Speech-language pathologists (SLPs) at our hospital currently rely on their best clinical judgment to establish a nectar viscosity for bottle feedings because rheological equipment is not readily available. Therefore, clinicians use a 3:1 ratio of infant formula and/or breast milk to oat cereal to achieve a nectar viscosity. The main purpose of this study was to use a cone and plate rheometer to objectively quantify the viscosity of the five-thickened bottle-feeding conditions currently used at our hospital: infant formulas (19, 22, and 24 calorie) and breast milk (alone and a 50:50 mixture with 22-calorie formula) in a 3:1 ratio with oat cereal. A second goal was to determine the effects of time (over a 30-minute feeding duration) at body versus room temperature on viscosity outcomes for each thickened liquid condition. Results revealed that none of the thickened liquid bottle feeding conditions consistently remained in the nectar thick viscosity range. Specifically, several trials for each of the three thickened infant formula recipes rose *above* the nectar limit (i.e., into the honey thick range), whereas both thickened breast milk recipes (with and without 22-calorie formula) fell *below* the nectar limit (i.e., into the thin liquid range). Outcomes were significantly affected by temperature, but in an inverse manner for the three formulas (i.e., higher at body temperature) versus two breast milk (i.e., lower at body temperature) conditions. We are currently adjusting the 3:1 ratio for each of these conditions to achieve reliably stable low and high viscosity levels in the nectar thick range throughout a 30-minute feeding duration. Our ultimate goal is to standardize the viscosity of thickened liquid bottle feedings for infants across all levels of care for improved health care outcomes.

CHAPTER I: BACKGROUND

INTRODUCTION

Dysphagia, a medical term for feeding and swallowing impairment, affects 25-45% of typically developing infants per year and 33-80% of infants born with developmental disorders per year (Lefton-Greif, 2008). Dysphagia in infants can result from multiple etiologies including prematurity, neurological deficits, congenital anomalies, and birth defects. The most at-risk population is infants born with anatomic birth defects, which occurs in 1 out of every 33 live births (120,000 babies) annually; 80% (96,000) of those babies suffer from dysphagia. Infants who are born prematurely are also at high-risk, as 26% of this population develops dysphagia or show signs of dysphagia, especially those born at a low gestational age and birth weight (Jadcherla, 2016).

Dysphagia in infants can have a variety of consequences, the foremost being aspiration pneumonia due to liquid entering the lungs while bottle or breast feeding (Arvedson, Rogers, Buck, Smart, & Msall, 1994). During infant feeding, liquid flow from the nipple is turbulent, resulting in numerous eddies and vortices within the oral cavity before the liquid is swallowed. Liquid flow from the nipple is measured by calculating how many milliliters of formula are expressed from the bottle per minute. Infants who are considered medically fragile are often given “slow flow” rate nipples to slow the rate in which formula is expressed from the bottle to accommodate the infant’s ability to maintain physiologic stable state during feeding (Pados, Park, Thoyre, Estrem, & Nix, 2015). Moreover, liquid flow from the nipple is rapid and aimed in the direction of the larynx (entrance to the lungs). While healthy babies can tolerate these factors and safely swallow liquid from the nipple all the way to the stomach without aspiration, infants with dysphagia cannot (Lau, 2015). In fact, the thinner the infant formula or breast milk, the higher the aspiration risk for dysphagic infants. “Thickening liquids” to administer via bottle is, therefore, the primary strategy used in health care to reduce the risk of aspiration in dysphagic infants (Gosa & Dodrill, 2016; Hollin, 2011). The rationale is that thickened liquids flow more slowly from the bottle nipple and through the oral cavity, allowing the infant more time to initiate swallowing, which in turn provides sufficient time for the larynx to close and prevent aspiration as the liquid flows through the pharynx to enter the esophagus.

Assessment and treatment of dysphagia requires the knowledge of several health care providers that together form an interdisciplinary dysphagia team. Key team members typically include a primary care pediatric physician, pediatric specialists (e.g., otolaryngologist, pulmonologist, etc.), dietician, speech-language pathologist (SLP), occupational therapist, and nurses. Evaluation of the infant’s feeding/swallowing function and determining the safest thickness of liquid for the infant to consume falls within the scope of the SLP, under the direction of the primary care pediatric physician and/or the referring pediatric specialist (Silverman, 2010).

CONSEQUENCES OF LIQUID ASPIRATION DURING FEEDING

Aspiration of liquid into the lungs during infant feeding can result in aspiration pneumonia, an infection in the lungs that contributes to significant morbidity and mortality. In fact, infants with aspiration pneumonia have more medical complications resulting in increased intensive care transfers, longer hospitalizations, higher hospital costs, and higher 30-day hospital readmission rates compared to infants with bacterial or viral pneumonia due to non-aspiration causes (e.g., community acquired pneumonia) (Thomson et al., 2016). These negative health outcomes associated with infant dysphagia highlight the need for aggressive measures to reduce aspiration pneumonia risk in this vulnerable patient population.

While infants in the hospital can be susceptible to other types of pneumonia (i.e., community-acquired pneumonia, aspiration pneumonitis), aspiration pneumonia is caused by an inhalation of liquids into the lower airway *during feeding*. Thus, aspiration pneumonia is indicative of dysphagia-related aspiration and consequently the need for intervention by an SLP to reduce the risk of aspiration pneumonia (Marik, 2001). Infants are especially susceptible to aspiration pneumonia because they have weaker immune systems. Even more so, premature infants are at a high risk for aspiration pneumonia due to an underdeveloped or immature aerodigestive system (Colin, McEvoy, & Castile, 2010; Gallacher, Hart, & Kotecha, 2016; Marcus et al., 2009).

Aside from aspiration during feeding, there are several other causes of pneumonia in infants. For example, community-acquired pneumonia is an inflammation in the lung tissue caused by pathogens and bacteria entering the lungs, leading to an infection. In children and infants, community-acquired pneumonia is a common and serious infection (Ostapchuk, Roberts, & Haddy, 2004). Aspiration pneumonitis is inflammation of the lung tissue caused by gastric contents being regurgitated from the stomach into the airway, which is often caused by gastrointestinal reflux disease (GERD) (Marik, 2001). In fact, 50% of infants under three months of age experience regurgitation at least once a day. The prevalence of GERD at seven months of age falls to 21%, and it continues to decline with age throughout childhood (Nelson, Chen, Syniar, & Christoffel, 1997). For this study, we are focusing on aspiration risk specifically from dysphagia rather than other causes of pneumonia.

Infants with dysphagia leading to aspiration pneumonia have longer hospital stays and correspondingly higher medical costs than healthy infants (Capilouto & Cunningham, 2016; Lessen, 2011). Moreover, the average hospital cost of infants with aspiration pneumonia is $11, 594, which is double the cost for infants with non-aspiration pneumonia ($5,162). In addition, infants with aspiration pneumonia have 20% greater odds of hospital readmission within 30 days, compared to infants with non-aspiration pneumonia (Thomson et al., 2016). When hospitalized for pneumonia, mortality rates are higher for infants with aspiration pneumonia, especially those with a neurological impairment, compared to other causes of pneumonia (Dop et al., 2015; Thomson et al., 2016). These findings highlight the need for better intervention strategies to reduce the risk of aspiration pneumonia in dysphagic infants, with the ultimate goal to improve health care outcomes and reduce associated medical costs.

The signs of dysphagia in infants can be quite variable. Examples include oxygen desaturation (i.e., drop in the level of oxygen in the blood), bradycardia (i.e., lower than normal heart rate), coughing, gagging, arching the back and irritability (i.e., signs of gastrointestinal pain during feeding), refusal to feed, and failure to thrive. These signs may continue long after discharge from the hospital. Infants who show signs of dysphagia are also more at risk for chronic lung problems (e.g., chronic lung disease) and compromised nutritional status (Hawdon, Beauregard, Slattery, & Kennedy, 2000). Any infant discharged from the hospital must be able to sufficiently consume oral feedings and maintain/gain weight (Jadcherla, 2016). Dysphagia can affect both these discharge qualifications, resulting in a longer hospital stay and associated medical costs.

ECONOMIC AND HEALTH IMPACT OF INFANT DYSPHAGIA

The length of stay and cost of health care for infants with dysphagia varies greatly, depending upon the cause of the feeding and swallowing problem. The most crucial factor is age and timing of onset (Lefton-Greif, 2008). For preterm infants, the effect is immediate due to an underdeveloped gastrointestinal tract, which includes the structures of the oral cavity, pharynx, and esophagus that are essential for normal feeding and swallowing. While not every infant that is born prematurely develops dysphagia, they are the most at risk population. It is reported that approximately 37% to 40% of all infants diagnosed with a feeding or swallowing disorder are born prematurely. The number of infants born preterm has increased by 20% since 1990 (Lefton-Greif, 2008), and the rise in the survival rate of premature infants has led to an increase length of hospitalization and corresponding increase in health care costs.

Infants born without at-risk complications (e.g., prematurity, developmental disorders, etc.) have an average hospital stay of only one to two days, with a cost of approximately $1,500 (Lessen, 2011). The average length of stay for infants who are premature or have a low birth weight ranges from 14 to 17 days at an average cost of $21,500 to $76,700 (Kowlessar, Jiang, & Steiner, 2013). This is a drastic increase from those infants without medical complications. Premature infants are required to stay in the hospital for an extended time for several reasons, the primary reason being insufficient oral motor ability to coordinate sucking and swallowing during feedings. Sufficiently treating dysphagia by enhancing feeding progression in this vulnerable population could decrease the length of stay in hospitals. Annually, this could save hospitals over two billion dollars (Lessen, 2011).

For infants with severe dysphagia, a feeding tube may be necessary to maintain nutrition and growth. Within the first year of life for an infant on a feeding tube, the health care cost is approximately $46,785. The placement of feeding tubes occurs in 127 out of every 100,000 infants under one year of age (Fox et al., 2014). By avoiding surgical placement of feeding tubes and instead using other feeding strategies, such as an appropriate thickened liquid diet to reduce aspiration risk, the health care cost savings could be nationally reduced by $2.1 million in the first year and to $9.1 million at five years (Jadcherla, 2016).

TREATMENT TO REDUCE ASPIRATION RISK IN INFANTS WITH DYSPHAGIA

The predominant treatment approach for infants with dysphagia is to increase the viscosity (i.e., thickness) of liquid administered via bottle feeding. However, there are a variety of methods for measuring viscosity. Moreover, there are many variables that affect the viscosity of liquid, including the type of liquid used (i.e., infant formula or breast milk), type of thickening agent used, temperature of the liquid, and setting time before/during consumption (Dion, Duivestein, St Pierre, & Harris, 2015). Methods of measuring viscosity and the variables that impact viscosity are described below.

Viscosity measurements*:* Thickening bottle feedings as a treatment approach for dysphagic infants entails thickening infant formula or breast milk to increase the viscosity (i.e., thickness) to a nectar or honey consistency. The National Dysphagia Diet (NDD) has set viscosity guidelines for thin (1-50 centipoise or cP), nectar (51-350 cP), and honey (351-1750 cP) consistencies (ADA., 2002). However, the viscosity ranges for each category are extremely wide and are not yet backed by evidence-based research for use with dysphagic infants. Instead, they are only a “best guess” guide for SLPs to follow. Moreover, commercially available thickening agents (described below) do not provide instructions for thickening infant formula or breast milk to nectar or honey consistency for bottle feedings (Gosa & Dodrill, 2016).

Current standard practice for thickening bottle feedings for infants with dysphagia entails mixing various thickening agents (e.g., grain cereals) with infant formula or breast milk to achieve the target viscosity of a nectar or honey consistency (Frazier et al., 2016; Rubin, 2011). However, the majority of health care providers rely on an “eyeball” method to estimate the viscosity of the liquid. As a result, there is high variability in the thickness of liquids fed to dysphagic infants (J. A. Cichero, Jackson, Halley, & Murdoch, 2000).

In order for clinicians to have a quick and simple tool to categorize thickened liquids into thin, nectar, or honey consistency ranges, subjective tests have been developed, including a line spread test and a fork drip test (Nicosia & Robbins, 2007; Park, Kim, & Lee, 2016). The line spread test is an inexpensive and efficient way to estimate the viscosity of liquids. It is performed by placing liquid on a level Plexiglas surface and allowing it to spread on the surface over 60 seconds (Budke, Garcia, & Chambers, 2008). Thus, it is used to determine the consistency of liquid by determining the gravitational spread of liquid across a flat surface. A cylinder is used to place the liquid at the center of the Plexiglas board marked with 0.5-centimeter lines. These lines are used to measure the spread of liquid after a minute (Lund, Garcia, & Chambers, 2013). This test provides a broad categorization of liquid (i.e., thin, nectar, or honey consistency); however, it does not provide a specific measure of viscosity, so it cannot discriminate within a category (i.e., one nectar consistency may be more viscous than another nectar consistency) (Nicosia & Robbins, 2007).

The fork test was developed to measure the consistency of liquids based on the flow of material on a fork. Three levels of consistencies are described using the fork test: grade 1 refers to liquids that remain on the fork; grade 2 refers to liquid that partially flows between the tines of a fork; and grade 3 refers to liquids that do not remain on the fork. Liquids were categorized into the three grades based on viscosity measurements: grade 3 = 0-300 cP, grade 2 = 300-10,000 cP, and grade 1 = greater than 10,000 cP (Park et al., 2016). The measurements used for the fork test do not align with measurements from the NDD. Although the fork test has proven to be valid and reliable for distinguishing between three consistency levels, it can only provide an approximate viscosity measurement. The exact thickness of liquids needs to be reported so clinicians can readily reproduce mixtures needed for a thickened diet (Park et al., 2016). The only way to accurately measure viscosity is with a rheometer.

