FEEDING THE MEDICALLY FRAGILE INFANT:
EFFECTS OF FEEDING METHOD AND MILK FLOW ON PHYSIOLOGY AND BEHAVIOR

Britt Frisk Pados

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Approved by:

Suzanne M. Thoyre

Katherine E. Gregory

George Knafl

Gail C. McCain

Marcia Van Riper

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ABSTRACT

Britt Frisk Pados: Feeding the Medically Fragile Infant:
Effects of Feeding Method and Milk Flow on Physiology and Behavior
(Under the direction of Dr. Suzanne M. Thoyre)

Background: Oral feeding is a challenge for medically fragile infants, particularly those born preterm and with hypoplastic left heart syndrome (HLHS). Rate of milk flow from the bottle nipple affects physiologic stability during feeding in preterm infants, but little data is available on the flow rates of nipples used for feeding hospitalized infants. Changes in milk flow rate likely also affect physiologic stability of infants with HLHS during feeding, however no studies have evaluated responses of infant with HLHS to different feeding methods. Feeding interventions aim to reduce feeding stress in fragile infants to promote growth. Outcome measures that sensitively measure stress are needed.

Purpose: This dissertation is composed of three studies. Chapter two presents milk flow rates of nipples used for feeding hospitalized infants. Chapter three examines the physiologic and behavioral responses of an infant with HLHS to variations in milk flow rate. Chapter four evaluates heart rate variability (HRV) as a feeding intervention outcome measure in the preterm infant.

Methods: In chapter two, milk flow rates of ten each of 29 nipple types (n=290) were tested using a breast pump. In chapter three, a single-subject with HLHS was evaluated during feeding with either a slow-flow or standard-flow nipple. In chapter four, a secondary analysis of heart rate variability indices was conducted from a test of a co-regulated approach to feeding preterm infants (n=14).

Results: In chapter two, flow rates varied widely between nipple types. Chapter three found that oral feeding was distressing for an infant with HLHS, regardless of flow condition. In chapter four, only SD12, a non-linear index of HRV, was found to significantly differentiate between feeding methods.

Conclusions: Data on milk flow rates from nipples used in hospitals will guide clinicians in nipple selection. Information on flow rates of nipples used after discharge is needed. Further study of how infants with HLHS respond to oral feeding is necessary to identify supportive strategies. Research is also warranted to further evaluate the use of HRV, particularly non-linear indices, during feeding interventions.

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LIST OF ABBREVIATIONS

ANS	Autonomic nervous system
Ao	Aorta
Base	Baseline (this abbreviation was only used in tables 3.7 and 3.9)
bpm	Beats per minute
C-CHEWS	Cardiac Children's Hospital Early Warning Score
CHD	Congenital heart disease
ChOMPS	Child Oral and Motor Proficiency Scale
CLD	Chronic lung disease
cm	centimeters
CoReg	Coregulated approach to feeding preterm infants; name of the feeding intervention in the study presented in chapter 4
CV	Coefficient of variation
DMNX	Dorsal motor nucleus
DOL	Day of life
ECG	Electrocardiogram
FaMM Feed	Family Management Measure: Feeding
Feed	Feeding (this abbreviation was only used in tables 3.7 and 3.9)
FR	Flow restrictor
GI	Gastrointestinal
HF	High frequency
HLHS	Hypoplastic left heart syndrome
HR	Heart rate
HRV	Heart rate variability
Hz	Hertz
IBI	Interbeat interval
IVH	Intraventricular hemmorhage
LA	Left atrium
LF	Low frequency
LMM	Linear mixed modeling

LV	Left ventricle
min	minute
mins	minutes
mL	milliliters
mmHg	millimeters of mercury
MPA	Main pulmonary artery
ms^2	milliseconds squared
NA	Nucleus ambiguous
NBRS	Neurobiological Risk Score
NG	Nasogastric
No	Number
0_2	Oxygen
Pedi-EAT	Pediatric Eating Assessment Tool
PMA	Post-menstrual age
PNS	Parasympathetic nervous system
Qp:Qs	Pulmonary to systemic blood flow ratio
RA	Right atrium
RDS	Respiratory distress syndrome
Recover	Recovery (this abbreviation was only used in tables 3.7 and 3.9)
RIP	Respiratory inductance plethysmography
RR	Respiratory rate
rr_i	R wave to R wave interval
RSA	Respiratory sinus arrhythmia
RV	Right ventricle
RV-PA	Right ventricle to pulmonary artery
S1P	Stage 1 palliation
S2P	Stage 2 palliation
SA	Sinoatrial
SD	Standard deviation
SD1	Length of the transverse axis of the ellipse in a Poincaré plot

SD12	Ratio of the length of the transverse axis to the length of the longitudinal axis of the ellipse in a Poincaré plot
SD2	Length of the longitudinal axis of the ellipse in a Poincaré plot
secs	seconds
SNS	Sympathetic nervous system
Sp02	Oxygen saturation

CHAPTER 1: INTRODUCTION

Background and Significance

Feeding is one of the most basic tasks for sustaining life once an infant is no longer being nourished in utero. While in utero, the infant rehearses sucking, swallowing, and breathing behaviors but nutrition and oxygenation are supported by the placenta. As the infant transitions to the extra-uterine environment, survival is dependent on effective coordination of fluid management (sucking and swallowing) with respiration in order to take in enough milk for adequate growth while also sustaining oxygenation. Feeding is essential for survival, but it is not simple. It is extremely complex and its success is dependent on a variety of factors both internal and external to the infant. The infant must have the anatomic structure and neurologic capacity to perform the physical act of feeding as well as the physiologic support to maintain stability during this act. All of this must happen within an environment that is safe and supportive of the task of feeding.

Given the complexity of feeding, even the healthy infant often encounters difficulty early on. Feeding difficulty is the inability to safely and/or effectively intake adequate nutrition for appropriate growth. These difficulties usually manifest as physiologic instability during feeding or early cessation of feeding prior to the ingestion of adequate nutrition. In the healthy infant, these difficulties are usually manageable given a supportive environment and attentive caregiver. As the infant matures in the first days and weeks of life, these feeding difficulties are typically overcome. On the other hand, the medically fragile infant, whose anatomic, physiologic, and/or neurologic systems are not supportive of feeding, is likely to encounter significant difficulty with feeding, which is much more challenging to manage and which may further compromise their already vulnerable systems. Infants with congenital heart disease (CHD) and infants born preterm (prior to 37 weeks post-menstrual age (PMA)) are two examples of medically fragile infants who frequently encounter difficulty feeding.

Etiology of Feeding Difficulties

The etiology of feeding difficulties in infants born preterm and those with CHD is slightly different, although there are overlapping features. In this dissertation, chapter 3 focuses on a subset of infants with CHD who have hypoplastic left heart syndrome (HLHS) and chapter 4 focuses on infants born preterm. HLHS is a defect of the left side of the heart that results in a hypoplastic left ventricle. Survival is dependent on either heart transplantation or a series of three palliative reconstructive surgeries of the cardiac anatomy that results in a single right ventricle (RV) providing blood flow to both the pulmonary and systemic circulations (Feinstein, et al., 2012); the latter is the more common course of action. The first surgical procedure (stage 1 palliation (S1P)) typically occurs in the first week of life and the second procedure occurs around 4-6 months of age. The inter-stage period between S1P and stage 2 palliation (S2P) is a time associated with high mortality (Hehir, Cooper, Walters, & Ghanayem, 2011) and feeding difficulties have been implicated in contributing to inter-stage death (Hehir, et al., 2011). Infants with HLHS were chosen at the focus of chapter 3 because this particular group of infants is at high risk for feeding difficulty.

Common risk factors for oral feeding difficulty among preterm infants and those with HLHS include prolonged periods of intubation or respiratory support and prolonged nasogastric tube feedings in conjunction with periods of time without oral feeding (Barlow, 2009; Dodrill, et al., 2004; Einarson & Arthur, 2003). Both groups also frequently have elevated respiratory rates at rest; preterms as a result of respiratory distress syndrome and infants with HLHS as a result of pulmonary overcirculation. Studies of nutritive sucking in infants have shown that ventilation is markedly reduced during the sucking phase of feeding, then stabilizes when the infant pauses to breathe (Mathew, 1991b). Physiologically normal infants are able to increase ventilation during these pauses by increasing respiratory rate and/or tidal volume (Mathew, 1991b). However, in physiologically compromised infants, the change in ventilation during the initial continuous sucking phase may be too great to recover from and/or the challenge of increasing ventilation to recover may interfere with their ability to continue nutritive sucking. Increased ventilation needs at rest results in limited capacity for ventilatory interruption and also increases the risk of aspiration associated with mistiming of the swallow (Barlow, 2009). Finally, both groups frequently experience gastroesophageal reflux disease, which contributes to risk for feeding aversion (Hyman,

1994), and are at risk for swallowing dysfunction. Infants with HLHS are at risk for swallowing dysfunction resulting from manipulation of the left recurrent laryngeal nerve during aortic arch reconstruction (Sachdeva, et al., 2007). Preterm infants who have undergone surgical closure of a patent ductus arteriosis are also at high risk for swallowing dysfunction (Benjamin, et al., 2010).

In addition to these common risk factors for oral feeding difficulty, infants who are born preterm often encounter difficulty sucking and creating a latch to the bottle or breast as a result of immature oral musculature. They also experience difficulty coordinating sucking, swallowing, and breathing as a result of immature neurologic function (Barlow, 2009). Infants with HLHS have reduced oxygen levels as a result of mixing of oxygenated and deoxygenated blood in the common atrium, which limits their capacity for managing further decline in oxygen level resulting from ventilatory interruptions with oral feeding. Infants with HLHS also typically have some cardiac dysfunction, which limits their ability to respond to the activity of feeding, and both congenital and acquired neurologic abnormalities (Glauser, Rorke, Weinberg, & Clancy, 1990a, 1990b), which influence the coordination of sucking, swallowing, and breathing.

Milk Flow

When medically fragile infants experience difficulty with bottle-feeding in the hospital, a common strategy employed by nurses is to change the bottle nipple. This strategy is used across populations of infants and results in changes in milk flow rate, as flow rates have been found to vary considerably between different nipple types (Jackman, 2013; Mathew, 1988).