A rheometer is a computer-controlled laboratory device used to measure the viscosity of liquid. The term rheometer comes from the Greek word *rheo*, which means flow. Hence, a rheometer is a device for measuring flow, or more specifically, for measuring *resistance to flow* (i.e., viscosity). A rheometer can control variables that a line spread test cannot, such as the temperature of the liquid sample being tested, shear rate (i.e., rate of liquid deformation), and shear stress (i.e., the amount of force or friction acting on the liquid). These variables are used by the rheometer to automatically calculate the viscosity of the liquid being tested, where viscosity = shear stress / shear rate at a given temperature. While the shear rate on a rheometer can be set anywhere between 1-100 inverse seconds, it is typically set to 50 inverse seconds for dysphagia research because this shear rate most closely resembles the flow of liquid in the oral cavity during swallowing (J. A. Cichero et al., 2000; Steele, Van Lieshout, & Goff, 2003; Stuart

& Motz, 2009). Although rheometers are highly accurate in viscosity measurements, their high cost, as well as the need for specialized training, renders this technology impractical for widespread clinical use (Kim & Yoo, 2015).

Variables That Impact Viscosity:

Type of liquid: In the United States, 81% of mothers begin to breastfeed their babies at birth, and by six months of age approximately 52% of mothers are still breastfeeding (Center for Disease Control National Center for Chronic Disease Prevention and Health Promotion - Division of Nutrition, 2016). Numerous health benefits are associated with breast milk consumption by infants, especially preterm infants (Campos, Repka, & Falcão, 2013). For example, breast milk helps preterm infants fight infection, helps provide better neurological development outcomes, and prevents long term cardiovascular and metabolic disease (Hair et al., 2016). For infants with dysphagia, breast milk must be collected in advance and thickened immediately before use for bottle feedings. The problem is that thickened breast milk does not maintain a stable viscosity during a standard feeding time (i.e., up to 30 minutes) because breast milk contains salivary amylase that rapidly breaks down the added thickening agent, resulting in a thin liquid consistency that increases the risk of aspiration (Almeida, Almeida, Moreira, & Novak, 2011). There are currently no standards for mixing thickening agents with breast milk to achieve a stable nectar or honey consistency during infant bottle feedings.

An alternative to breast milk is infant formula, for which there are several variables to consider. Examples include brand (e.g., Enfamil, Similac), form (i.e., powder or liquid), caloric density (e.g., 19, 20, 22, 24 calories per serving), and nutrient content. Reasons for choosing certain infant formulas over others are largely dependent upon hospital preference, resulting in large variability between hospital settings (Dion et al., 2015). All infant formulas, regardless of powder or liquid form, have a viscosity of less than 10 cP, which puts them in the thin liquid viscosity range established by the NDD (Frazier et al., 2016). However, powdered infant formulas contain bacteria and are therefore not recommended for use for at-risk infants such as those born preterm or immune-compromised (Agostoni et al., 2004; Dion et al., 2015).

Thickening agents: Prior to 2011, xanthan gum-based thickeners (e.g., Simply Thick) were widely used to thicken infant formula and breast milk for bottle feedings with dysphagic infants. However, in 2011, the Food and Drug Administration (FDA) warned against using xanthan gum-based thickeners with infants under one year of age, due to 22 reported cases of necrotizing enterocolitis, 21 of which occurred in preterm infants. Fourteen of these cases required surgery, which resulted in seven deaths (Beal, Silverman, Bellant, Young, & Klontz, 2012; FDA, 2016; Woods, Oliver, Lewis, & Yang, 2012). As a result, hospitals have resorted to using grain (i.e., rice or oat) cereals as a thickening agent for use with dysphagic infants. However, grain cereals, specifically oat, are much more variable than gum-based thickeners in achieving nectar and honey viscosities (Stuart & Motz, 2009). Regardless of which grain cereal is used, there are no standard “recipes” to achieve specific viscosities for infant formula and breast milk bottle feedings.

To further complicate matters, the FDA issued a warning in April 2016 to inform the medical community of the safety risk of feeding rice cereal to infants. Prior to this warning, rice cereal was a more popular thickening agent than oat cereal for dysphagic infants (Dion et al., 2015; Madhoun, Siler-Wurst, Sitaram, & Jadcherla, 2015). However, rice contains inorganic arsenic which has recently been linked to reduced performance on developmental tests by infants who consumed rice cereal in high quantities ((FDA), 2016). Therefore, the FDA set a limit of inorganic arsenic ingestion at 100 parts per billion for infants ((FDA), 2016), which is equivalent to only three bottle feedings of rice-thickened formula or breast milk daily (Carignan, Punshon, Karagas, & Cottingham, 2016). Given that infants typically feed every two-three hours, all infants receiving thickened bottle feedings would exceed the FDA dietary exposure limit for arsenic. As a result, oat cereal is currently the only remaining safe option for thickening bottle feedings and is therefore the thickening agent of choice for this thesis project. However, there are currently no standards for using oat cereal to thicken infant formula or breast milk for consumption by dysphagic infants.

With no clinical standards in place to achieve the desired viscosity for thickened bottle feedings, clinicians often resort to increasing the amount of thickener added until the infant appears to have no signs or symptoms of dysphagia (September, Nicholson, & Cichero, 2014). The major problem faced by this approach is that liquids that are too thick result in significantly increased feeding fatigue and caloric expenditure from overzealous suckling to express the thickened liquid from the bottle nipple, which may reduce nutritional intake and result in poor growth and development of dysphagic infants (Gosa & Dodrill, 2016; September et al., 2014). In contrast, liquids that are inadvertently too thin result in an increased risk of aspiration pneumonia and associated negative health care outcomes (Yoon & Yoo, 2017). Moreover, due to variation between as well as within providers, the viscosity of thickened bottle feedings may be highly variable between feedings. The goal of this study is to create a standard recipe to thicken infant formula and breast milk to nectar or honey consistency using oat cereal.

Temperature: Viscosity of liquids can also be affected by temperature. Viscosity and temperature have an inverse relationship such that when temperature increases, viscosity decreases (Garcia, Chambers, Matta, & Clark, 2008). Infant formula at body temperature is less viscous than room temperature formula, which is an important factor to consider when thickening infant bottle feedings (J. Cichero, Nicholson, & Dodrill, 2011). Breast milk feedings are naturally provided at body temperature; however, infant formula or expressed breast milk cools to room temperature over the duration of the bottle feeding (J. A. Cichero, Nicholson, & September, 2013). Stuart & Motz (2009) noted in their clinical settings there is no standard way to achieve body temperature for bottle feedings. Health care providers typically place bottles of infant formula into bags or bowls containing hot water until the desired temperature is reached; however, these temperatures vary between health care providers and settings (Stuart & Motz, 2009).

Although infant bottle feedings are typically warmed to body temperature before consumption, the majority of research studies to date have tested the viscosity of infant formula only at room temperature (Frazier et al., 2016). However, not all studies were conducted at room temperature. Several studies were carried out at body temperature to most closely replicate thickened infant feedings given in the hospital (J. Cichero et al., 2011; Gosa & Dodrill, 2016; September et al., 2014). Cichero et. al. noted that formulas that were tested at room temperature were more viscous than those at body temperature and when temperatures fell naturally, viscosity increased (J. Cichero et al., 2011). While studies of room temperature thickened infant formula do provide useful guidelines about how liquids thicken, it is not comparable to thickened infant formulas at body temperature. Therefore, additional research needs to be conducted with body temperature bottle feedings (Garcia et al., 2008), which is a goal of this thesis proposal.

Setting time: Viscosity of infant formula and breast milk with a thickening agent added is dependent upon the time that the mixture is allowed to set (i.e., the amount of time before the thickened liquid is tested or consumed). The setting time may impact overall thickness of infant formula or breast milk (Dion et al., 2015). While thickened breast milk thins over time (Almeida et al., 2011), starch-based thickened infant formulas tend thicken over time (Madhoun et al., 2015; September et al., 2014). Although starch–based thickeners continue to thicken over time and the highest increase in viscosity is seen in the first ten minutes of mixing with liquid, it is considered stable after these initial ten minutes. Grain thickeners (i.e., rice or oat cereal) contain particles which take some time to dissipate and stabilize, however, 30 seconds of shaking the formula and grain cereal was found to be plenty of time to dissolve the cereal (September et al., 2014).

Given that setting time changes the viscosity of thickened liquids, this factor should be considered by health care providers when bottle feeding infants in clinical settings, 75% of infant dysphagia providers report feeding infants within a specific time after mixing was an important factor in consumption of thickened liquids; however, the time was not specified (Dion et al., 2015). Therefore, it is crucial to control setting time for bottle feedings, which is yet another goal of this thesis project.

EVALUATION OF DYSPHAGIA

Lack of viscosity standards for bottle feedings of infants with dysphagia also impacts dysphagia evaluation by SLPs. Although dysphagia evaluation is not the focus of this thesis project, it is important to discuss how the proposed work and findings could have a beneficial impact on the evaluation of infants with dysphagia because the viscosity of liquids given during a swallow study must closely reflect an infant's typical diet (Frazier et al., 2016). For this reason, a brief overview of dysphagia evaluation is provided below.

Videoflouroscopic Swallow Studies (VFSS) is an X-ray procedure that is considered the gold standard diagnostic test to detect the presence and physiological cause of dysphagia and aspiration in all patient populations, including infants (Frazier et al., 2016). This test is performed by a team of medical professionals, including at the very least an SLP and a radiologist or radiology technician. During testing, a barium- or iodine-based contrast agent is mixed with unstandardized amounts of grain cereal to subjectively achieve a nectar or honey consistency liquid for bottle feeding. However, the viscosities used for daily bottle feedings with infants are not the same viscosities used during VFSS assessment. Liquid barium is more viscous and dense than liquids given during mealtimes, therefore cannot be compared (J. Cichero et al., 2011; Stuart & Motz, 2009).

Recent studies have compared the viscosities of barium compared to the viscosity of formula and thickened formula. Rheological differences (the flow of liquid) of thickened formula were compared to barium at thin, nectar and honey consistencies. A mixture of barium and rice cereal thinned over time due to the separation of the two ingredients. This is due to the heterogeneous nature of the mixture (Gosa & Dodrill, 2016). Liquid and powder barium of the same brand and concentration were found to have vastly different viscosities, liquid barium being more viscous than powder barium. This demonstrates the great variability in viscosities of similar products, therefore making it a great risk to assume that these products are affected the same way when paired with a thickening agent (i.e., grain cereal). Formulas of different caloric densities also show different behaviors when mixed with barium, 20 calorie formula thinned over time while 24 calorie formula thickened over time. This suggests that clinicians need direct guidance on how to mix formula with contrast agent to achieve the desired viscosity for both VFSS and bedside treatment (Frazier et al., 2016).

The lack of viscosity standards during VFSS risks misidentification or incorrect diagnosis of dysphagia (Frazier et al., 2016). Not only are there no standards for mixing infant formula or breast milk with grain cereals, there are also no standards for mixing contrast agents with grain cereals. As a result, the viscosities of daily bottle feedings versus bottle feedings during VFSS testing are likely not the same (J. Cichero et al., 2011). Therefore, we currently cannot compare the viscosity of these two recipes. Thickened formulas at evaluation and treatment must be comparable to ensure the safety of the infant and proper treatment and management. Formulas used for assessment are often more viscous than liquids given during treatment (i.e., mealtime), therefore the thickened formula for treatment may not be thick enough to prevent aspiration (J. Cichero et al., 2011; Gosa & Dodrill, 2016).

While the purpose of this study was not to test contrast agents paired with formulas or breast milk and grain cereals, it is important to highlight the differences between evaluation and treatment so that this can be considered for future research. In order to achieve proper thickened consistencies during evaluation, a standard recipe for mixing grain cereals with formula for treatment is needed to have a foundation for what is considered a standard to reach nectar and honey thick consistencies. Improving thickened infant formula will improve evaluation in the future; however, this is beyond scope of this study.

PURPOSE OF THE STUDY

Infants born preterm or with other health conditions are at a high risk for dysphagia and aspiration pneumonia. There is no current standardized way to clinically treat dysphagia in this vulnerable population. Several thickening agents such as gum-based thickeners have been banned from use with infants under one year of age, due to several cases of necrotizing enterocolitis. Due to the limited number of products available to safely thicken liquids for dysphagic infants, it is difficult to clinically treat this patient population. Even with the products available, standard recipes have not been established for mixing thickeners with infant formula or breast milk. The goal of this study is to create a standard recipe to thicken infant formula or breast milk to achieve a stable nectar consistency using oat cereal, and to apply these findings at the University of Missouri Women's and Children's Hospital (WCH) in Columbia, Missouri. The ultimate goal is to standardize the viscosity of thickened liquids for infants across all levels of care at WCH, including the neonatal intensive care unit (NICU), pediatric medical floors, outpatient clinic, and home health care setting, as well as during VFSS testing in the radiology department. The immediate goal of the study is to enhance the safety of thickened liquid bottle feedings for dysphagic infants at WCH.

The SLPs at WCH currently use a 3:1 ratio of infant formula and/or breast milk to oat cereal to presumably achieve a nectar consistency for bottle feedings. Without rheological equipment readily available for clinical use at WCH, SLPs have relied on their best clinical judgement to establish this 3:1 ratio, which is mixed as anywhere from 30 to 180 milliliters or more for a single bottle, feeding depending on the infant's weight and nutritional needs. The purpose of our study is to determine if this 3:1 ratio results in a thickened liquid that truly falls within the nectar viscosity range (51-350 cP) established by the NDD. Moreover, we determined if the viscosity remains stable for 30 minutes, which is the maximum bottle-feeding duration for dysphagic infants at WCH; the typical range is five to 30 minutes. A final goal was to determine what modifications to the 3:1 ratio are needed to establish standardized recipes to thicken infant formula and/or breast milk to three different thickness levels spanning the wide nectar viscosity range: low (60 +/- 5 cP), middle (200 +/- 5 cP) and high (340 +/- 5 cP). We accomplished this by using a rheometer to objectively quantify the viscosity of all bottle-feeding conditions currently used at WCH: infant formula (19, 22, and 24 calorie) and breast milk (alone and a 50:50 mixture with 22 calorie formula) in a 3:1 ratio with oat cereal.

The following questions were investigated:

1) Do the thickened liquid recipes (3:1 ratio) currently used at WCH truly fall within the nectar viscosity range (51- 350 cP) at body temperature?

a. I hypothesized that higher caloric infant formulas are most likely to be in the nectar thick viscosity range after thickening with oat cereal. This rationale is based on the general assumption that higher calorie formulas are inherently thicker than lower calorie formulas before adding the thickening agent.

b. I hypothesized that recipes containing breast milk will have lower viscosities compared to recipes without breast milk. In addition, I expect that recipes containing breast milk will become less viscous during the 30-minute test period, perhaps even falling into the thin viscosity range (1-50 cP). This rationale is based on previous studies showing that breast milk contains salivary amylase that rapidly breaks down thickening agents (Almeida et al., 2011).

2) How stable are the WCH thickened liquid recipes at body temperature throughout a 30-minute test time?

I hypothesized that thickened liquid recipes will not remain stable over a 30-minute test time. Specifically, as demonstrated in previous research, infant formulas mixed with oat cereal will gradually thicken (i.e., become more viscous) over time as the water content continues to be absorbed by the thickening agent (September et al., 2014). In contrast, breast milk mixed with oat cereal will become thinner (i.e., less viscous) over time because the salivary amylase found in breast milk is known to degrade thickening agents over time (Almeida et al., 2011).

3) Is viscosity significantly higher for thickened liquid recipes at room temperature compared to body temperature?

I hypothesized that liquids tested at room temperature will be more viscous than liquids tested at body temperature. This rationale is based on previous research showing that temperature and viscosity have an inverse relationship for thickened liquids (i.e., as temperature increases, viscosity decreases) (J. Cichero et al., 2011; Garcia, Chambers, Matta, & Clark, 2008).

4) Based on the findings from the three research questions above, what modifications to the 3:1 ratio of liquid (i.e., infant formula and/or breast milk) and oat cereal will be necessary to achieve a low, middle, and high viscosity level within the nectar consistency range (51-350 cP) to facilitate individualized treatment planning for dysphagic infants?

We expect the findings from the previous three research questions will provide sufficient information to allow us to easily adjust the 3:1 ratio of liquid to oat cereal to achieve the following three levels of nectar consistency bottle feedings: low (60 +/- 5 cP), middle (200 +/- 5 cP) and high (340 +/- 5 cP).

CHAPTER II: METHODS

INFANT FORMULAS AND THICKENING AGENT

All products included in this study are the products regularly used in the NICU at WCH. For infant formulas, we used three Similac brand formulas that consist of three different caloric densities: 19-calorie, 22-calorie, and 24-calorie. Each formula was paired with Gerber single grain oat cereal as a thickening agent. Currently, WCH uses the same 3:1 ratio, regardless of the caloric density of the formula. Furthermore, WCH uses only oat cereal as a thickener for bottle feedings, due to the FDA-identified health risk associated with other thickening agents. It was hypothesized that the higher the caloric density of infant formula, the less thickener (i.e., oat cereal) is needed to achieve the desired viscosity range (i.e., nectar consistency).

BREAST MILK SAMPLE COLLECTION

An institutional review board (IRB) study was approved in order to collect breast milk samples from willing donors between the ages of 18 and 35 who are currently breast feeding their infants. Breast milk samples were collected from five participants recruited from WCH and the local community. Breast milk donation kits were provided to each participant in the study. Donation kits included six 30 mL sterile containers, each marked at the 20-mL line to collect a total of 120 mL of breast milk. Over an eight-hour period, participants poured 20 mL of breast milk into the provided containers immediately after pumping. Each sample was frozen immediately after filling the container. Within 24 hours after collection, frozen breast milk samples were transported by a member of the research team to Dr. Lever's lab at MU, where they were stored at -20 °C until needed for testing.

Samples of breast milk were transported to the rheometer in an ice bucket to maintain frozen until ready for use. Samples of breast milk were thawed using a bottle warmer. To do this, the container with breast milk was placed in the bottle warmer containing water. The bottle of breast milk remained in the bottle warmer until body temperature (37 °C) was reached. Samples of thawed breast milk were mixed with oat cereal. Three conditions were tested: breast milk alone, breast milk thickened with oat cereal, and breast milk combined with 22-calorie formula and thickened with oat cereal. Viscosity measurements (described below) of each sample were acquired via rheometer immediately after thawing and/or mixing.

VISCOSITY MEASUREMENTS

Viscosity of the liquid test solutions were measured at room temperature (25 °C +/- 2 °C) and body temperature (37 °C +/- 2 °C), which are the two standard temperatures for infant bottle feedings (J. Cichero et al., 2011; Frazier et al., 2016). While WCH provides thickened bottle feedings at body temperature, it is important to investigate viscosity at room temperature because it is likely that mothers or other care providers may give thickened bottle feedings at room temperature for convenience. Thus, including both temperatures (room and body) provided information for hospital as well as home settings.

Viscosity measurements were acquired using a cone and plate rheometer (HAAKETM RheoStressTM 100 5 N cm viscometer with a 35-mm diameter, 4° cone). The rheometer's software (HAAKE RheoWin 1.97) is programmed to run at a shear rate of 50.00 s-1 (inverse seconds), which is an infant's typical swallow rate (J. A. Cichero et al., 2000; Stuart & Motz, 2009). RheoWin software can control for multiple variables such as time, temperature, shear rate and number of data points collected per sample.

RECIPES FOR THICKENING BOTTLE FEEDINGS

The thickened infant formula and/or breast milk was initially prepared according to instructions from SLPs on our research team who are employed in the NICU at WCH. These SLPs currently use a 3:1 ratio of formula to oat cereal to achieve a nectar consistency: 15 mL of formula and 5 mL of grain cereal. This ratio was based on experience and their "best guess" to achieve nectar consistency. For research purposes, a digital scale was used to weigh 5 mL of oat cereal to 1.4 grams to maintain a precise measurement of 5 mL. To control for a "level" 5 mL scoop, six students in our lab measured and weighed 5 mL of oat cereal 10 times each. The average across these 60 measurements was 1.4 grams. Weighing the oat cereal provided more exact measurement and ensured that each sample in our study is mixed with the same amount of thickening agent.

Infant formula and breast milk were transferred from their original containers into a 30-mL graduated cylinder using a 10-mL manual pipette. The pipette was calibrated to test the accuracy of its measurements. To do this, 15 mL was measured exactly by extracting 10 mL then 5 mL using the 10-mL pipette then transferred to the 30-mL graduated cylinder to ensure that each sample contains the same amount of liquid. The 30-mL lidded container with infant formula and oat cereal was continuously and vigorously shaken by hand for 10 seconds to ensure that oat cereal particles have been fully mixed throughout the liquid. Samples with breast milk and oat cereal were continuously and vigorously mixed by swirling the container in a circular motion rather than shaking, in order to prevent breakdown of the breastmilk during mixing.

All samples of infant formula and/or breast milk were mixed with oat cereal immediately before viscosity testing so that samples were fresh, because this replicates the mixing procedure used at WCH. Each test run required a 1.5 mL sample, which was placed on the plate of the rheometer using a 10mL manual pipette. After testing, the sample was removed from the plate using Kimwipes. The cone and plate system of the rheometer was rinsed by placing deionized water on the plate and using a Kimwipe to remove all residue from the previous sample. When samples containing breast milk were tested, an ethanol wipe was used to remove any residue.

SETTING TIME

Setting time was controlled for all samples that were collected; however, we did not investigate the effect of setting time. Samples were placed on the rheometer cone and plate system immediately after mixing. We have controlled for setting time by allowing the rheometer to steadily reach 50 inverse seconds for 30 seconds before data collection began. Each test sample was allowed to stabilize for the initial two minutes of data collection, then we used the remaining 30 minutes (i.e., maximum feeding duration) for our data in this study.

TEST PROTOCOL

TABLE 1. Sample size per condition.

CONDITION	Room temperature (25 °C +/- 2 °C)	Body Temperature (37 °C +/- 2 °C)
19 calorie formula	5	5
19 calorie formula with oat cereal	5	5
22 calorie formula	5	5
22 calorie formula with oat cereal	5	5
24 calorie formula	5	5
24 calorie formula with oat cereal	5	5
Breast Milk	5	5
Breast Milk with oat cereal	5	5
Breast Milk with 22 calorie formula and oat cereal	5	5

Samples were tested both at room and body temperature. Although infants at WCH are fed thickened infant formula and breast milk at body temperature, it may cool to room temperature during feeding. Samples were collected at both temperatures in order to assess if there are differences in viscosity between these two temperatures.

Each week, five-minute deionized (DI) water trials were run in order to check the calibration of the rheometer before samples were collected. Frequent calibration was necessary to ensure that the rheometer was working properly. (DI) water was used to calibrate thin liquids and Brookfield 200 cP general purpose oil was used for nectar thick liquids. To calibrate the rheometer, 1.5 mL of DI water or Brookfield 200 general purpose oil was placed on the cone and plate. Samples ran for 32 minutes, with the initial two minutes allowed for the sample to stabilize, to examine how stable samples were over the maximum feeding duration. These samples were consistent over time; therefore, we used elected to reduce the calibration time to only five minutes.

To measure the viscosity of test samples, each trial was programmed to run for 32 minutes in order to account for the initial two-minute ramp time required to reach viscosity equilibrium. Data collection began at two minutes (i.e., 120 seconds) and continued for 30 consecutive minutes, which is the maximum infant bottle-feeding duration at WCH. Data points were collected every 15 seconds. With four data points collected per minute for a 30-minute duration, we collected a total of 120 data points per sample times five trials per condition.

Preliminary results provided information as to whether the current 3:1 ratio of liquid (i.e., infant formula and/or breast milk) and thickening agent (i.e., oat cereal) is truly a nectar consistency. From there, we began to alter this ratio to achieve a true nectar consistency in order to develop a standardized protocol to achieve specific viscosities for nectar consistency bottle feedings at WCH. Since nectar consistency has a wide viscosity range (51-350 cP), we planned to target a low, medium, and high viscosity to provide a variety of standardized nectar thick consistency options for SLPs to use in clinical practice. For example, more oat cereal may need to be added to formula and/or breast milk to increase the viscosity and achieve a higher nectar consistency. The results from this study may provide SLPs at WCH with better understanding of the current ratios used for bottle feeding. The future goal is to provide SLPs at WCH with standard recipes and individualized treatment plans to reduce the risk of aspiration in dysphagic infants.

CHAPTER III: RESULTS

Our initial goal was to perform 10 trials for each of the five thickened liquid conditions under investigation in this study. However, due to unexpected technical and mechanical problems that each took months to resolve, we adjusted our goal to only five trials per condition for timely study completion. Reported sample sizes in other research in this field of study included between three (Garcia, Chambers, Matta, & Clark, 2008) and six (J. A. Cichero, Jackson, Halley, & Murdoch, 2000) trials per condition. Thus, making our sample size of five trials per condition is in line with published literature.

Our first major set-back occurred when the desktop computer controlling the rheometer malfunctioned, which corrupted the data files and did not allow them to be re-opened for data assessment. Software re-installation eventually resolved this issue. The second major set-back was mechanical failure of the rheometer, which required months to successfully resolve. To briefly explain, our rheometer operates pneumatically and was originally powered by in-line compressed air supplied by the building where the rheometer is housed. However, when ambient air becomes compressed, water vapor condensation forms over time. As a result, the internal pneumatic components of the rheometer, unbeknownst to us, became filled with condensation that eventually waterlogged the entire airline and rheometer. We began noticing the rheometer’s rod and cone intermittently stopped turning during testing, which resulted in inaccurate (higher) viscosity measurements. To solve this issue, we disconnected the rheometer from the airline, and then ran compressed nitrogen through the machine overnight to flush out the water. We chose nitrogen because over 70% of ambient air is composed of nitrogen, and unlike ambient air, nitrogen's low reactivity makes it extremely hydrophobic. For this same reason, we converted the rheometer to run on compressed nitrogen (using the same regulated pressure as with compressed air), which prevented further line condensation buildup. After this conversion to nitrogen, the rheometer was successfully recalibrated, and no additional issues arose throughout the remainder of this study. To maintain scientific rigor, only samples collected after this nitrogen conversion step were included in statistical analysis.

A total of 90 samples were tested to answer our research questions: five thickened liquid conditions X five trials X two temperatures (n=50 samples), plus four thin liquid conditions X five trials X two temperatures (n=40 samples). Additionally, numerous calibration trials were run throughout this study, typically on a weekly basis. A single bottle of formula was used for up to two trials of the same condition. A single box of oat cereal was used to thicken all liquids for this study.