Milk flow is the rate at which milk transfers from the bottle to the mouth during feeding (Mathew, 1991b). Milk flow rate is one variable external to the infant that can affect the infants' ability to safely coordinate swallowing and breathing, and therefore the degree of stress associated with oral feeding (al-Sayed, Schrank, & Thach, 1994; Mathew, 1991a). Given the common anatomical structures for swallowing and breathing, swallowing requires closure of the airway and therefore a pause in respiration (Barlow, 2009). In order to recover from this pause in respiration, it is necessary for the infant to increase respiration in between swallows. It has been found, however, that some infants are not capable of increasing respiration during the pause and therefore have decreased minute ventilation during feeding, primarily as a result of decreased respiratory rates (al-Sayed, et al., 1994).

When milk flow is high, the infant is forced to either swallow at a frequency adequate to clear the oropharynx from fluid to prevent aspiration (at the expense of breathing) (al-Sayed, et al., 1994) or divert the milk away by allowing it to drool out his mouth or stop feeding altogether. Healthy, full-term infants have some capacity for self-regulating the flow of milk by changing sucking rate (Schrank, Al-Sayed, Beahm, & Thach, 1998) or pressure (Colley & Creamer, 1958; Mathew, Belan, & Thoppil, 1992). On the other hand, premature infants, with immature neurologic and respiratory systems, have limited ability to self-regulate flow (Mathew, et al., 1992). Unable to self-regulate milk flow, the premature infant exposed to higher flow during bottle-feeding exhibits greater reduction in ventilation than full-term infants (Mathew, 1991a). Premature infants have also been found to drool more with high flow rates than full-term infants (Kao, Lin, & Chang, 2010; Schrank, et al., 1998).

Jackman's (2013) study is the only study of milk flow rates of currently available nipples. Her findings were limited in the number of nipples tested and the methods used. Additional data on milk flow rates between different types of nipples and variation in flow rate within a given type of nipple is needed to guide clinicians in making decisions about nipple selection for supporting physiological stability during oral feeding of medically fragile infants. The study presented in chapter 2 describes milk flow rates of bottle nipples used for feeding hospitalized infants.

Although there is fairly good evidence that slower milk flow is more appropriate for infants who are preterm (Kao, et al., 2010; Mathew, 1991a), it remains unknown how infants with HLHS respond to changes in milk flow and what capacity they have to self-regulate flow as they attempt to integrate fluid management and respiration despite both cardiac and respiratory compromise. No studies have examined the physiologic or behavioral responses of infants with HLHS to the challenge of oral feeding. More information is needed about this particularly fragile population of infants to identify strategies to support them during oral feeding. Chapter 3 presents a study of the effects of differing milk flow rates on an infant with HLHS.

Theoretical Framework

Physiologic homeostasis is coordinated by the autonomic nervous system (ANS), which is responsible for distributing resources, such as blood, oxygen, and nutrients, to meet the demands of the organism (Porges, 1992). In response to the challenge of feeding, physiologic changes in respiratory and

cardiac indices are expected. Physiologic changes can also manifest as behavioral changes during oral feeding (Thoyre & Carlson, 2003). Polyvagal theory (Porges, 1995) provides a theoretical basis for understanding the relationship between physiologic responses to stress and emotional, cognitive, and behavioral regulation as an infant faces the dynamic challenge of oral feeding. An overview of the key concepts of Polyvagal Theory will be presented as well as a conceptualization of feeding within the theory.

Polyvagal Theory describes the physiologic response of mammals to stress as a function of the two pathways of the vagus nerve (Porges, 1995). Stress is defined as a disruption in homeostasis, where homeostasis is the stable state of an organism when internal needs are met (Porges, 1992). The Polyvagal Theory states that the evolutionary development of the vagus nerve resulted in two pathways: the myelinated nucleus ambiguous (NA) and the unmyelinated dorsal motor nucleus (DMNX) (Porges, 1995, 2009). While the NA controls the muscles of the supradiaphragmatic structures, such as the larynx, pharynx, esophagus, soft palate, heart, head, and face, the DMNX controls the structures below the diaphragm, particularly regulating the digestive functions of the gastrointestinal (GI) tract (Porges, 1995; Rinaman, 2006). In addition to controlling the supradiaphragmatic structures, the NA is also responsible for the coordination of sucking, swallowing, and breathing during feeding (Porges, 1995).

Polyvagal Theory explains the mammalian response to both low stress and high stress states. Mammals are distinguished from reptiles by high baseline vagal tone with temporary decreases in response to stress (Porges, 1995). During times of low stress, there is high vagal tone via the NA, resulting in low heart rate (HR); variability of the heart rate around baseline; increased tone in the inner ear for differentiation of human voices; preservation of metabolic resources for growth and restoration; coordination of sucking, swallowing, and breathing for feeding; and increased tone of the muscles of the head and face for social communication (Porges, 2007). Simultaneously, minimal input from the DMNX during times of low stress encourages digestion and absorption of nutrients from the GI tract (Porges, 2001).

Conversely, with elevated stress levels, there is a hierarchical activation of the two stress response systems. First, the sympathetic nervous system (SNS) responds by mobilizing resources to meet the demands of the situation, resulting in increased HR, decreased heart rate variability (HRV),

activation of the stress response system of the hypothalamic pituitary axis (e.g., release of cortisol), stimulation of the immune system (e.g., release of cytokines), and diversion of blood away from the GI tract to the more vital organs such as the heart, brain, and lungs (Porges, 1992, 2009). HRV is the fluctuation in the interval between consecutive normal heart beats and reflects the balance of input from the sympathetic and parasympathetic divisions of the ANS (Schroeder, et al., 2004). High HRV, or a wide range around baseline, indicates a well-functioning and adaptable ANS, while low HRV signifies inability to adapt to increased physiologic demands (Verklan & Padhye, 2004).

If the SNS response is not able to reestablish homeostasis, the DMNX, the secondary system, is activated resulting in disengagement, hypotonia, apnea, and bradycardia (Porges, 2003). The unmyelinated DMNX is the portion of the vagus that is common to both mammals and reptiles and its purpose is to conserve resources during stressful events (Porges, 1995). In reptiles, this response is functional, allowing them to freeze in response to predators (Porges, 1995). Unfortunately, in mammals who have relatively high oxygen needs, activation of the DMNX response and the resulting apnea and bradycardia can result in life-threatening oxygen depletion (Porges, 2007). The three different functions of the vagus allow mammals to not only thrive in safe environments, but survive in dangerous and life-threatening ones as well (Porges, 2009).

Conceptualization of Feeding within the Polyvagal Theory

Polyvagal Theory states that the perception of an event as stressful is subjective and dependent on the vulnerability of the individual at the time of the event (Porges, 1992; Porges, Doussard-Roosevelt, Stifter, McClenny, & Riniolo, 1999). An event may be perceived as stressful if it is environmentally or metabolically demanding or if it requires mental effort, attention, or social interaction (Porges, et al., 1999). Feeding has the potential for being perceived as stressful by an infant for a number of reasons, especially if the infant is physiologically compromised at rest. If the infant enters the feeding with unstable physiology, this is compounded by the environmental, metabolic, and social interactional stresses of feeding.

Feeding may be environmentally stressful because of light or noise. Feeding may also precipitate metabolic stress if the infant experiences pain or if feeding competes with the infant's ability to maintain physiologic stability (Porges, 1992). The degree of ventilatory disruption associated with oral feeding,

combined with the respiratory needs of the infant at baseline, contribute to the degree of physiologic stress associated with feeding. Finally, feeding may be stressful because it requires a great deal of mental effort, attention, and social interaction, particularly if the infant is inexperienced with feeding, is immature, or if the feeder does not adequately or appropriately respond to the infant's needs (Porges, 2003).

When stressed during feeding, Polyvagal Theory suggests that the infant would respond by withdrawal of vagal input from the NA, which would inhibit their ability to effectively coordinate sucking, swallowing, and breathing and to accurately give the feeder facial cues about their hunger and/or satiety, their level of fatigue, or their need to pause for respiration. Unknowingly, this may lead the feeder to either end a feeding before the infant is satiated or to push the infant to continue to feed despite the infant's exhaustion or respiratory instability, which may further compromise an already physiologically vulnerable infant. These theoretical changes are consistent with evidence of behavioral disorganization seen during feeding of preterm infants (Pickler, Frankel, Walsh, & Thompson, 1996; Thoyre & Carlson, 2003) and infants with CHD (Lobo & Michel, 1995).

Simultaneous with withdrawal of input from the NA, activation of the SNS would result in increased HR and decreased HRV (Verklan & Padhye, 2004). Although an increase in HR is expected with an activity such as feeding, the higher the HR, the more energy is expended to maintain physiologic homeostasis and the less energy is available for growth. Additionally, as the HR rises above approximately 180 beats per minute, ventricular filling time is diminished and oxygen consumption by the myocardium is increased (Gupta, 2014). This may be tolerated in a healthy heart, but is extremely problematic for infants with HLHS who are recovering from cardiac surgery and have reduced cardiac function at rest.

Diversion of blood away from the GI tract inhibits the infant's digestion and possibly places them at risk for developing necrotizing enterocolitis, a disease of the bowel that is initiated by damage to the intestinal mucosa from a hypoxic event and results in bacterial invasion, bowel necrosis, sepsis, and possibly death (Giannone, Luce, Nankervis, Hoffman, & Wold, 2008; McElhinney, et al., 2000).

When feeding is supported in a manner that reduces the level of stress experienced by the infant, vagal input from the NA is supported, which allows the infant to communicate with caregivers, coordinate

sucking, swallowing, and breathing for feeding, and to digest and absorb what they have eaten. At the same time, a reduction in stress during feeding minimizes the risk of activation of the SNS and DMNX stress pathways and therefore conserves energy and minimizes oxygen-depleting events such as apnea and bradycardia. Since feeding is a frequent event, usually occurring approximately every three hours during early infancy, and because the first several years of life are a critical period in the development of the nervous system, the potential effects of the level of stress experienced during feeding go beyond each individual feeding and may have long-term effects (Beauchaine, Gatzke-Kopp, & Mead, 2007).

If feeding is persistently stressful and accompanied by activation of the SNS and/or DMNX, the developing nervous system may be trained to remain in a state appropriate for dangerous situations even when the conditions are safe (Beauchaine, et al., 2007). This persistent activation may result in immune system dysfunction, respiratory inefficiency, and psychosocial disorder (Porges, 2003). In medically fragile children, immune system impairment and respiratory dysfunction may be further compromising and even life-threatening.