For each experimental trial (i.e., sample) and calibration standard, the rheometer software generated report (.txt file) that included centiPoise (cP) and temperature (°C) at 15-second time increments over a 30-minute test period. All data were entered into IBM SPSS (version 23) database for subsequent statistical analysis.

Calibration Results:

Calibration was performed repeatedly throughout the project duration to ensure reliability of viscosity measurements using two standards: deionized (DI) water and a 200 cP oil-based liquid (Brookfield 200). DI water consistently clustered around the expected viscosity range of 1 cP for the 30-minute test run duration, at both body and room temperatures. The 200 cP calibration standard consistently clustered tightly around 200 cP at room temperature and 100 cP at body temperature; both remained stable for the entire 30-minute test run. These calibration results confirmed that our rheometer was producing reliable viscosity data for this study. Representative calibration results for room temperature are shown in **FIGURE 1**.

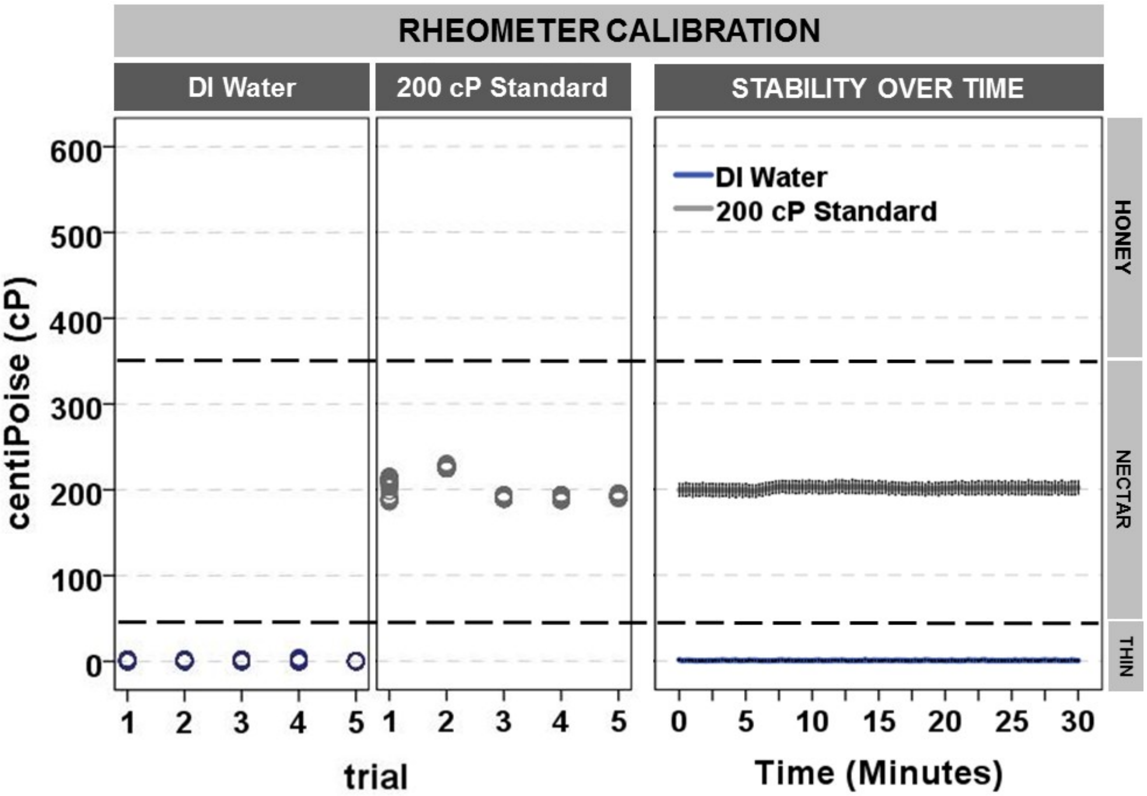

FIGURE 1: Rheometer calibration. Two different liquids of known viscosity were used for system calibration at room temperature: distilled water (1 cP) as a *thin liquid standard*, and a Brookfield 200 cP oil-based solution as a *nectar thick liquid standard*. Results are shown for representative trials collected throughout the study period. **Left:** 5 individual trials are shown for each calibration fluid. **Right:** Average viscosity for each of the 5 trials over the 30-minute test run. Viscosity measurements were tightly clustered around the expected viscosities for each standard and remained stable over 30 minutes. Dashed lines indicate the viscosity ranges established by the NDD for thin, nectar, and honey thick liquids.

Unthickened Liquid Results: The viscosity of unthickened formula and breast milk was measured at room and body temperature before proceeding to thickened liquid conditions. As expected, all unthickened liquids were in the thin liquid viscosity range (1-50 cP) established by the NDD, regardless of temperature (**FIGURE 2**).

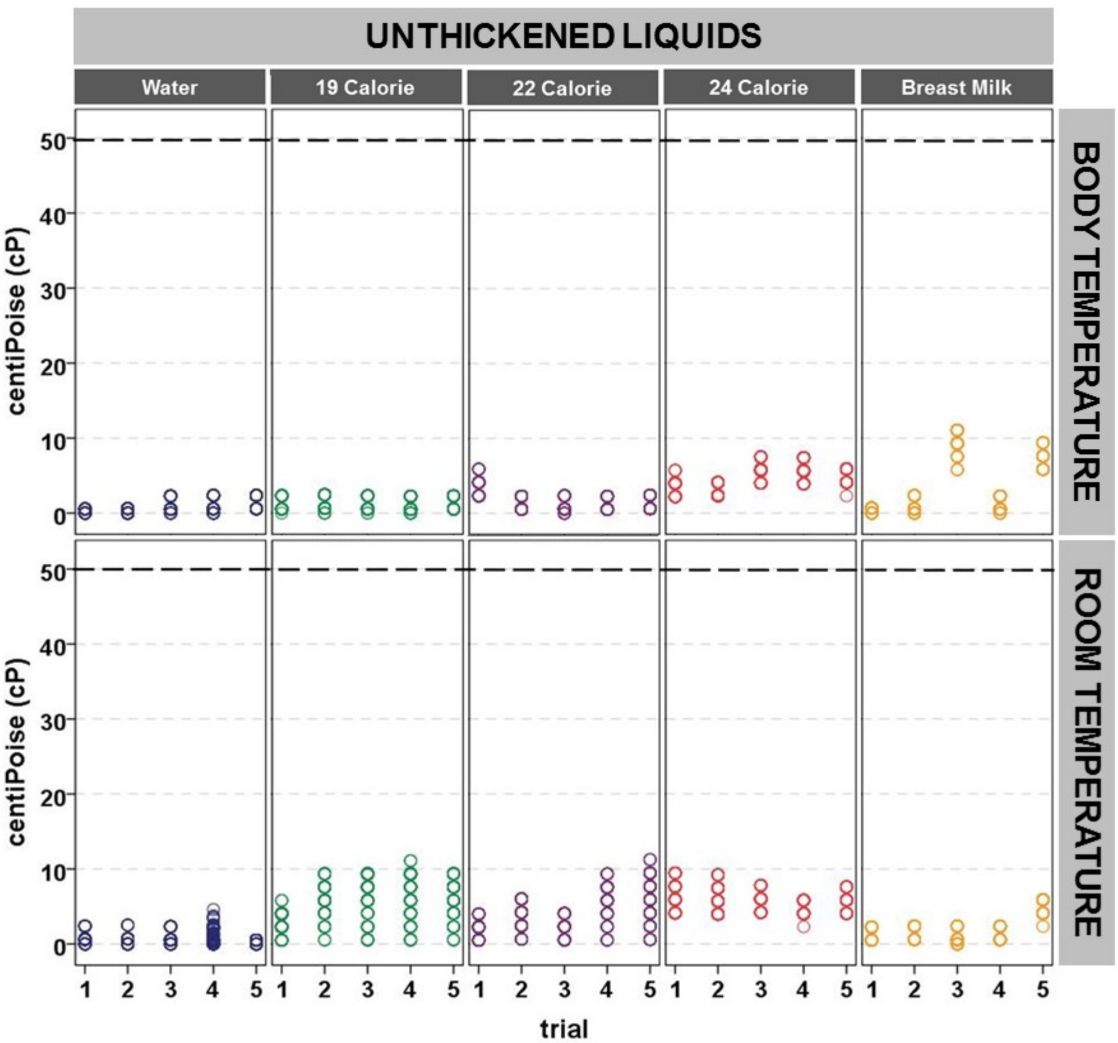

FIGURE 2: Individual trial data for thin liquids at body versus room temperature. The 120 data points recorded during each 30-minute trial are shown for water (control) and the 4 thin liquids routinely used at our hospital to create thickened bottle feedings: 19, 22, and 24 calorie infant formula and breast milk. The dashed line at 50 cP indicates the high viscosity limit established by the NDD for thin liquids. All trials, regardless of temperature, remained within the thin liquid viscosity range. Room temperature formulas had higher viscosities than formula tested at body temperature. In contrast, breast milk had higher viscosity measurements at body temperature compared to room temperature.

Research Question 1: Do the thickened liquid recipes (3:1 ratio) currently used at WCH truly fall within the nectar viscosity range (51- 350 cP) at body temperature?

When comparing the mean viscosities of the five conditions, only one (breast milk + 22-calorie formula) was *not* within the nectar thick viscosity range established by the NDD (51 - 350 cP), as shown in **FIGURE 3**. In this graph, a dichotomy between the three thickened infant formula conditions (19, 22, and 24 calorie) and the two breast milk conditions was obvious. Specifically, the viscosity of the two thickened breast milk conditions (i.e., with and without 22 calorie formula) was significantly lower than the three infant formula conditions ($p<0.0001$, one-way ANOVA comparing mean viscosity across formula versus breast milk grouping conditions). Whereas the mean viscosity of the three thickened infant formula conditions fell near the middle of the nectar thick range, thickened breast milk was closer to the lower limit of the nectar range, and thickened breast milk + 22-calorie formula fell within the *thin* liquid range. In addition, a significant difference in mean viscosity was found across the five conditions ($p<0.0001$, one-way ANOVA), with post-hoc t-tests revealing that each condition was significantly different from the others ($p<0.05$). The following high to low rank order was identified, based on mean viscosities of the five thickened liquid conditions: 24 calorie (252 cP), 19 calorie (238 cP), 22 calorie (210 cP), breast milk (71 cP), and breast milk + 22 calorie (31 cP).

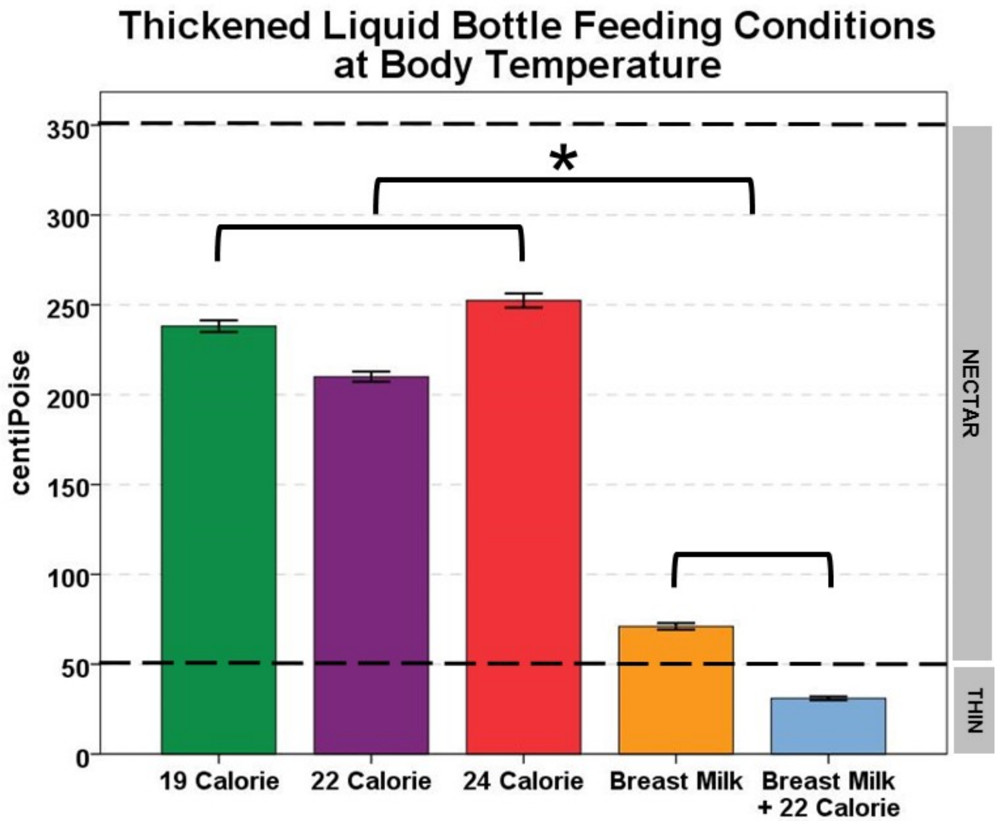

FIGURE 3: Mean viscosity of thickened liquid bottle feeding conditions at body temperature. All samples were thickened using a 3:1 ratio of liquid to oat cereal. Dashed lines at 51 and 350 cP indicate the low and high viscosity limits established by the NDD for nectar thick liquids. The three thickened infant formula conditions (19, 22, and 24 calorie) had a significantly higher viscosity compared to the two breast milk conditions (p<0.0001, asterisk). Whereas the mean viscosity of the three thickened infant formula conditions was well within the nectar thick range, the mean viscosity of thickened breast milk was near the lower limit of the nectar range. The mean viscosity for thickened breast milk + 22-calorie formula was within the *thin* liquid range. Error bars represent ±1 standard error of the mean.

A much different, and perhaps more clinically relevant, story emerged when comparing the viscosity values of the five *individual trials* (rather than the mean viscosity) for each thickened liquid condition at body temperature, as shown in **FIGURE 4**. Of the 15 thickened liquid trials across the 19, 22, and 24 calorie infant formula conditions, seven (i.e., nearly half) exceeded the nectar thick viscosity *upper limit* (350 cP) set by the NDD. In addition, 8 of the 10 trials (i.e., 80%) across the two thickened breast milk conditions fell below the nectar thick viscosity *lower limit* (51 cP) set by the NDD. Descriptive statistics for the maximum and minimum viscosity values for the five individual trials across the five thickened liquid conditions are summarized in **TABLE 2**. Visualization of the raw data in this manner demonstrates the wide variation in viscosity measurements within and between trials of the same condition, as well as across conditions. Based on this individual trial data, it can be concluded that the 3:1 ratio of liquid and oat cereal at body temperature does not consistently meet the NDD viscosity criterion for nectar thick liquid for any of the five-thickened liquid bottle-feeding conditions currently in use at our hospital.

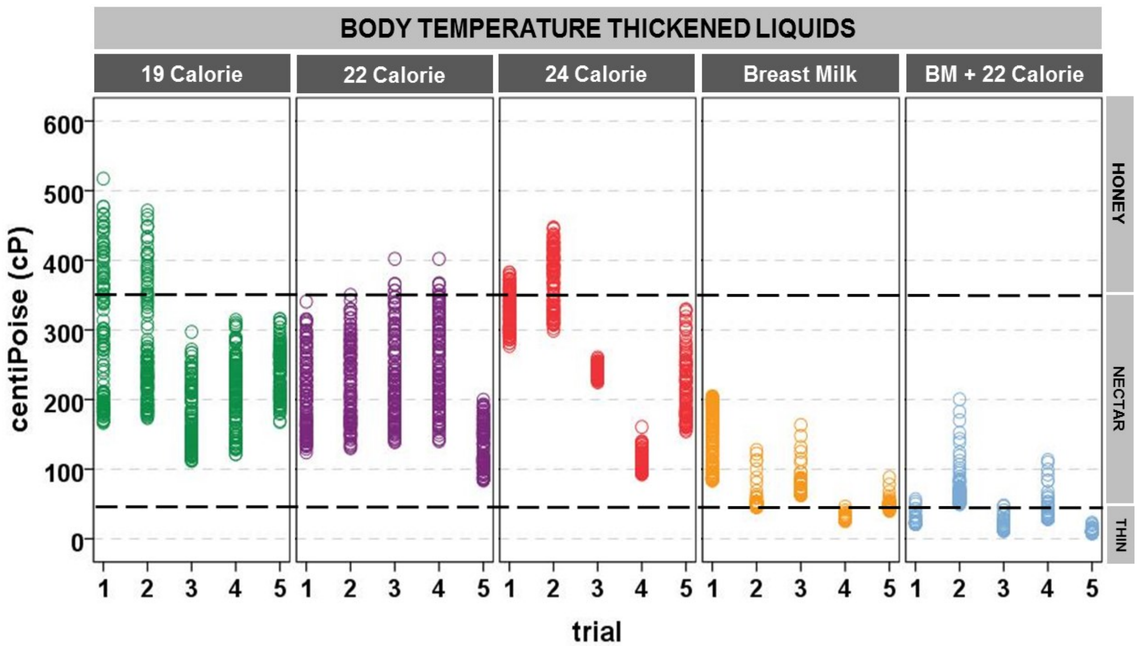

FIGURE 4: Individual trial data for thickened liquids at body temperature. The 120 data points recorded during each 30-minute trial (605 per condition) are shown for the five-thickened liquid bottle-feeding recipes at body temperature. All samples were thickened using a 3:1 ratio of liquid to oat cereal. Dashed lines at 51 and 350 cP indicate the low and high viscosity limits established by the NDD for nectar thick liquids. Nearly 50% of the trials for the three thickened infant formula conditions (19, 22, and 24 calorie) exceeded the *upper limit* of the nectar thick viscosity range, whereas 80% of the thickened breast milk trials fell below the *lower limit* of this range. BM = breast milk.

Table 2. Descriptive statistics for individual trials of the five thickened liquid conditions at body temperature

Thickened Liquid Conditions	Trial	Minimum Viscosity (cP)	Maximum Viscosity (cP)	Range (cP)	Average (cP)	StdDev
19 Calorie Formula	1	166.5	517.3	350.8	290.9	98.2
	2	173.5	471.4	297.9	173.7	45.5
	3	112.0	297.1	192.6	211.2	49.8
	4	121.7	314.3	147.9	240.2	35.6
	5	167.8	315.7	302.1	379.6	77.8
22 Calorie Formula	1	124.2	340.7	216.5	208.9	55.3
	2	130.1	350.3	220.2	223.2	58.1
	3	138.7	366.3	227.6	228.5	63.1
	4	140.3	402.1	261.8	250.1	64.9
	5	83.89	199.9	115.7	133.9	31.4
24 Calorie Formula	1	276.9	382.4	105.5	326.9	27.6
	2	298.9	447.2	148.3	373.0	41.7
	3	224.7	260.4	35.7	241.1	7.5
	4	92.4	160.4	68.2	109.8	11.7
	5	154.3	329.5	175.2	211.3	44.6
Breast Milk	1	84.3	204.8	120.5	144.1	41.9
	2	45.9	127.4	81.5	53.6	14.1
	3	62.7	163.4	100.7	78.8	15.3
	4	25.1	46.3	21.2	29.8	2.6
	5	39.9	88.2	48.3	48.9	5.9
Breast Milk + 22 Calorie Formula	1	21.6	56.7	35.2	24.4	5.8
	2	49.9	200.3	150.3	68.5	26.7
	3	11.1	48.0	36.9	15.9	6.2
	4	28.8	113.3	84.5	36.1	15.8
	5	7.4	22.9	15.6	10.2	2.2

Color coding indicates data out of nectar viscosity range: Red indicates honey viscosity and orange indicates thin viscosity. cP = centipoise, StdDev = standard deviation.

Research Question 2: How stable are the WCH thickened liquid recipes at body temperature throughout a 30-minute test time?

To investigate the stability of the five thickened liquid conditions at body temperature over time, we graphically displayed the mean and variance (± 1 standard error of the mean) of each condition over the 30-minute test duration (**FIGURE 5**), which corresponds with the maximum bottle-feeding duration for dysphagic infants at our hospital. At the start of testing, the mean viscosity for each of the three thickened infant formulas was near the mid-level of the nectar range, but then gradually increased (i.e., thickened) over the 30-minute test duration, eventually exceeding the upper limit of the nectar viscosity range established by the NDD. In contrast, the mean viscosity for each of the two thickened breast milk conditions started below mid-level of the nectar range, and then steadily declined (i.e., thinned) for approximately the first six minutes of testing. After this point, breast milk slowly increased in viscosity for the remainder of the 30-minute test duration, whereas breast milk + 22 calorie formula (remained remarkably stable, albeit in the thin liquid (instead of nectar) viscosity range.

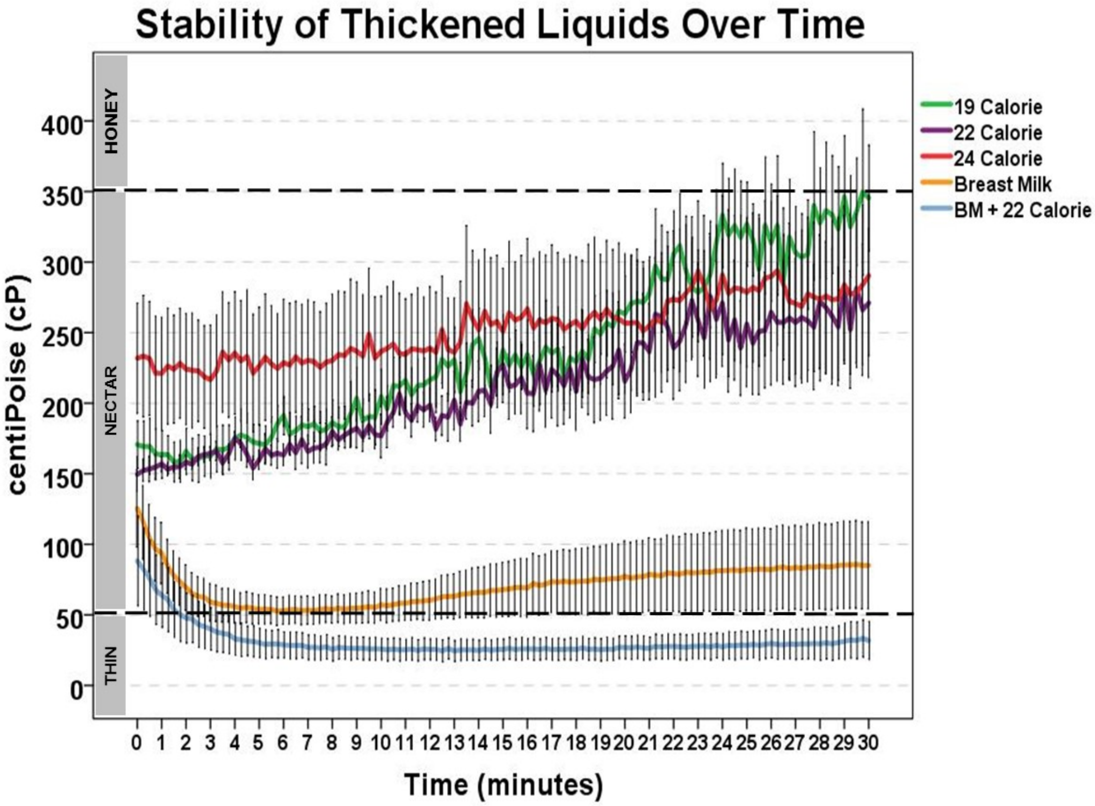

FIGURE 5: Sample variability of all thickened liquid conditions at body temperature. Colored lines represent the mean viscosity (centiPoise) of the five different thickened liquid conditions over time (30 minutes), with error bars representing +/- 1 standard error of the mean at each 15-second data collection interval. Dashed lines indicate the viscosity ranges established by the NDD for thin, nectar, and honey thick liquids. Note that thickened breast milk + 22-calorie formula has the lowest variability (i.e., highest stability) over the 30-minute test run compared to the other four thickened liquid conditions. BM = breast milk; 19, 22, and 24 calorie indicates the caloric density of the corresponding infant formula.

To quantify the change in viscosity over time, we used only the start and end viscosity values to compute the change scores and corresponding percent change for individual trials within each of the five thickened liquid conditions at body temperature, as shown graphically in **FIGURE 6** and summarized in **TABLE 3**. Change scores were calculated by taking the difference between start and end viscosity values. The percent change in viscosity was calculated using the following formula: ((end viscosity – start viscosity) / start viscosity) X 100. The absolute value of the percent change was then used for rank ordering the five conditions relative to stability of viscosity over time, where 1 is the most stable and five is the least stable, as summarized in **TABLE 4**. The least stable condition over the 30-minute test run was 19-calorie formula (105% change in viscosity), and the most stable was 24-calorie formula (24% change in viscosity). Between these bookends were 22-calorie formula (89% change), thickened breast milk + 22-calorie formula (65% change) and thickened breast milk (36% change).

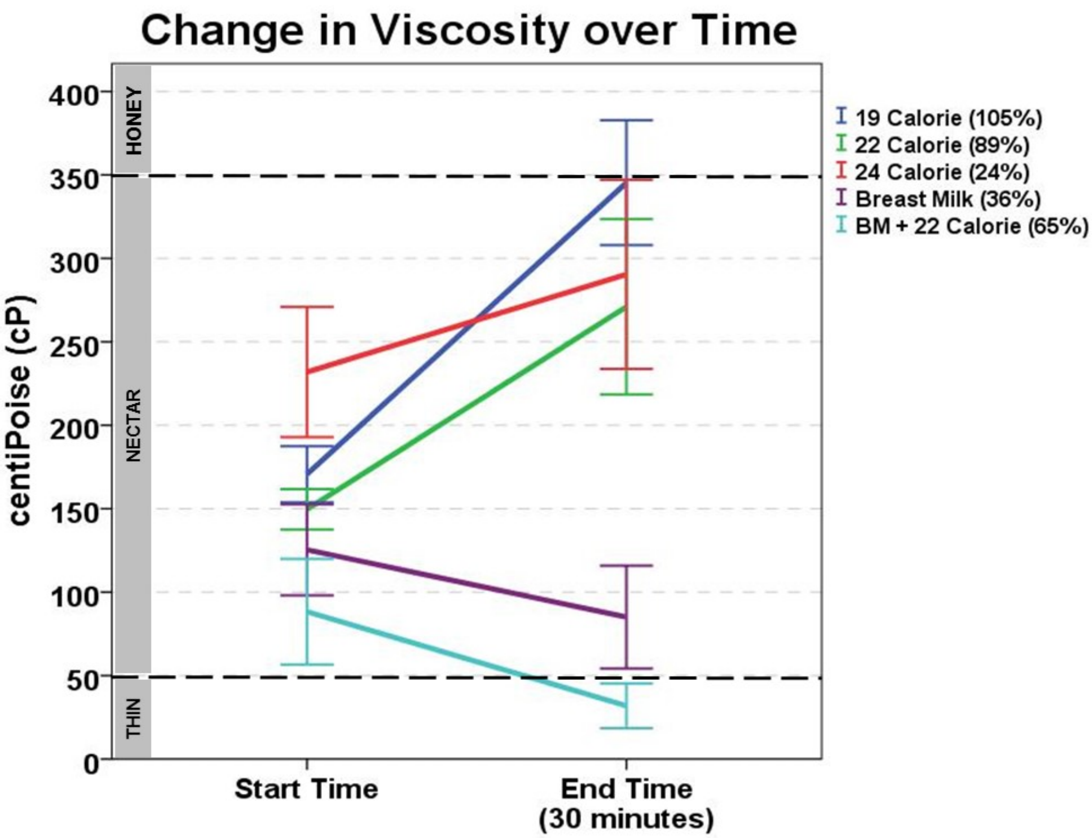

FIGURE 6. Change in thickened liquid viscosity over time at body temperature. Sample stability was determined by comparing the average viscosity (centiPoise) values for each recipe at the start and end time for each 30-minute test run. Dashed lines indicate the viscosity ranges established by the NDD for thin, nectar, and honey thick liquids. Error bars represent +/- 1 standard error of the mean. BM = breast milk; 19, 22, and 24 calorie indicates the caloric density of the corresponding infant formula; % = percent change (absolute value) in mean viscosity start versus end times for each thickened liquid condition.