Persistent activation of the SNS and/or DMNX and inability to appropriately alter vagal tone may be potentially damaging to the developing nervous system and contribute to development of psychiatric disorders associated with difficulties in social behavior, such as autism spectrum disorder (Porges, 2003). An over-responsive nervous system has been found to be associated with high trait anxiety, which when combined with poor vagal adjustment has been linked to anxiety and panic disorders (Beauchaine, et al., 2007). Additionally, evaluation of a situation as being safe or dangerous is learned and if feeding is consistently determined to be dangerous, the child may identify feeding as unsafe long after their physiologic state has improved to make feeding safe, resulting in long-term difficulty with eating.

On the other hand, if feeding is consistently a non-stressful event and the environment is perceived as safe, the nervous system is exercised to support social behavior, growth, and restoration (Porges, 2003). Infants with appropriate vagal tone responses have been found to have less temperamental difficulty (Stifter & Fox, 1990), better emotion regulation (Porter, Wouden-Miller, Silva, & Porter, 2003), better attachment (Izard, et al., 1991), better social competence (Eisenberg, et al., 1995), and more empathy towards others (Fabes, Eisenberg, & Eisenbud, 1993). Vagal activity from NA input has also been found to support weight gain in infants by increasing gastric motility and release of food

absorption hormones and has been correlated with shorter hospital stay (DiPietro & Porges, 1991; Field & Diego, 2008).

Polyvagal Theory offers a theoretical framework for conceptualizing the complexity of infant feeding and the relationships between stress and emotional, cognitive, and behavioral regulation. The degree of stress experienced during oral feeding has the potential for profound short-term and long-term effects. As more research is done to evaluate feeding interventions to reduce feeding-related stress in medically fragile infants, outcome measures are needed that will measure stress sensitively and, ideally, provide early indicators of distress. Polyvagal Theory introduces HRV as a potential outcome measure of stress. While several studies have evaluated HRV during feeding (Brown, 2007; Cohen, Brown, & Myers, 2009; Harrison, 2011; Harrison & Brown, 2012; Lappi, et al., 2007; McCain, Fuller, & Gartside, 2005; McCain, Knupp, Fontaine, Pino, & Vasquez, 2010; Portales, et al., 1997; Suess, et al., 2000), only McCain's (2005) study has used HRV to evaluate the degree of stress experienced by infants during feeding. Further research is needed to determine whether HRV is a sensitive enough measure to detect alterations in the degree of stress related to different feeding strategies. The study presented in chapter 4 explores the use of HRV as a feeding intervention outcome measure in preterm infants.

Aims

This dissertation is composed of three studies that each contribute to the literature with regards to feeding medically fragile infants. Specifically, the aims were to:

1. Present data on the milk flow rates and variability in flow of bottle nipples used for feeding hospitalized infants.
2. Examine the physiologic and behavioral responses of an infant with HLHS to variations in milk flow rate.
3. Evaluate the usefulness of HRV as a feeding intervention outcome measure in the preterm infant.

Prepared Manuscripts

The three-manuscript option was chosen in lieu of a traditional dissertation. Chapter one has been an introduction to the problem of feeding medically fragile infants and the theoretical framework within which the author conceptualizes this problem. Chapters two through four of this dissertation present three manuscripts, which have been prepared for publication. Please note that references to

appendices will be removed prior to submission for publication. Chapter five provides a discussion of the manuscripts, clinical implications of the findings, and presents plans for future study.

Chapter two is titled “Milk flow rates from bottle nipples used for feeding hospitalized infants.” The purpose of this study was to evaluate milk flow rates from bottle nipples commonly used in hospitals for feeding medically fragile infants in order to provide clinicians with evidence with which to base decisions about nipple selection.

Chapter three is a presentation of a single-case experiment of the effects of milk flow on the response to feeding in an infant with HLHS. This manuscript is titled “Effects of milk flow on the physiologic and behavioral response to feeding in an infant with hypoplastic left heart syndrome.” The purpose of this study was to examine the physiologic changes and observational indicators of distress that occur when an infant with HLHS is bottle-fed with either a standard-flow nipple or a slow-flow nipple.

Chapter four is titled “Heart Rate Variability as a Feeding Intervention Outcome Measure in the Preterm Infant.” The purpose of this study was to investigate the use of heart rate variability as a measure of physiologic stress during feeding in a group of medically fragile preterm infants born at less than 35 weeks post-menstrual age.

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CHAPTER 2: MILK FLOW RATES FROM BOTTLE NIPPLES USED FOR FEEDING HOSPITALIZED INFANTS

Overview

Medically fragile infants often experience physiologic compromise during oral feeding. Milk flow is an easily manipulated variable that may contribute to the degree of physiologic instability experienced. Very little evidence is currently available to guide the selection of a bottle nipple for these infants. This study tested the milk flow rates and the variability in flow of currently available nipples used for bottle-feeding hospitalized infants. Clinicians in three countries were informally surveyed regarding nipples used for feeding hospitalized infants. Twenty-nine nipple types were identified and 10 nipples of each type were tested by measuring the amount of infant formula expressed in one minute using a breast pump. Mean milk flow rate (mL/min) and coefficient of variation (CV) were used to compare nipples within brand and within category (i.e., Slow, Standard, Premature). Flow rates varied widely between nipples, ranging from 2.10 for the Enfamil Cross-cut to 85.34 mL/min for the Dr. Brown's Y-cut. Variability of flow rates among nipples of the same type ranged from a CV of 0.05 for Dr. Brown's Level 1 Standard- and Wide-Neck to 0.42 for the Enfamil Cross-cut. Mean CV by brand ranged from 0.08 for Dr. Brown's to 0.36 for Bionix. Given the wide range of flow rates and variability of nipples used for feeding hospitalized infants, nipple selection is an important decision in supporting the medically fragile infant during feeding. This study provides clinicians with information for choosing the best available nipple to support oral feeding in fragile infants.

Keywords: bottle feeding, infant, premature infant, feeding methods

Introduction

Feeding can be physiologically challenging for premature and medically fragile infants who are learning to orally feed. While breast-feeding may be the ultimate goal, most hospitalized infants will receive some bottle-feedings. Many variables contribute to the infant's ability to bottle-feed safely and effectively, but one easily manipulated variable is the rate of milk flow from the bottle nipple. Milk flow is defined as the rate of transfer of milk from the bottle into the mouth during sucking. The rate of milk flow can affect infants' ability to integrate fluid management with respiration and the degree of physiologic instability associated with feeding (al-Sayed, Schrank, & Thach, 1994; Mathew, 1991a). When an infant swallows, the airway is closed for about one second to prevent aspiration of milk (Mathew, 1991b). As milk flow increases and requires increased swallowing frequency, ventilation is increasingly interrupted and respiratory rate decreases (al-Sayed, et al., 1994). When milk flow slows, the swallow is delayed until a critical volume is accumulated (al-Sayed, et al., 1994), allowing the infant to breathe more and better maintain physiologic stability during feeding.

Rate of milk flow varies considerably between different brands and types of nipples (Jackman, 2013; Mathew, 1988). Healthy, full-term infants are typically resilient feeders and are able to alter sucking rate (Schrank, Al-Sayed, Beahm, & Thach, 1998) and pressure (Colley & Creamer, 1958; Mathew, Belan, & Thoppil, 1992) in order to regulate milk flow. On the other hand, medically fragile infants, such as those born preterm, have a limited ability to self-regulate flow (Mathew, 1991a). When milk flow is too high, the infant must either swallow at a frequency adequate to clear the oropharynx from fluid to prevent aspiration (at the expense of ventilation) (al-Sayed, et al., 1994); allow the milk to pool in the oropharynx (and risk aspiration); divert the milk away by allowing it to drool out their mouth (Schrank, et al., 1998); or stop feeding.

Clinicians caring for hospitalized infants are faced with decisions about nipple selection to support medically fragile infants in learning to orally feed, however there is only one recently published study of flow rates from currently available nipples to support these decisions (Jackman, 2013). Jackman (2013) conducted a study of flow rates from 23 types of nipples, six of which she identified as used in neonatal intensive care units. Jackman (2013) found wide variability in the flow rates of nipples tested, ranging from 6 to 60 mL/min. Nipples marketed as "slow-flow" were not consistent in the flow rates delivered, with

some having three times the flow as others (Jackman, 2013). Finally, significant variation was reported between nipples of the same type (Jackman, 2013). Given the variability between nipples of the same type, Jackman's study was limited in that only one nipple per type was tested for nipples intended for multiple use and three nipples per type were tested for single-use nipples. To account for the variability between nipples and determine an accurate mean flow rate of each nipple type, more tests were needed. No statistical analysis was presented in the report of this study.

More information is needed to support clinicians in decision-making regarding nipple selection for feeding hospitalized infants. Without this information, infants are often exposed to multiple types of nipples in an effort to find a good match. The variability in nipples during early oral feeding may contribute to the length of time required to successfully feed and ultimately, to length of stay. This comparative, descriptive study tested the milk flow rates and variability of nipples used for bottle-feeding hospitalized infants.

Methods

Clinicians from the United States, Netherlands, and Australia were informally surveyed regarding nipples available to them for feeding infants in the hospital. Twenty-nine nipples were identified and tested (Table 2.1). A power analysis revealed that ten of each type of nipple was sufficient to compare flow rates between the nipple types with 80% power at an alpha of 0.05.

All of the nipples except the Dr. Brown's Level 1 Wide-Neck fit on a 60 mL Grad-U-Feed Nurser (Mead Johnson & Co, Glenview, IL) and were tested with this bottle. Bottles were filled with Similac Advance Stage 1 (20 calories/ounce) ready-to-feed formula (Abbott Laboratories, Abbott Park, IL). To ensure equal levels of hydrostatic pressure, the height from the level of the liquid surface to the tip of the nipple was maintained at 2.5 cm (Figure 2.1), requiring 50 mL of formula for nipples tested with the Grad-U-Feed Nurser and 70 mL for the Dr. Brown's Wide-Neck bottle. The formula was changed every ten tests to prevent increased viscosity as a result of denaturation of proteins from prolonged exposure to air.

The bottle and nipple unit being tested were attached to a breast shield of a breast pump using a layer of plastic paraffin film followed by a silicone-based polymer to create a seal. The bottle and nipple unit were held at a 30 degree angle (Figure 2.2). A negative pressure system was created using a Pump in Style Advanced breast pump (Medela, Inc., McHenry, IL). The stimulation phase suction pattern with a

suction pressure of 180 mmHg was used for all tests. Given the opportunities for loss of suction from the pump to the nipple, negative pressure within the bottle was tested every 50 tests using the Samba 201 Micro Pressure Measurement System (BIOPAC Systems, Inc., Goleta, CA). Mean suction rate was 110 cycles per minute and mean negative pressure within the bottle was 14 mmHg.