TABLE 3. Change scores and percent change for individual trials of the five thickened liquid conditions at body temperature

Thickened Liquid Conditions	Trial	Start cP	End cP	Change Score (cP)	Percent Change
19 calorie Formula	1	182.8	466.5	283.7	155.2
	2	201.7	386.9	185.2	91.1
	3	124.6	248.1	123.5	99.1
	4	137.5	310.2	172.7	125.6
	5	206.7	315.0	108.3	52.4
22 Calorie Formula	1	124.2	308.2	184	148.1
	2	120.1	307.4	177.3	136.3
	3	145.9	402.1	256.2	175.6
	4	192.9	253.4	60.8	31.6
	5	155.5	83.9	-71.6	-46.0
24 Calorie Formula	1	305.3	373.8	68.5	22.4
	2	322.3	435.6	113.3	35.2
	3	237.2	229.9	-7.3	-3.1
	4	111.8	110.3	-1.5	-1.3
	5	182.9	302.4	119.5	65.3
Breast Milk	1	201.5	203	1.5	0.7
	2	127.4	52.9	-74.5	-58.5
	3	163.4	87.0	-76.4	-46.8
	4	46.3	30.4	-15.9	-34.3
	5	88.2	52.1	-36.1	-40.9
Breast Milk + 22 Calorie Formula	1	56.8	21.6	-35.2	-62.0
	2	200.3	83.6	-116.7	-58.3
	3	48.0	14.6	-33.4	-69.6
	4	113.3	30.6	-82.7	-73.0
	5	23	9.1	-13.9	-60.4

Color coding indicates data out of nectar viscosity range. Red indicates honey viscosity and orange indicates thin viscosity; cP = centiPoise

Table 4: Percent change in mean viscosity and stability rank order for the five thickened liquid conditions at body temperature

Thickened Liquid Conditions	Percent change in mean viscosity (absolute value)	[1]Stability rank order
19 Calorie Formula	105%	5
22 Calorie Formula	89%	4
24 Calorie Formula	24%	1
Breast Milk	36%	2
Breast milk + 22 Calorie Formula	65%	3

[1]Stability rank order: 1 = most stable; 5 = least stable

Research Question 3: Is viscosity significantly higher for thickened liquid recipes at room temperature compared to body temperature?

To answer this question, we performed a one-way analysis of variance (ANOVA) comparing each thickened liquid recipe across temperature (room versus body), followed by post-hoc t-tests with Bonferroni correction for multiple comparisons. As shown in **TABLE 5**, temperature significantly affected the viscosity of all five thickened liquid conditions. The three thickened infant formula conditions had a significantly higher mean viscosity (i.e., were thicker) at body temperature compared to room temperature. In contrast, the two thickened breast milk samples (i.e., with and without 22-calorie formula added) had a significantly lower mean viscosity (i.e., were thinner) at body temperature compared to room temperature samples. These opposing findings are shown graphically in **FIGURE 7** for added clarity.

TABLE 5. The effect of temperature on mean thickened liquid viscosity over time.

Condition	Temperature	n	Data points	Mean	F	*p* value	Direction of effect
19 Calorie Formula	Room	5	605	196.8	112.3	.0001	B > R
	Body	5	605	238.1			
22 Calorie Formula	Room	5	605	137.0	474.1	.0001	B > R
	Body	5	605	210.0			
24 Calorie Formula	Room	5	605	238.6	10.9	.001	B > R
	Body	5	605	252.4			
Breast Milk	Room	5	605	137.2	267.8	.0001	**B < R**
	Body	5	605	71.9			
Breast Milk + 22 Calorie Formula	Room	5	605	115.3	374.4	.0001	**B < R**
	Body	5	605	31.0			

B = body temperature (37 ± 2 °C); R = room temperature (25 ± 2 °C); n=sample size; Data points = number of viscosity measurements per 30-minute trial; F = F statistic; Direction of temperature effect: bold text denotes opposing direction for breast milk samples compared to formula alone.

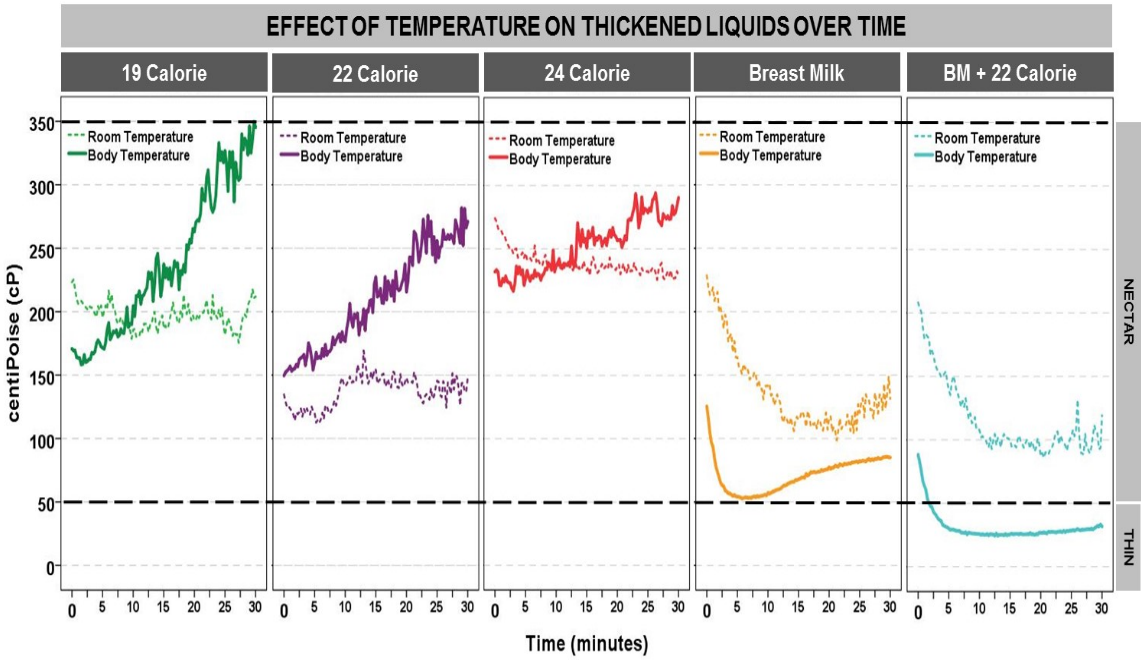

FIGURE 7. Effect of temperature on thickened liquids over time. These graphs show the comparison of the mean viscosity at room versus body temperature over time for each of the five conditions. The three thickened infant formulas (19, 22, and 24 calorie) were significantly more viscous at *body temperature*, while the two thickened breast milk conditions were significantly more viscous at *room temperature*.

Individual trials for each of the five thickened liquid conditions are shown in **FIGURE 8**. The most obvious finding here was that the viscosity of thickened infant formulas was not only lower, but it became more stable (i.e., less variable) within and across trials at *room temperature*. As a result, all 15 trials remained within the nectar thick consistency range. However, the opposite effect occurred for thickened breast milk conditions. Specifically, room temperature resulted in not only a higher viscosity for thickened breast milk conditions, but they also became less stable (i.e., more variable) within and across trials at room temperature. Thus, thickened infant formulas are more stable at room temperature, whereas thickened breast milk conditions are more stable at body temperature.

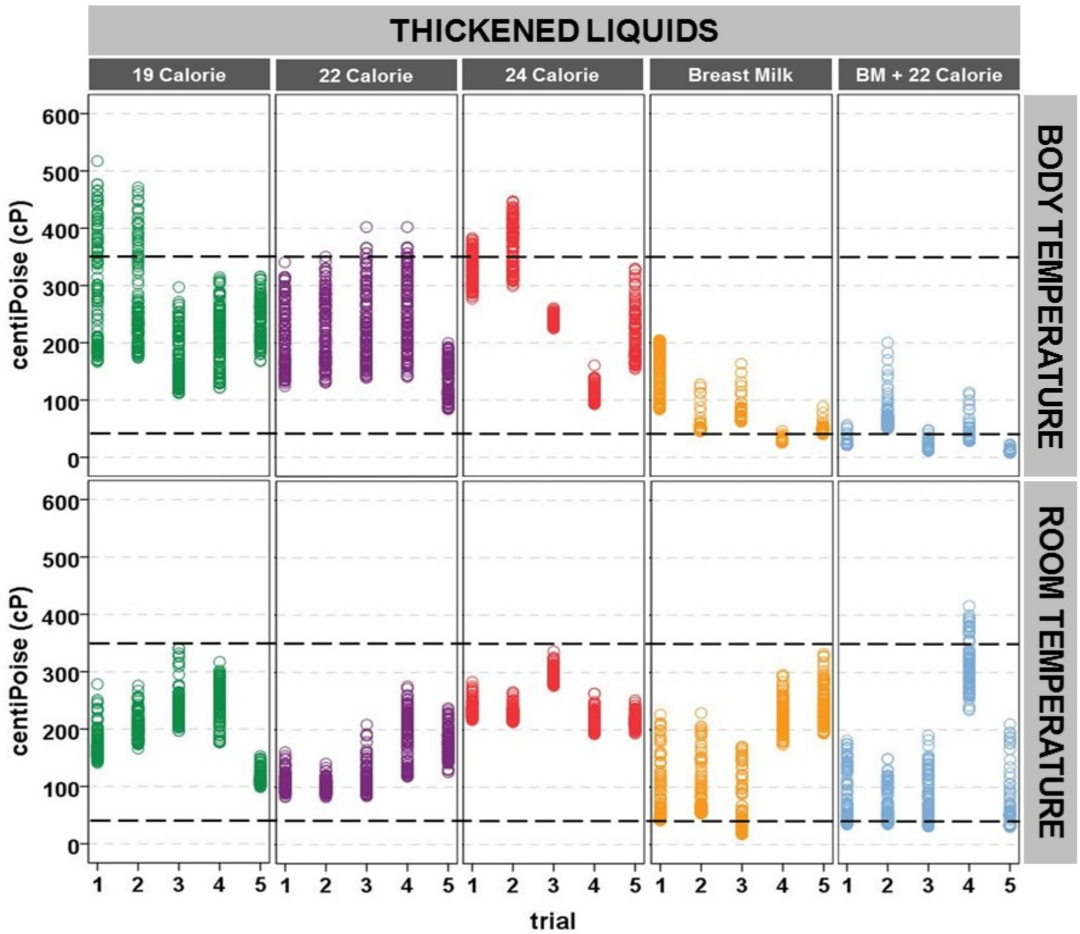

FIGURE 8. Comparison of individual trials of the five thickened liquid conditions at room and body temperature. Individual trial data is displayed for each of the five thickened liquid conditions. Dashed lines indicate the high and low nectar viscosity limits.

Research Question 4: Based on the findings from the three research questions above, what modifications to the 3:1 ratio of liquid (i.e., infant formula and/or breast milk) and oat cereal will be necessary to achieve a low, middle, and high viscosity level within the nectar consistency range (51-350 cP) to facilitate individualized treatment planning for dysphagic infants?

Based on the variability in viscosity measures that we identified within and between trials for each thickened liquid condition, we expect it may be difficult to target three different viscosity levels within the nectar range for each condition. However, targeting two distinct nectar viscosities that divide the nectar consistency range in half may be more feasible; specifically, low (51 - 200 cP) versus high (201 - 350 cP) nectar viscosity levels.

Given our finding that 24-calorie formula was the most stable of the five thickened liquid conditions over time at body temperature, we are now focusing our efforts on recipe modifications using only 24-calorie formula. To do this, we are adjusting the amount of oat cereal that is added to 15 mL of formula. For the thickened 24-calorie formula condition, the viscosity began in the high nectar thick range but increased into the honey thick range over time. Thus, to prevent thickened 24-calorie formula from becoming too thick, we expect that very little oat cereal will need to be removed from the current 3:1 ratio to maintain the viscosity in the high nectar range. Even more oat cereal would need to be removed from this ratio to maintain the viscosity in the low nectar range.