Formula was expressed for one minute into a 500 mL beaker situated on a calibrated platform scale (Thermo Fisher Scientific, Inc, Waltham, MA), accurate to 0.01 grams. At the conclusion of one minute, the weight of formula expressed was recorded. Outliers were re-tested to ensure accuracy of the measurement. Tests were video-recorded and measurements were confirmed by video review. Milk flow rates (mL/min) were calculated using the density of Similac Advance formula of 0.97 mL/gram (AVCalc, 2014).

The Bionix Controlled Flow Baby Feeder consists of two parts that may contribute to variability in milk flow: the nipple with the silicone inner channel and the flow restrictor (FR) system, consisting of the yellow flow restrictor, purple seal, and green flow adjuster (Bionix Medical Technologies, 2014). Since the nipple and FR system may contribute to flow in different ways, ten nipples were tested using the same FR system and separately ten FR systems were tested using the same nipple. For both the nipple and FR tests, the Bionix was tested on each of the five flow levels, resulting in a total of 100 tests. Also of note, the Dr. Brown's nipples were tested with the venting system in place, which is how the nipple is intended to be used. The venting system comprises the cream colored vent insert and the blue vent resevoir (Handi-Craft Company, 2014a). The Medela SpecialNeeds Feeder was tested without the white circular valve membrane or the yellow circular disc (Medela Inc., 2014). The method used in this study for applying negative pressure to the nipple could not work with the valve membrane in place. The SpecialNeeds Feeder is intended to have three flow levels: zero flow, medium flow, and maximum flow, depending on the position of the slit opening in the infant's mouth when positive pressure (i.e., compression) is applied by the infant's mouth (Medela, Inc., 2014). In this study, no positive pressure was applied. In the presence of negative pressure only, the slit opening should, theoretically, respond similarly regardless of positioning, but nipples were tested in the same position for consistency.

The methods used in this study were designed to test nipples under standardized conditions. The flow rates established by this method are not necessarily the flow rates that an infant will achieve when

feeding. Infants feed with varying sucking rates and pressures and will achieve different flow rates within and between feedings. Thus, this data should be interpreted only as a means to compare flow rates between nipples.

Statistical Analysis

Mean milk flow rate (mL/min) and SD were calculated for each nipple type. Variability within nipple types was assessed using the coefficient of variation (CV; SD/mean). To compare variability between nipple types, CV was categorized into three levels: low (< 0.1), moderate (0.1 – 0.2), and high (> 0.2).

The Shapiro-Wilk statistic was used to assess nipples for normality with an alpha of 0.05 considered significant. Comparisons between nipple types were made within brand and within category (Slow, Standard, and Premature) using one-way ANOVA when normally distributed; non-parametric one-way ANOVA was used otherwise. Multiple comparison tests for the post-hoc analysis of one-way ANOVA utilized Duncan's multiple range test, with an alpha of 0.05 being significant. When non-parametric one-way ANOVA was utilized, pairwise comparisons were made using the Wilcoxon Rank Sum Test and the alpha was adjusted using a Bonferroni adjustment.

For the purpose of comparing nipples within the categories of "Slow," "Standard," and "Premature," nipples were categorized by name, with a few exceptions. The Bionix Level 1 is intended to "introduce taste" and the Level 2 is intended to deliver a slow flow (Bionix Medical Technologies, 2014); these two levels were categorized as "Slow." The Bionix Level 5 is intended to deliver flow "at or near a flow rate of a Stage 1 nipple" (Bionix Medical Technologies, 2014) so this was categorized as "Standard." For comparisons within category, the Bionix nipple and flow restrictor tests were combined for each level. Dr. Brown's Preemie and Ultra-Preemie were included in the categories of both "Slow" and "Premature." Dr. Brown's Level 1 Wide- and Standard-Neck were categorized as "Standard." The Medela SpecialNeeds Feeder was categorized as "Slow."

Results

Flow rates varied widely between nipples, ranging from 2.10 for the Enfamil Cross-cut to 85.34 mL/min for the Dr. Brown's Y-cut (Figure 2.3). Variability of flow rates among nipples of the same type

ranged from a CV of 0.05 for Dr. Brown's Level 1 Standard- and Wide-Neck to 0.42 for the Enfamil Cross-cut (Figure 2.4). Mean CV by brand ranged from 0.08 for Dr. Brown's to 0.36 for Bionix (Figure 2.5).

Comparisons Within Brand

Bionix Controlled Flow Baby Feeder. This system was tested to evaluate the flow and variability of the nipples (indicated by an "N" after the level in the text) and the flow restrictor (FR) systems (indicated by a "FR" after the level in text and figures) separately. For both the nipple and FR tests, milk flow increased overall in the direction intended (level 1 being the slowest and level 5 being the fastest). Within the nipple tests, each level provided a significantly different flow rate (p<0.001), with the exception of levels 2N and 3N, which were not significantly different. Within the FR tests, 2FR and 3FR were not significantly different and 4FR and 5FR were not significantly different.

Comparing the FR tests to the nipple tests, level 1N and 1FR were comparable and levels 2N and 2FR were comparable. For all levels above 2, the nipple tests were significantly (p<0.001) slower than the FR tests. For all levels above 1, there was overlap between levels. Levels 2N, 2FR, and 3N were all similar to one another. Level 3FR was comparable to Levels 4N and 5N. At each level, the CV was higher for the FR tests than the nipple tests. Bionix Levels 3N and 5N were the only levels with CV < 0.1.

Dr. Brown's. All levels of Dr. Brown's nipples were found to be significantly different (p<0.005), with the exception of the Preemie and Level 1 Wide-Neck, which were found to be comparable. Dr. Brown's Ultra-Preemie performed as intended with the lowest flow (3.39 mL/min) of all the nipples by this brand; this nipple was the second lowest flow of the 29 nipple types tested in this study. Dr. Brown's Y-cut had the highest flow of all the nipples tested (85.34 mL/min) and was moderately variable with a CV of 0.13. All of the other Dr. Brown's nipples had a CV < 0.1.

Enfamil. All levels of Enfamil nipples were found to be significantly different (p<0.05), with the Cross-Cut being the slowest and the Preemie nipple being the fastest. Enfamil Standard was the only nipple with a CV < 0.1.

Similac. Slow and Standard nipples did not have significantly different flow rates. Similac Slow ranged from 6.16 to 9.38 mL/min (CV 0.1 – 0.2) while Similac Standard ranged from 3.8 to 12.0 mL/min (CV > 0.2). Similac Orthodontic was significantly faster (p<0.05) than Slow or Standard and was highly variable (CV > 0.2). Similac Premature was the fastest of all Similac products (p<0.05).

Pigeon. All levels of Pigeon nipples were significantly different ($p<0.05$). The No-Drip was the slowest, but also the most variable (CV > 0.2).

Comparisons Within Category

Slow Flow Nipples. Nine of the 29 nipples tested were categorized as "Slow" and flow ranged from 3.39 to 14.68 mL/min (Figure 2.6). Dr. Brown's Ultra-Preemie, Bionix Level 1, and Medela SpecialNeeds Feeder were comparable to one another; these were all significantly slower ($p<0.001$) than the other "Slow" nipples. Bionix Level 2, Dr. Brown's Preemie, NUK Slow, and Similac Slow all delivered comparable flow. Enfamil Slow was significantly faster ($p<0.001$) than all other "Slow" nipples. Pigeon Slow was significantly slower than Enfamil Slow ($p<0.001$) but significantly faster ($p<0.05$) than all other "Slow" nipples.

Standard Flow Nipples. Seven nipples were categorized as "Standard" and flow ranged from 6.61 to 25.07 mL/min (Figure 2.7). Similac Standard, Difrax, and Dr. Brown's Level 1 Wide-Neck and Standard-Neck nipples were comparable to one another; these were all significantly slower than the other "Standard" nipples ($p<0.05$).

Premature Nipples. Four nipples were categorized as "Premature" and flow rates ranged from 3.39 to 22.68 mL/min (Figure 2.8). All four "Premature" nipples delivered significantly different flow rates ($p<0.05$).

Discussion

Choosing a nipple for feeding a medically fragile infant is an important decision given the wide range of flow rates found in this study. The name of a nipple (e.g., "Slow") is not always an accurate indicator of the flow rate. Additionally, variability in flow rate between and within nipple types is an added challenge that may contribute to feeding difficulty.

Within the Bionix brand, for all levels above Level 1, there was overlap in flow rates between levels, suggesting that the Bionix Controlled Flow Baby Feeder may not perform as expected when the user increases the flow adjuster from one level to the next. The FR systems were particularly variable, with 4 of the levels having CV > 0.2. Changing the nipple or the FR may inadvertently change the flow rate delivered, even if the user sets the flow adjuster to the same position.

Bionix does provide a Flow Rate Comparison chart on their website (Bionix Medical Technologies, 2014). According to Bionix, a similar, but not completely transparent, method was used to test flow rates for 50 seconds using a Medela Classic Breast Pump (S. Herzig, e-mail communication, March 2014). Our results were consistent with theirs for the increase in flow between levels 1 and 2; they found a 75% increase while we found 77%. Both our tests and theirs found the greatest increase in flow to be between levels 3 and 4. Bionix also tested nipples made by other companies, but it is difficult to make comparisons because the names of the nipples have changed and because the methods may have been different. In the current study, Bionix Level 1 was among the slowest of the nipples tested and may be useful for feeding infants who require a very slow flow.

Dr. Brown's markets the Ultra-Preemie nipple as being 35% slower than their Preemie nipple (Handi-Craft Company, 2014b). In our tests, the Ultra-Preemie was 54% slower than the Preemie nipple. Dr. Brown's brand was the most consistent brand, with the lowest mean CV of all brands (Figure 2.5).

The Enfamil Cross-cut was the slowest of all nipples tested. The cross-cut has two slits that form a cross at the tip of the nipple. Enfamil advertises this nipple as having a faster flow than their standard nipple (Amazon.com, 2014), which is not consistent with our findings. Cross-cut nipples are described as varying in flow, with increasingly faster flow as the infant applies suction and opens the cross wider (Start & St James-Roberts, 2000). Two clinical studies have evaluated the physiologic effects of feeding with either a cross-cut or a single-hole nipple and found that, at sucking pressures established by preterm infants, the cross-cut yielded slower flow than the single hole (Chang, Lin, Lin, & Lin, 2007; Kao, Lin, & Chang, 2010). These studies did not use Enfamil nipples, but may support further investigation of our findings and how the cross-cut performs in practice.

For both the Enfamil and Similac brands, the Premature nipple was faster than the Slow or Standard flow nipples. This is important for clinical practice as many clinicians assume that a nipple labeled "Premature" indicates a slower flow rate. Premature infants typically generate lower sucking pressures than full-term infants (Medoff-Cooper, McGrath, & Shults, 2002) and may become fatigued early in the feeding before adequate volume is ingested. There was a previously held popular belief that increasing the flow rate for these infants would make it easier for them to transfer milk given low sucking pressures (Mathew, 1990) and that faster feedings would allow them to intake volume before becoming

fatigued. Certain premature nipples may have been designed based on these assumptions. More current evidence supports slower flow for maintaining physiologic stability during feeding for these infants, allowing them to breathe more (al-Sayed, et al., 1994; Mathew, 1991a; Park, Thoyre, Knafl, Hodges, & Nix, 2014), maintain better oxygenation, and endure oral feeding longer.

Another clinically significant finding was that the Similac Slow and Standard nipples do not deliver significantly different flow rates. Compared to the Enfamil products, both the Similac Slow and Standard were slower than the Enfamil Slow. Anecdotally, we have heard from clinicians that the Enfamil Slow delivers a slower flow than the Similac Slow nipple. There may be other qualities of nipples, such as the mechanical stiffness of the nipple material, that affect flow from the nipple or the infant's sucking during feeding that could not be detected using our methods (Barlow, 2009). Our findings, however, are consistent with Jackman's (2013) findings for these nipples.

This study had some limitations. The method used in this study applied only negative pressure to nipples. Nipples with a slit opening as opposed to a hole opening likely perform differently when positive pressure is applied during feeding, changing the shape of the opening. The two nipples in this study with slit openings were the Enfamil Cross-Cut and the Medela SpecialNeeds Feeder; caution should be used when interpreting these results as the flow rates may be different in practice. Additionally, it should be noted that the Dr. Brown's Y-cut nipple was tested with standard thickness formula. In clinical practice, this nipple is typically used with thickened milk in medically fragile infants.

Conclusions

Milk flow is an important variable in the complex task of oral feeding for the medically fragile infant. This study confirmed results of previous studies (Jackman, 2013; Mathew, 1988), which found a wide range in milk flow rates from different nipple types. This study has built on previous work by testing additional nipples that are currently available for feeding hospitalized infants, further exploring variability within nipple types, and by improving upon the testing and analysis methods.

Clinicians may use this data to guide nipple selection for medically fragile infants by comparing the flow rates and variability of the nipples that are available within their institution. Evidence-based decisions regarding nipple selection may support optimal oral feeding for these fragile infants and facilitate earlier discharge home. Given the importance of milk flow in fragile infants, manufacturers of

nipples should consider providing information on nipple packaging that reflects flow rate and variability of each nipple type; this would help to reduce any confusion related to the naming of nipples (i.e, premature, slow, standard), which may not accurately reflect flow rate. Manufacturers could also use the information from this study to improve upon nipple construction to reduce variability, particularly in nipples intended for fragile infants.

Researchers should use this data to make decisions about nipples used in tests of feeding interventions and select nipples with low variability in order to ensure consistency of flow. The specific nipple(s) used, flow rate, and variability of nipples should be documented in reports of feeding intervention studies.

Testing of flow rates of the nipples in this study will need to be repeated periodically to reflect currently available nipples. Additionally, testing of milk flow rates of nipples used for feeding infants after discharge is currently underway and will provide clinicians with information to guide parents in selecting nipples for use at home that are comparable in flow to that with which the infant has been successful in the hospital. Future research should consider testing the effect of flow rate and variability in bottle nipples on the sucking parameters (e.g., sucking pressure and rate) of fragile infants. Evaluation of the feeding patterns of fragile infants while being fed with various bottle nipples may provide information to tailor nipple selection to sucking pattern to further support these fragile infants as they learn to eat.

Table 2.1.

Nipples Tested

Brand Name	Company & Location	Nipples Tested
Bionix	Bionix Medical Technologies Toledo, OH	Controlled Flow Baby Feeder Levels 1-5; nipples and flow restrictor (FR) systems tested separately
Difrax	Difrax BV Bilthoven, Netherlands	Teat Natural Standard-Neck Small (0+ months)
Dr. Brown's	Handi-Craft Co. St. Louis, MO	Level 1 Standard-Neck Level 1 Wide-Neck Ultra Preemie Preemie Y-cut
Enfamil	Mead Johnson & Co. Glenview, IL	Standard-Flow (royal blue collar) Slow-Flow (turquoise collar) Preemie (light blue collar) Cross-Cut (yellow collar)
Medela	Medela Inc. McHenry, IL	SpecialNeeds Feeder (formerly Haberman Feeder)
NUK	NUK USA LLC Hackensack, NJ	Orthodontic Silicone Slow-Flow Standard-Neck
Pigeon	Pigeon Tokyo, Japan	Standard-Flow Slow-Flow No-Drip
Similac	Abbott Nutrition Lake Forest, IL	Standard-Flow (yellow nipple, clear collar) Slow-Flow (yellow nipple, yellow collar) Orthodontic (yellow nipple, clear collar) Premature (red nipple, clear collar)

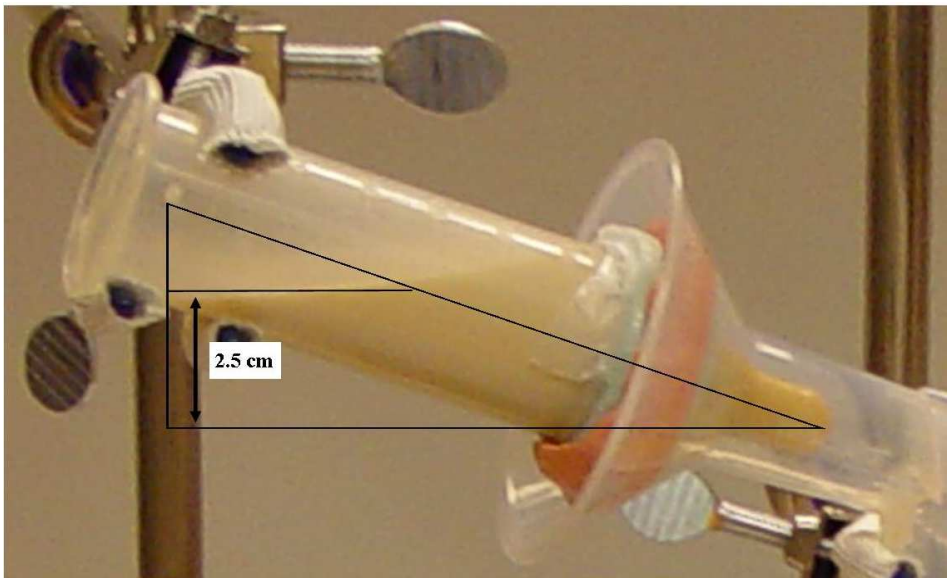

Figure 2.1. Hydrostatic pressure measured as the height from the level of the nipple opening to the height of the level of fluid.

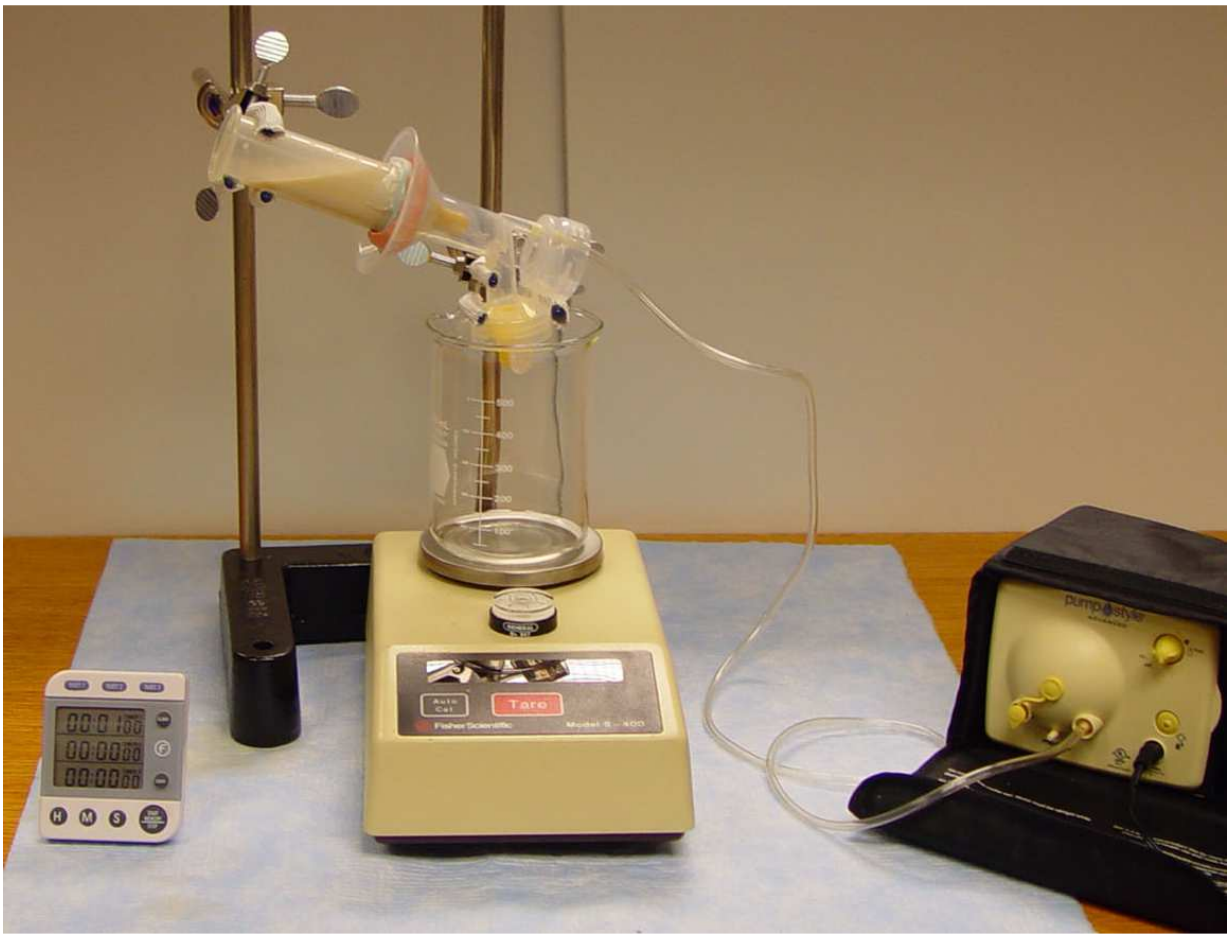

Figure 2.2. Nipple testing equipment.