Modifications to thickened breast milk should also be considered. Given that three of the five thickened breast milk trials fell slightly below the nectar limit into the thin liquid range, we propose that only a small increase in the amount of oat cereal is needed to consistently maintain a low nectar range. However, to reach a high nectar range, a much larger amount of oat cereal will need to be added to breast milk. For modifications to the thickened breast milk + 22-calorie formula condition, we expect that substituting 24-calorie formula is a logical solution to achieve a more stable low nectar range, instead of adding more oat cereal. This approach takes advantage of the inherent stability of 24-calorie formula over other caloric density formulas. However, to achieve a high nectar range with breast milk + 24-calorie formula, additional oat cereal will be needed. We expect several iterations of modification will be necessary to establish stable low and high levels of nectar thick liquid for both breast milk conditions (i.e., with and without 24-calorie formula).

CHAPTER IV: CONCLUSION

There are currently no standardized recipes for speech-language pathologists (SLPs) to use when providing thickened liquid bottle feedings to treat infants with dysphagia. SLPs at our hospital currently use a 3:1 ratio of infant formula and/or breast milk to oat cereal, which is mixed as a volume of 30 to 180 milliliters or more for a single bottle feeding, depending on the weight and nutritional needs of each infant. Without rheological equipment readily available for clinical use, our SLPs have relied on their best clinical judgment to establish this ratio, which they hypothesize produces thickened bottle feedings that fall within the nectar thick viscosity range (51- 350 cP) established by the NDD. The main purpose of this study was to use a cone and plate rheometer to objectively quantify the viscosity of the five-thickened bottle-feeding conditions currently in use at our hospital: infant formulas (19, 22, and 24 calorie) and breast milk (alone and a 50:50 mixture with 22-calorie formula) in a 3:1 ratio with oat cereal. These thickened bottle feedings are always warmed to body temperature immediately before use and then administered during a 30-minute maximum feeding time. Therefore, a second goal of this study was to determine the effect of feeding time on viscosity outcomes for each thickened bottle-feeding condition in use at our hospital. A third goal was to determine the effect of temperature on viscosity outcomes, as we expect that bottle feedings may cool to room temperature during the 30-minute feeding time. Therefore, we used body (37 ± 2 °C) and room (25 ± 2 °C) temperature as bookends for this purpose. Our final goal was to determine what modifications to our current 3:1 ratio of liquid and oat cereal are needed to achieve a low and high nectar thick viscosity range for better individualized treatment plans for infants with dysphagia.

For each of the five thickened liquid conditions, we collected viscosity measurements at 15 second intervals over a 30-minute test run using a shear rate of 50/s. Initially, we hypothesized that the thickened infant formula conditions and would fall within the nectar thick range and the thin liquid viscosity range respectively; however, when considering the *average* viscosity measurements for each of the five conditions, *all but one* (breast milk + 22-calorie formula) was within the nectar thick range established by the NDD (51 - 350 cP). However, results based on *individual trials* (rather than the mean viscosity) revealed much different, and perhaps more clinically relevant, information. Specifically, nearly half of the thickened infant formula trials rose *above the upper limit* of the nectar thick range (i.e., into the honey thick range), whereas 80% of thickened breast milk trials fell *below the lower limit* for nectar thick liquid (i.e., into the thin liquid range).

When considering the effect of time, thickened infant formula conditions did not remain in within the nectar think viscosity range for 30 minutes, in fact, nearly all increased into the honey thick viscosity range over time. We had hypothesized that thickened infant formulas conditions would not remain stable over a 30-minute test run, which was proven true by the gradual increase of all thickened infant formula conditions. Although, all thickened infant formula conditions increased over time, 24-calorie infant formula remained the most stable over time. Furthermore, we expected that thickened breast milk conditions would become thinner (i.e., less viscous) over time due to the salivary amylase found in breast milk that degrades thickening agents. On *average*, thickened breast milk conditions decreased into the thin liquid viscosity range.

On *average*, thickened breast milk conditions had a drastic decrease in viscosity for the first six minutes before these conditions stabilized. We hypothesize this lag in stability could be due to salivary amylase found in breast milk, which is known to degrade thickening agents (Almeida et al., 2011). From preliminary data, we suggested to clinicians at WCH that thickened breast milk conditions only be given for six minutes and then a new thickened bottle be given because after six minutes thickened breast milk decreased into the thin liquid range. To increase length and viscosity of thickened breast milk bottle feedings, we propose that once adjustments are made to the current ratio of thickened breast milk conditions, they be allowed at least six minutes to stabilize before bottle feeding begins.

Another goal of this study was to determine the effect of temperature on thickened liquid conditions. Our results contradicted our hypothesis that liquids at room temperature will be more viscous than thickened liquids at body temperature. For this study, the inverse relationship (i.e., as temperature increases, viscosity decreases) did not hold true for thickened infant formula conditions. Temperature significantly affected the viscosity of all five thickened liquid conditions. The three thickened infant formula conditions had a significantly higher mean viscosity (i.e., were thicker) at body temperature compared to room temperature. In contrast, the two thickened breast milk samples (i.e., with and without 22-calorie formula added) had a significantly lower mean viscosity (i.e., were thinner) at body temperature compared to room temperature samples.

Currently, all bottle feedings at WCH are provided at body temperature. However, room temperature thickened infant formula conditions are more stable and therefore should be considered by clinicians. Thickened breast milk conditions were more stable at body temperature and therefore, should continue to be given at body temperature with the adjustments of oat cereal volume made to maintain low and high nectar viscosity ranges. Due to the temperature effect on thickened liquid conditions, clinicians should also discuss with families whether body or room temperature feedings will be provided after hospital discharge. Since there is variance in room and body temperature of samples, this indicates the need to better understand the interaction between temperature, viscosity, and time.

Our final goal was to determine new recipes to achieve a low and a high nectar viscosity range. Based on results from this study, 24-calorie infant formula was the most stable over a 30-minute feeding duration. Therefore, initial medication should be made using 24-calorie infant formula compared to lower caloric densities due to the stability when thickened. Modifications need to be made in order to achieve a nectar thick range for both thickened infant formula and breast milk conditions. A good starting point would be to maintain 15 mL of 24-calorie formula and reduce oat cereal from 1.4 grams to 1.25 grams to determine if this modification maintains a high nectar consistency throughout the entire 30-minute run. To achieve a low nectar consistency for an entire 30-minute duration, oat cereal could be reduced to 1.0 gram as a starting point, followed by further adjustments as needed. For thickened breast milk, we are increasing oat cereal to 1.5 grams for a low nectar viscosity and 2.0 grams for a high nectar viscosity range. Thickened breast milk + 24-calorie infant formula is also being tested. We expect with these modifications to the current 3:1 ratio of liquid to oat cereal, will provide better results for our final research question to achieve two distinct nectar viscosities.

In summary, the findings from this study demonstrate that individual trials of thickened liquid conditions using our current 3:1 ratio of infant formula and oat cereal do not consistently maintain a nectar thick viscosity. Thickened infant formula that exceeds the nectar thick viscosity range may currently be placing infants at risk for increased feeding fatigue and caloric expenditure from overzealous sucking to express thickened liquid from the bottle nipple. In contrast, thickened breast milk conditions may place infants at a continued risk for aspiration because these conditions do not sufficiently maintain a nectar thick viscosity for 30 minutes; instead, falls within the thin liquid viscosity range. To achieve and maintain an appropriate nectar thick consistency, feeding times may need to be altered. As an example, breast milk conditions remained within the nectar thick viscosity range for the first six minutes of data collection before falling into the thin liquid viscosity range. Therefore, bottle feedings could be stopped at six minutes, then a new bottle could be made in order to ensure a nectar thick range is maintained for the duration of feeding.

LIMITATIONS

We were unable to provide a complete answer to our fourth research question pertaining to ratios needed to achieve a low and high viscosity level within the nectar viscosity range. However, our preliminary findings provided a logical starting point that focuses on 24-calorie formula (with and without breast milk), due to its stability advantage over other conditions. Our goal is to establish ratios for low and high viscosity levels for infant formula (with and without breast milk) within the nectar consistency range throughout a 30-minute viscosity range.

We are currently targeting a low and a high nectar viscosity range established by the NDD. However, viscosity ranges are not backed by evidence-based research. Although the nectar thick viscosity range is our intended range, it is arbitrarily set by the NDD and insufficient evidence exists to prove this range is thick enough to prevent dysphagia in infants.

While we strived to control numerous variables during this research project (e.g., precise measurements of liquid volume, oat cereal weight, temperature, run time, and shear rate), we recognize this may be impossible in clinical practice. Therefore, we have also been collecting data with less stringent controls to determine the impact on viscosity outcomes when compared to our well-controlled data. For example, we have run several samples at ambient room temperature (e.g., 20 and 22 °C), rather than setting the rheometer temperature control to 25 ±2 °C. Results will provide insight for which variables truly need to be strictly controlled in clinical practice.

One variable that was not controlled for this study was room humidity, which typically ranged from 30% to 60%, depending upon season and associated heating ventilation and air conditioning (HVAC) system settings. It is entirely likely that humidity fluctuations may have affected our viscosity data, as reported by published research outside of this field (Murakami et al., 2015). However, previous research in our field has not yet investigated humidity as a factor in viscosity measurements for thickened liquids. Although the effect of humidity on thickened liquids was not specifically investigated in our study, it is certainly worthy of future investigation.

Another source of variability pertains to the multiple students who ran the samples for this study. Therefore, inter-experimenter error may be contributing to sample variability. For example, it is highly unlikely that each student pipetted the 1.5 mL sample from the exact same location within the 30 mL container for loading on the rheometer plate.

From this study, we have evidence that temperature greatly impacts the viscosity level of thickened liquid conditions. We only looked at the two extreme bookends of temperature (i.e., room and body temperature) and these two temperatures were held constant throughout the 30-minute test run. In a clinical setting, temperature cannot be held constant. A bottle feeding that begins at body temperature will most likely decrease towards a room temperature over a 30-minute feeding duration. We did not investigate the effects of a decreasing temperature on thickened liquid condition's viscosity.

In this study we only tested trials at a controlled shear rate of 50/s, as that is considered the "industry standard" in our field. However, we fully recognize that outcomes may be very different when using other shear rates. Systematic investigation of the effect of shear rate on thickened bottle feedings has not yet been performed, and will certainly be a focus of our future studies, now that we have established the methodology in our lab to do so.

FUTURE DIRECTIONS

The Lever Lab is currently conducting research to change the ratios infant formula and/or breast milk to oat cereal in order to best make sample conditions that will fit the needs of infants at WCH. We are experimenting with 24-calorie infant formula for our initial recipes because it has the least change in viscosity over time. Specifically, we are testing 24-calorie infant formula, with and without breast milk, and varying the amount of oat cereal added per 15 mL liquid sample. This approach will allow us to determine the effect of increasing or decreasing the amount of thickener on the stability of each liquid. Stability must first be measured before new ratios of liquid and oat cereal can be suggested for clinical use with dysphagic infants. By first stabilizing each thickened liquid sample condition to the best of our ability by reducing the amount of oat cereal, there will be less variation in each sample, which will improve the safety of thickened bottle feedings provided to dysphagic infants at WCH by different clinicians and staff. Based on our results, all of the thickened liquid recipes demonstrated large variation; however, it is not yet determined what the clinical significance of variability is within an individual sample. Once our recipes based on 24-calorie infant formula targeting a low versus high level within the nectar consistency viscosity range are developed, we expect to submit a manuscript to the *Dysphagia Journal* to provide much needed evidence-based research on thickening liquids for infant use.

Further research needs to be conducted to study the physiological effects of room temperature bottle feedings. That is, what is the effect on the infant of swallowing a room temperature bottle compared to swallowing liquid that has already been warmed to body temperature? While WCH currently warms all liquids to body temperature before a feeding, the results of our study suggest a room temperature advantage – thickened liquids made with infant formula remain more stable over a 30-minute bottle feeding duration at room temperature than do body temperature liquids. Future studies should also determine the effect of a decreasing temperature throughout the duration of the 30-minute test run to replicate what truly happens to viscosity during an infant bottle feeding.

While the focus of this study was on nectar thick viscosity, WCH also thickens bottle feedings to a honey thick viscosity. Future studies should focus on ratios needed to achieve different viscosity levels of honey consistency that are compatible with bottle feedings. Honey thick liquid must still be able to flow through the bottle nipple without causing fatigue or unnecessary caloric expenditure for an infant as many infants with dysphagia are premature and therefore weight growth and development are necessary factors for discharge from the hospital.

Results from this study indicate that both thickened breast milk recipes are fairly stable over time; however, these recipes had an abrupt decrease in viscosity for approximately the first six minutes after mixing. This finding provides rationale for allowing thickened breast milk to sit for six minutes before beginning bottle feedings. However, a larger sample size would provide more data to best address the initial instability of thickened breast milk and to investigate the sample variability within and between donors. Such information is essential to developing standardized recipes for thickened bottle feedings for use in clinical practice. Another direction of interest for the Lever Lab is to develop methodology to naturally thicken breast milk bottle feedings for added stability. We propose to centrifuge breast milk to separate the thinner liquid from the solid particulates. Decanting the thin liquid supernatant would then leave a higher viscosity breast milk that would require less oat cereal to achieve nectar or honey thickness.