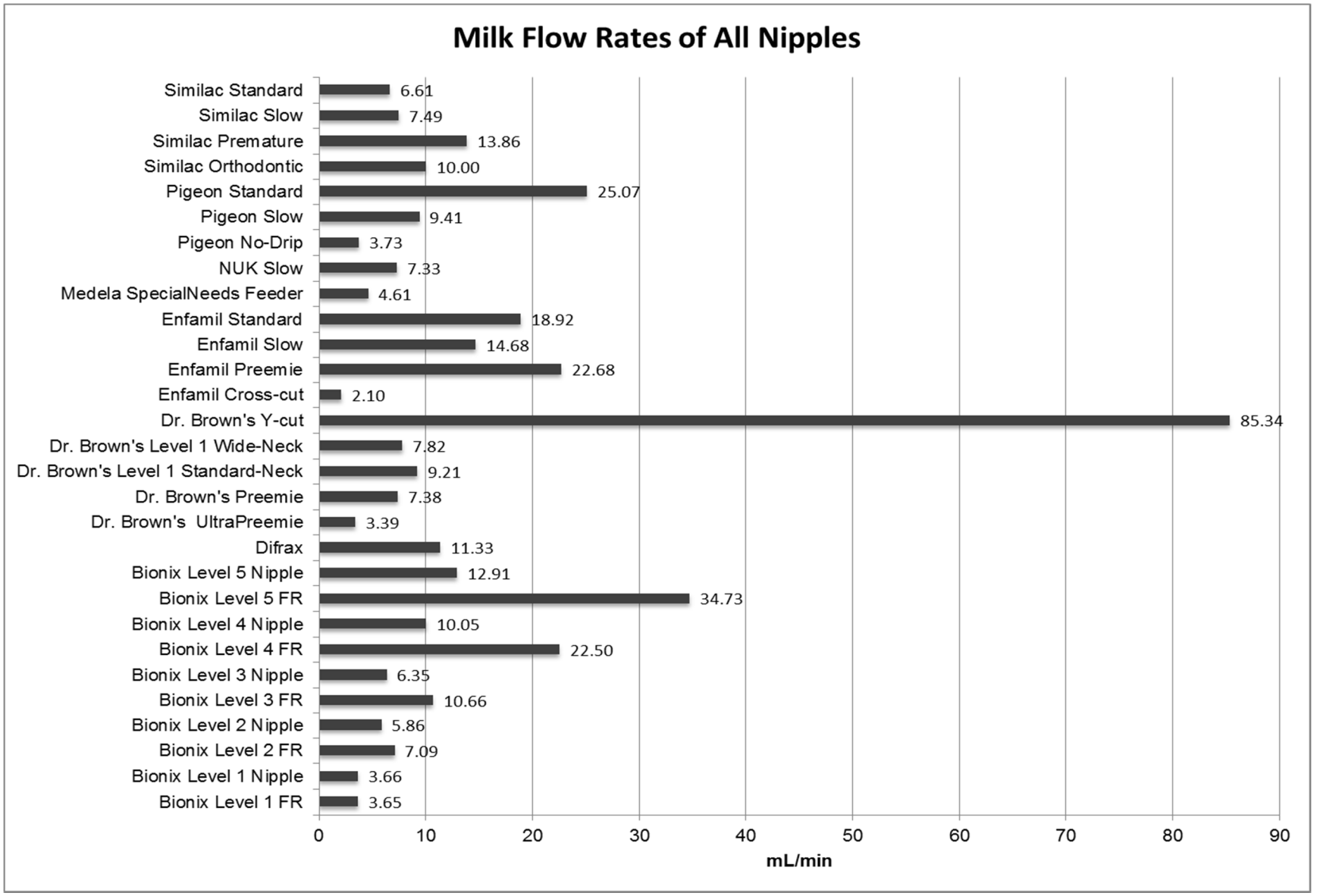

Figure 2.3. Milk flow rates of all nipples tested (mL/min). FR – flow restrictor.

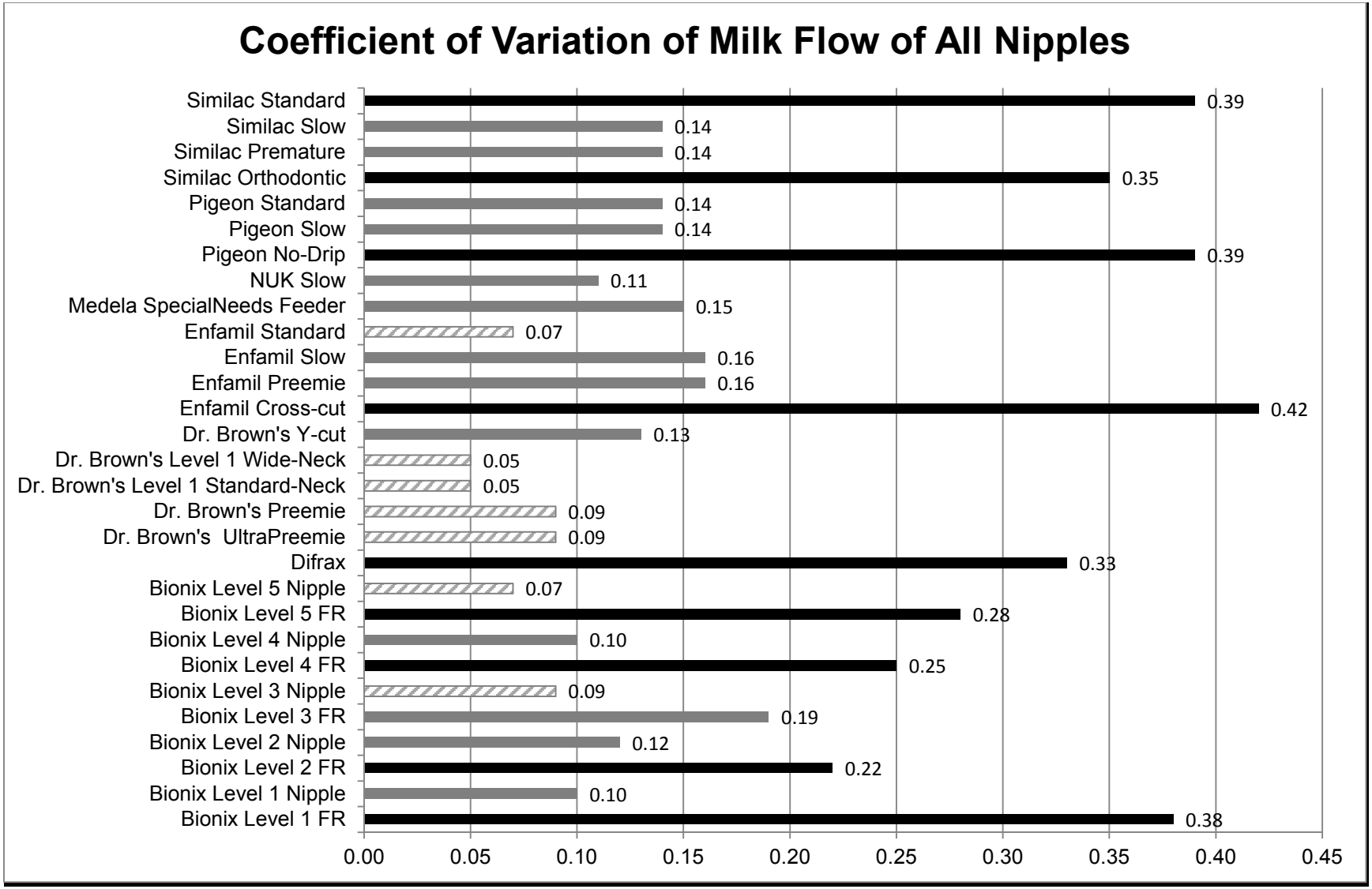

Figure 2.4. Coefficient of variation (CV) of milk flow of all nipples. Nipples are color coded by category of CV. Diagonal pattern indicates CV < 0.1, gray indicates CV 0. 1 – 0.2, and black indicates CV > 0.2. CV = mean/SD. FR – flow restrictor.