In order for viscosity measurements to be applicable in clinical practice, one option would be to use commercially available ”laboratory viscometers”. However, these devices require large sample volumes (typically >100 mL), cost several thousands of dollars, and are not equipped with consumable supplies to prevent contamination of samples that will be ingested by patients. An ideal solution would be development of a low-cost option viscometer designed for smaller sample volumes (i.e., congruent with bottle feedings) and single-patient use consumables (i.e., spindle sheath) for infection control in clinical settings. Such a device would allow for clinicians and other infant feeding staff to directly test the viscosity of each thickened bottle feeding immediately before it is provided to the infant. It would also provide immediate feedback to clinicians regarding the current versus target viscosity range. This actionable information would inform clinicians as to alterations to make, such as adding more oat cereal to increase thickness or add more liquid to decrease thickness.

The focus of this study was to investigate the viscosity of infant bottle feedings given for bedside treatment. Further research should expand to translate these findings in order to create recipes used for VFSS evaluation of dysphagia. During VFSS a contrast agent is added to the thickened formula in order to visibility see the liquid on X-ray. The contrast agent is likely to change the viscosity of the liquid; therefore, we currently cannot compare the viscosity of liquids used for evaluation and treatment. Clinicians need further guidance on how to mix formula with contrast agents to achieve the desired nectar and honey thick viscosities for VFSS evaluations. Thickened formulas at evaluation and treatment must be comparable to ensure the safety of the infant and proper treatment and management.

While all research in this study was done using the current ratios used at WCH, we believe that this work is applicable beyond our single hospital because we are addressing a universal issue. We are using standard infant formulas and thickening agents that are readily available for use. In the near future, we hope to collaborate with other SLPs beyond our hospital setting to facilitate translation of our bench work findings into clinical practice.

REFERENCES

Agostoni, C., Axelsson, I., Goulet, O., Koletzko, B., Michaelsen, K. F., Puntis, J. W., Nutrition, E. C. o. (2004). Preparation and handling of powdered infant formula: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr, 39*(4), 320-322.

Almeida, M. B., Almeida, J. A., Moreira, M. E., & Novak, F. R. (2011). Adequacy of human milk viscosity to respond to infants with dysphagia: Experimental study. *J Appl Oral Sci, 19*(6), 554-559.

Arvedson, J., Rogers, B., Buck, G., Smart, P., & Msall, M. (1994). Silent aspiration prominent in children with dysphagia. *Int J Pediatr Otorhinolaryngol, 28*(2-3), 173-181.

Beal, J., Silverman, B., Bellant, J., Young, T. E., & Klontz, K. (2012). Late onset necrotizing enterocolitis in infants following use of a xanthan gum-containing thickening agent. *J Pediatr, 161*(2), 354-356. doi:10.1016/j.jpeds.2012.03.054

Budke, J., Garcia, J. M., & Chambers, E. (2008). Comparisons of thickened beverages using line spread measurements. *J Am Diet Assoc, 108*(9), 1532-1535. doi:10.1016/j.jada.2008.06.434

Campos, L. F., Repka, J. C., & Falcão, M. C. (2013). Effects of human milk fortifier with iron on the bacteriostatic properties of breast milk. *J Pediatr (Rio J), 89*(4), 394-399. doi:10.1016/j.jped.2012.12.003

Capilouto, G. J., & Cunningham, T. J. (2016). Objective assessment of a preterm infant's nutritive sucking from initiation of feeding through hospitalization and discharge. *Neonatal Intensive Care, 29*(1), 40-45.

Carignan, C. C., Punshon, T., Karagas, M. R., & Cottingham, K. L. (2016). Potential exposure to arsenic from infant rice cereal. *Ann Glob Health, 82*(1), 221-224. doi:10.1016/j.aogh.2016.01.020

Center for Disease Control National Center for Chronic Disease Prevention and Health Promotion - Division of Nutrition, P. A., and Obesity. (2016). Breastfeeding Report Card - Progressing Towards National Breastfeeding Goals.

Cichero, J., Nicholson, T., & Dodrill, P. (2011). Liquid barium is not representative of infant formula: Characterisation of rheological and material properties. *Dysphagia, 26*(3), 264-271. doi:10.1007/s00455-010-9303-3

Cichero, J. A., Jackson, O., Halley, P. J., & Murdoch, B. E. (2000). How thick is thick? Multicenter study of the rheological and material property characteristics of mealtime fluids and videofluoroscopy fluids. *Dysphagia, 15*(4), 188-200. doi:10.1007/s004550000027

Cichero, J. A., Nicholson, T. M., & September, C. (2013). Thickened milk for the management of feeding and swallowing issues in infants: a call for interdisciplinary professional guidelines. *J Hum Lact, 29*(2), 132-135. doi:10.1177/0890334413480561

Colin, A. A., McEvoy, C., & Castile, R. G. (2010). Respiratory morbidity and lung function in preterm infants of 32 to 36 weeks' gestational age. *Pediatrics, 126*(1), 115-128. doi:10.1542/peds.2009-1381

Dion, S., Duivestein, J. A., St Pierre, A., & Harris, S. R. (2015). Use of thickened Liquids to manage feeding difficulties in infants: A pilot survey of practice patterns in Ccanadian pediatric centers. *Dysphagia, 30*(4), 457-472. doi:10.1007/s00455-015-9625-2

Dop, D., Gheonea, C., Stănescu, G. L., Moroşanu, A. E., Diaconu, R., Niculescu, E. C., Niculescu, D. (2015). Aspiration pneumonia in an infant with neurological sequelae - case report. *Rom J Morphol Embryol, 56*(3), 1191-1194.

Food and Drug Administration, (2016). FDA Expands Caution About SimplyThick. Retrieved from http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm256250.htm

Food and Drug Administration (FDA), F. a. D. A. (2016). FDA proposes limit for inorganic arsenic in infant rice cereal [Press release]

Fox, D., Campagna, E. J., Friedlander, J., Partrick, D. A., Rees, D. I., & Kempe, A. (2014). National trends and outcomes of pediatric gastrostomy tube placement. *J Pediatr Gastroenterol Nutr, 59*(5), 582-588. doi:10.1097/MPG.0000000000000468

Frazier, J., Chestnut, A. H., Jackson, A., Barbon, C. E., Steele, C. M., & Pickler, L. (2016). Understanding the Viscosity of Liquids used in infant dysphagia management. *Dysphagia, 31*(5), 672-679. doi:10.1007/s00455-016-9726-6

Gallacher, D. J., Hart, K., & Kotecha, S. (2016). Common respiratory conditions of the newborn. *Breathe (Sheff), 12*(1), 30-42. doi:10.1183/20734735.000716

Garcia, J. M., Chambers, E., Matta, Z., & Clark, M. (2008). Serving temperature viscosity measurements of nectar- and honey-thick liquids. *Dysphagia, 23*(1), 65-75. doi:10.1007/s00455-007-9098-z

Gosa, M. M., & Dodrill, P. (2016). Effect of time and temperature on thickened infant formula. *Nutr Clin Pract*. doi:10.1177/0884533616662991

Hair, A. B., Bergner, E. M., Lee, M. L., Moreira, A. G., Hawthorne, K. M., Rechtman, D. J., . . . Blanco, C. L. (2016). Premature Infants 750-1,250 g Birth Weight Supplemented with a Novel Human Milk-Derived Cream Are Discharged Sooner. *Breastfeed Med, 11*, 133-137. doi:10.1089/bfm.2015.0166

Hawdon, J. M., Beauregard, N., Slattery, J., & Kennedy, G. (2000). Identification of neonates at risk of developing feeding problems in infancy. *Dev Med Child Neurol, 42*(4), 235-239.

Hollin, R. (2011). Identification and management of dysphagia in children with neurological impairments. *Aust Nurs J, 18*(10), 31-34.

Jadcherla, S. (2016). Dysphagia in the high-risk infant: potential factors and mechanisms. *Am J Clin Nutr, 103*(2), 622S-628S. doi:10.3945/ajcn.115.110106

Kim, S. G., & Yoo, B. (2015). Viscosity of dysphagia-oriented cold-thickened beverages: effect of setting time at refrigeration temperature. *Int J Lang Commun Disord, 50*(3), 397-402. doi:10.1111/1460-6984.12141

Kowlessar, N. M., Jiang, H. J., & Steiner, C. (2013). Hospital stays for newborns, 2011. *Healthcare Cost and Utilization Project (HCUP) Statistical Briefs*

Lau, C. (2015). Development of suck and swallow mechanisms in infants. *Ann Nutr Metab, 66 Suppl 5*, 7-14. doi:10.1159/000381361

Lefton-Greif, M. A. (2008). Pediatric dysphagia. *Phys Med Rehabil Clin N Am, 19*(4), 837-851, ix. doi:10.1016/j.pmr.2008.05.007

Lessen, B. S. (2011). Effect of the premature infant oral motor intervention on feeding progression and length of stay in preterm infants. *Adv Neonatal Care, 11*(2), 129-139. doi:10.1097/ANC.0b013e3182115a2a

Lund, A. M., Garcia, J. M., & Chambers, E. (2013). Line spread as a visual clinical tool for thickened liquids. *Am J Speech Lang Pathol, 22*(3), 566-571. doi:10.1044/1058-0360(2013/12-0044)

Madhoun, L. L., Siler-Wurst, K. K., Sitaram, S., & Jadcherla, S. R. (2015). Feed-thickening practices in NICUs in the current era: Variability in prescription and implementation patterns. *J Neonatal Nurs, 21*(6), 255-262. doi:10.1016/j.jnn.2015.07.004

Marcus, C. L., Smith, R. J., Mankarious, L. A., Arens, R., Mitchell, G. S., Elluru, R. G., Blaisdell, C. J. (2009). Developmental aspects of the upper airway: report from an NHLBI Workshop, March 5-6, 2009. *Proc Am Thorac Soc, 6*(6), 513-520. doi:10.1513/pats.200905-024CB

Marik, P. E. (2001). Aspiration pneumonitis and aspiration pneumonia. *N Engl J Med, 344*(9), 665-671. doi:10.1056/NEJM200103013440908

Murakami, M., Nishi, Y., Fujishima, K., Nishio, M., Minemoto, Y., Kanie, T., & Nishimura, M. (2015). Impact of Types of Moisturizer and Humidity on the Residual Weight and Viscosity of Liquid and Gel Oral Moisturizers. *Journal of Prosthodontics, 25*(7), 570-575. doi:10.1111/jopr.12341

National Dysphagia Diet Task Force, ADA., N. D. D. T. F. (2002). *National Dysphagia Diet: Standardization for Optimal Care.* (D. Faulhaber Ed.). Chicago: American Dietetic Association.

Nelson, S. P., Chen, E. H., Syniar, G. M., & Christoffel, K. K. (1997). Prevalence of symptoms of gastroesophageal reflux during infancy. A pediatric practice-based survey. Pediatric Practice Research Group. *Arch Pediatr Adolesc Med, 151*(6), 569-572.

Nicosia, M. A., & Robbins, J. (2007). The usefulness of the line spread test as a measure of liquid consistency. *Dysphagia, 22*(4), 306-311. doi:10.1007/s00455-007-9086-3

Ostapchuk, M., Roberts, D. M., & Haddy, R. (2004). Community-acquired pneumonia in infants and children. *Am Fam Physician, 70*(5), 899-908.

Pados, B. F., Park, J., Thoyre, S. M., Estrem, H., & Nix, W. B. (2015). Milk flow rates from bottle nipples used for feeding infants who are hospitalized. *Am J Speech Lang Pathol*, *24*(4), 671–679. doi: 10.1044/2015_AJSLP-15-0011

Park, J. W., Kim, I. J., & Lee, H. J. (2016). Fork test: A new simple and reliable consistency measurement for the dysphagia diet. *Geriatr Nurs, 37*(4), 292-295. doi:10.1016/j.gerinurse.2016.04.011

Rubin, B. K. (2011). "The cruelest lies are often told in silence". *Chest, 140*(3), 567. doi:10.1378/chest.11-0667

September, C., Nicholson, T. M., & Cichero, J. A. (2014). Implications of changing the amount of thickener in thickened infant formula for infants with dysphagia. *Dysphagia, 29*(4), 432-437. doi:10.1007/s00455-014-9523-z

Silverman, A. H. (2010). Interdisciplinary care for feeding problems in children. *Nutr Clin Pract, 25*(2), 160-165. doi:10.1177/0884533610361609

Steele, C. M., Van Lieshout, P. H., & Goff, H. D. (2003). The rheology of liquids: a comparison of clinicians' subjective impressions and objective measurement. *Dysphagia, 18*(3), 182-195.

Stuart, S., & Motz, J. M. (2009). Viscosity in infant dysphagia management: comparison of viscosity of thickened liquids used in assessment and thickened liquids used in treatment. *Dysphagia, 24*(4), 412-422. doi:10.1007/s00455-009-9219-y

Thomson, J., Hall, M., Ambroggio, L., Stone, B., Srivastava, R., Shah, S. S., & Berry, J. G. (2016). Aspiration and non-Aspiration pneumonia in hospitalized children With neurologic impairment. *Pediatrics, 137*(2), e20151612. doi:10.1542/peds.2015-1612

Woods, C. W., Oliver, T., Lewis, K., & Yang, Q. (2012). Development of necrotizing enterocolitis in premature infants receiving thickened feeds using SimplyThick®. *J Perinatol, 32*(2), 150-152. doi:10.1038/jp.2011.105

Yoon, S. N., & Yoo, B. (2017). Rheological behaviors of thickened infant formula Prepared with Xanthan Gum-Based Food Thickeners for Dysphagic Infants. *Dysphagia, 32*(3), 454-462. doi:10.1007/s00455-017-9786-2