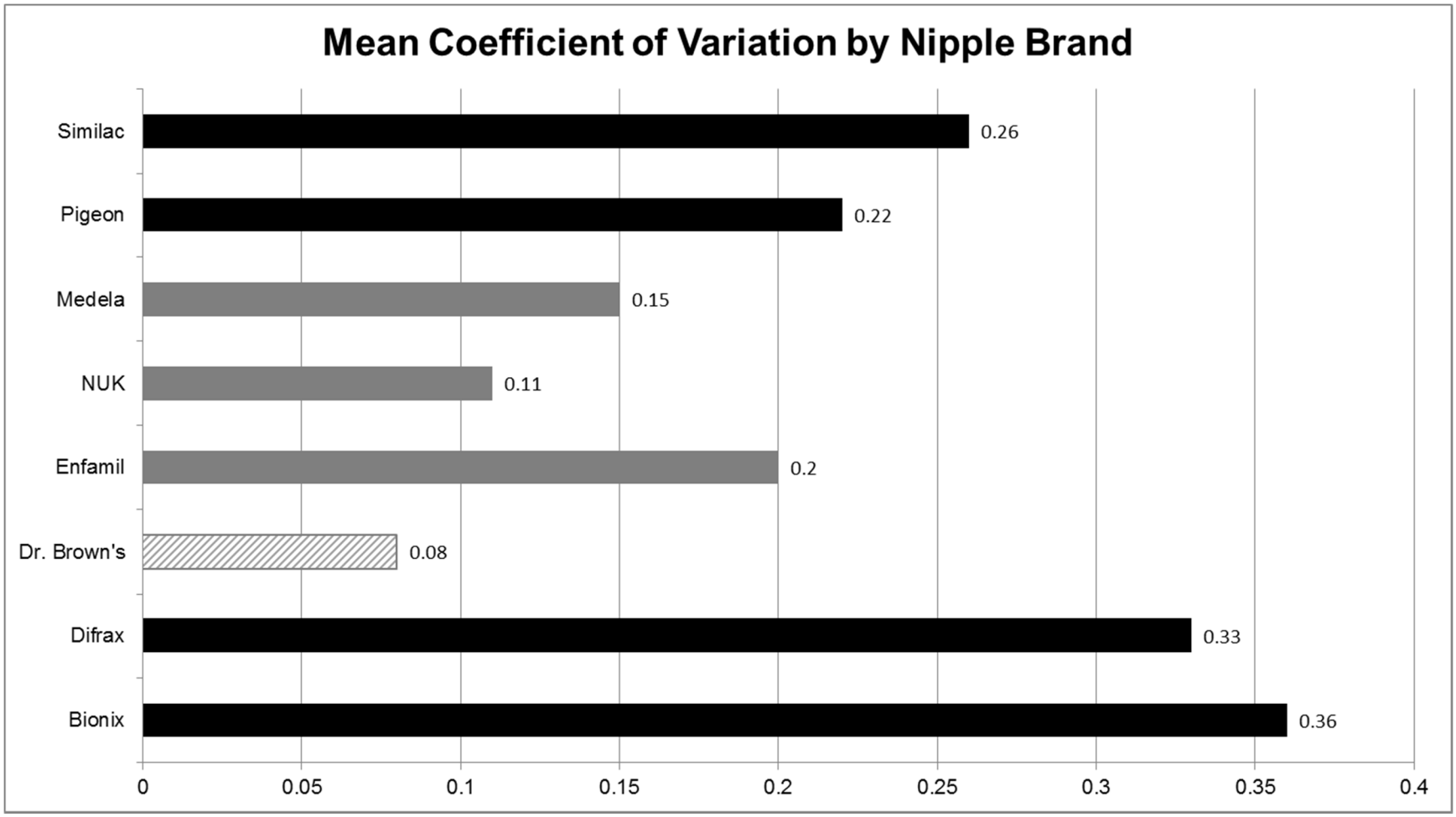

Figure 2.5. Mean coefficient of variation (CV) of milk flow rates by nipple brand. Calculated as the mean of the CV of each nipple type by each brand. Brands are color coded by category of CV. Diagonal pattern indicates mean CV < 0.1, gray indicates CV 0.1 – 0.2, and black indicates CV > 0.2.

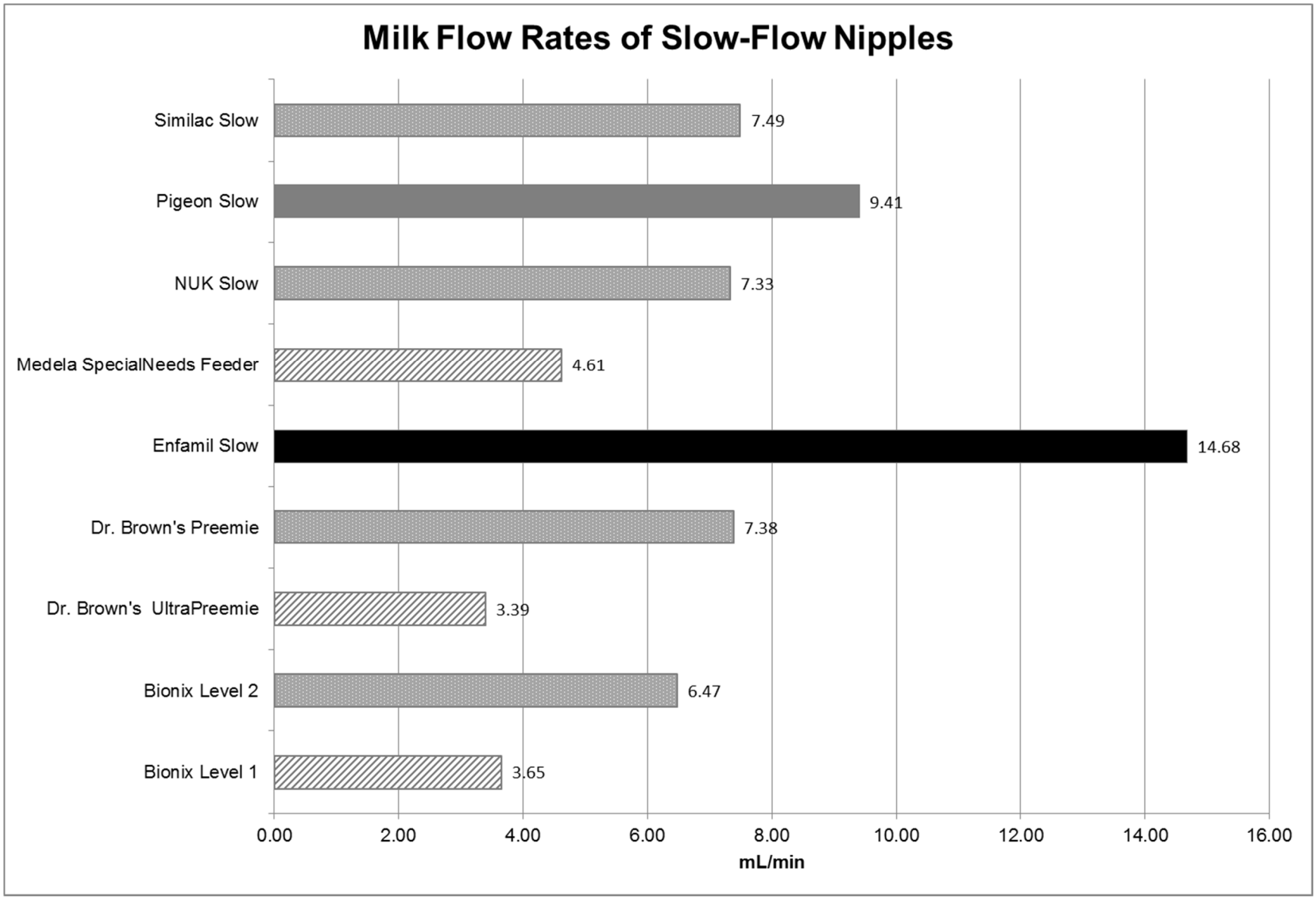

Figure 2.6. Milk flow rates of slow-flow nipples (mL/min). Nipples of the same color/pattern indicate that they are comparable in flow rate.

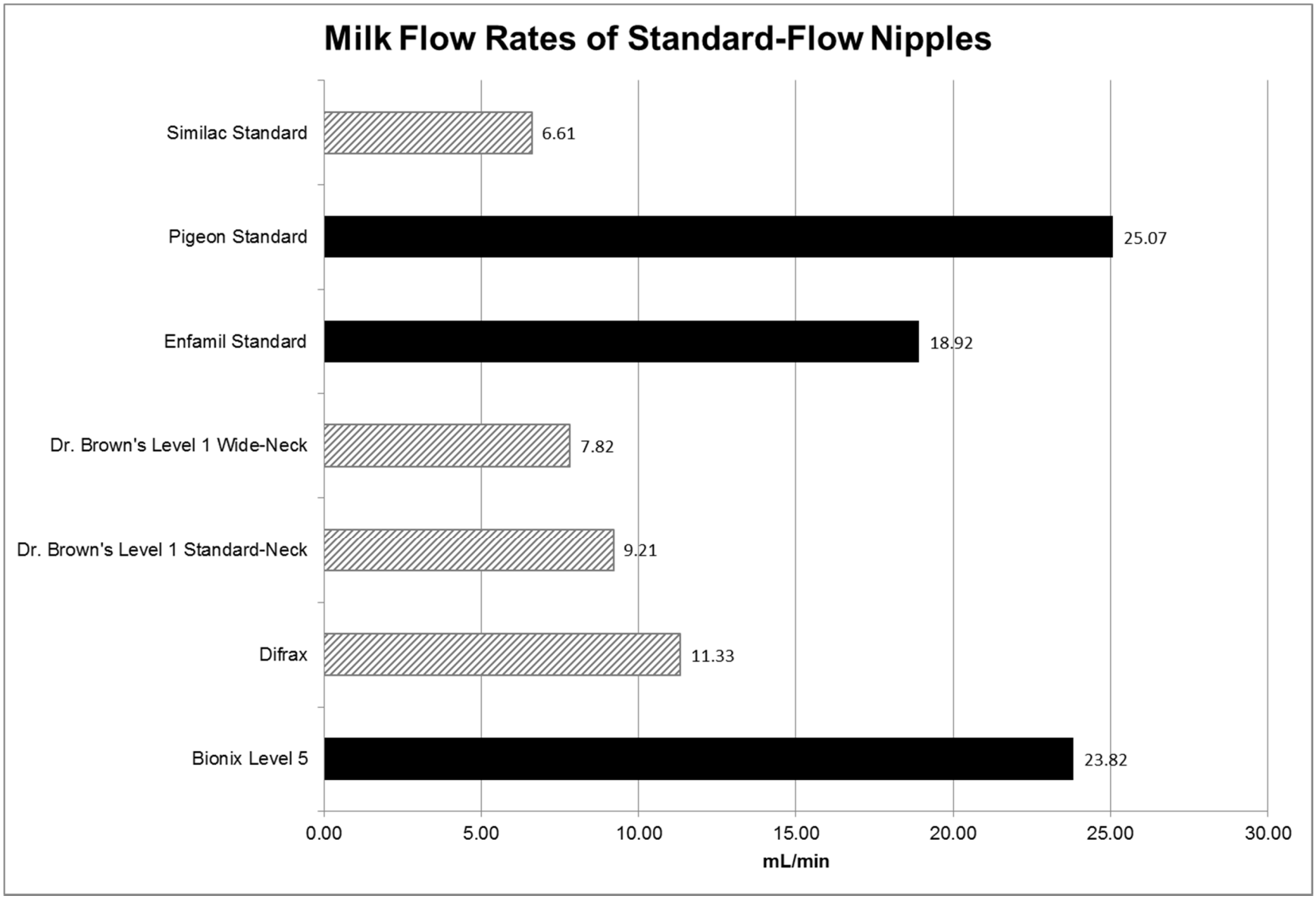

Figure 2.7. Milk flow rates of standard-flow nipples (mL/min). Nipples of the same color/pattern indicate that they are comparable in flow rate.

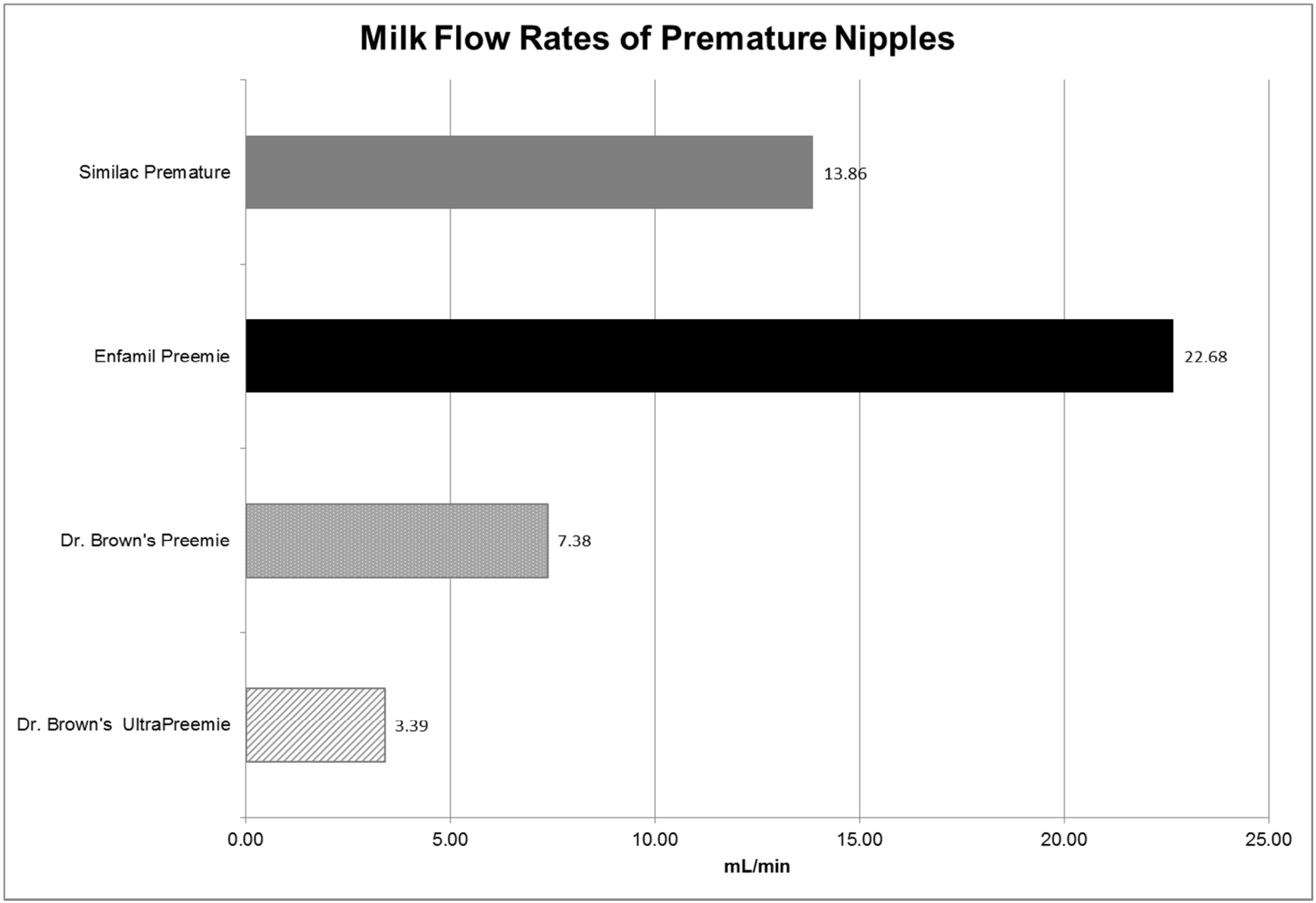

Figure 2.8. Milk flow rates of premature nipples (mL/min). Nipples of the same color/pattern indicate that they are comparable in flow rate.

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CHAPTER 3: EFFECTS OF MILK FLOW ON THE PHYSIOLOGIC AND BEHAVIORAL RESPONSES TO FEEDING IN AN INFANT WITH HYPOPLASTIC LEFT HEART SYNDROME

Overview

Infants with hypoplastic left heart syndrome often experience difficulty with oral feeding, which contributes to growth failure, morbidity, and mortality. In response to feeding difficulty, clinicians often change the bottle nipple (i.e., milk flow rate). Slow-flow nipples have been found to reduce the stress of feeding in other fragile infants, but no research has evaluated the responses of infants with hypoplastic left heart syndrome to alterations in milk flow. The purpose of this study was to evaluate the physiologic and behavioral responses of an infant with hypoplastic left heart syndrome to bottle-feeding with either a slow-flow (Dr. Brown's Preemie) or standard-flow (Dr. Brown's Level 2) nipple. A single infant was studied for three feedings: two slow-flow and one standard-flow. Oral feeding, whether with a slow-flow or standard-flow nipple, was distressing for this infant. During slow-flow feeding, she experienced more coughing events while during standard-flow, she experienced more gagging. Disengagement and compelling disorganization was most common during feeding 3 (Slow-flow), which occurred two days after surgical placement of a gastrostomy tube. Clinically significant changes in heart rate, oxygen saturation, and respiratory rate were seen in all feedings. Heart rate was higher during standard-flow and respiratory rate was higher during slow-flow. Further research is needed to examine the responses of infants with hypoplastic left heart syndrome to oral feeding and to identify strategies that will support these fragile infants as they learn to feed. Future research should evaluate an even slower flow nipple along with additional supportive feeding strategies.

Keywords: hypoplastic left heart syndrome, bottle feeding, feeding methods

Introduction

Each year, approximately 1,000 infants in the United States are born with hypoplastic left heart syndrome (HLHS) (Hoffman & Kaplan, 2002; Reller, Strickland, Riehle-Colarusso, Mahle, & Correa, 2008). HLHS is one of the most severe cardiac defects amenable to surgical palliation, and accounts for up to 25% of neonatal deaths associated with cardiac disease (Grossfeld, 2007). Infants with HLHS often experience profound feeding difficulties, which contribute significantly to growth failure, morbidity, and mortality (Davis, et al., 2008; Einarson & Arthur, 2003; Jadcherla, Vijayapal, & Leuthner, 2009; Skinner, et al., 2005). The phenomenon of feeding difficulties in this population is not well understood, however, and no research has examined the physiologic and behavioral responses of these infants to different feeding methods.

Feeding is a physiologically expensive event requiring coordination of sucking and swallowing with respiration to achieve nutritional intake while maintaining adequate oxygenation. When infants experience difficulty with bottle-feeding, one of the most commonly used strategies involves changing the type of bottle nipple (i.e., rate of milk flow). Altering the rate of milk flow has been shown to affect physiologic state during feeding in healthy, term infants (al-Sayed, Schrank, & Thach, 1994) and infants born prematurely (Kao, Lin, & Chang, 2010; Mathew, 1991a), but it remains unknown how infants with HLHS respond to different flow rates.

In healthy infants, increased milk flow results in increased rate of consumption at the expense of decreasing ventilation (al-Sayed, et al., 1994). During swallowing, the airway must close to prevent aspiration of fluid into the lungs. As flow rate increases and swallowing necessarily increases, the duration of airway closure also increases (al-Sayed, et al., 1994). The healthy infant with normal cardiorespiratory functioning is able to quickly recover from this decrease in ventilation during swallowing by increasing respiratory rate and tidal volume to maintain adequate oxygenation (Mathew, 1991b). The healthy, full-term infant is also capable of self-regulating the rate of milk flow during bottle-feeding by altering sucking rate (Schrank, Al-Sayed, Beahm, & Thach, 1998) and pressure (Colley & Creamer, 1958; Mathew, Belan, & Thoppil, 1992), allowing them to regulate the effect of milk flow on ventilation.

Premature infants, who have compromised respiratory function and immature neurologic and respiratory systems, have been found to have limited ability to self-regulate milk flow during bottle-feeding

and limited capacity to recover from the reduction in ventilation (Mathew, 1991a). Slowing the rate of milk flow during bottle-feeding has been found to reduce ventilatory compromise in preterm infants (Kao, et al., 2010; Mathew, 1991a). It is theorized that reducing the rate of milk flow delays swallowing until a critical volume of milk accumulates, therefore reducing the number of swallows and the time the airway is closed to ventilation (al-Sayed, et al., 1994; Lau & Schanler, 2000).

Infants with HLHS after stage 1 palliation are extremely fragile. They have decreased oxygenation at rest as a result of mixing of oxygenated and deoxygenated blood in a common atrium. They also frequently experience decreased cardiac output due to a single functioning ventricle and tachypnea as a result of increased pulmonary blood flow through the artificial systemic-pulmonary artery shunt (Pearl, Nelson, Schwartz, & Manning, 2002; Tweddell, et al., 2000). A further decline in oxygenation as a result of disruption of ventilation during swallowing, however minor, may cause considerable physiologic distress and given their poor cardiac output and tachypnea, their ability to recover is likely limited.

Slowing the rate of milk flow may enable the infant with HLHS to better maintain baseline ventilation requirements. Additionally, infants with HLHS are at risk for aspiration as a result of vocal cord injury (Sachdeva, et al., 2007) and swallowing dysfunction (Skinner, et al., 2005). Slowing the transit time of the bolus to the pharynx may allow the infant more time to coordinate a safe swallow and better protect the airway (Goldfield, Smith, Buonomo, Perez, & Larson, 2013). The effects of variations in milk flow on infants with HLHS remain unknown, and because their cardiac physiology is unique, these effects cannot be assumed based on other medically compromised infants. The purpose of this study was to examine the physiologic state changes (HR, oxygen saturation (Sp02), and respiratory rate (RR)) and behavioral indicators of distress (coughing, gagging, behavioral disorganization and disengagement) that occur during feeding and in the recovery period after feeding when an infant with HLHS is fed with either a slow-flow or standard-flow nipple.

Theoretical Framework

Polyvagal Theory (Porges, 1995) was used to conceptualize the physiologic and behavioral responses of an infant to stress during feeding. Polyvagal Theory explains that during times of low stress, the nucleus ambiguous (NA) pathway of the vagus predominates and allows for social communication by

providing tone to the muscles of the face, head, and ears (Porges, 1995). Input from the NA also supports feeding by coordinating sucking, swallowing, and breathing and preserves metabolic resources for growth by maintaining a low heart rate (HR) (Porges, 2007). Simultaneously, at times of low stress, limited input from the dorsal motor nucleus (DMNX) pathway of the vagus aids in digestion and absorption of nutrients from the gastrointestinal (GI) tract (Porges, 2001).

Conversely, in response to stressful situations, there is a hierarchical activation of the two stress response systems. Initially, the sympathetic nervous system (SNS) mobilizes resources to meet physiologic demands, resulting in increased HR, decreased heart rate variability (HRV), release of stress hormones, release of cytokines, and diversion of blood away from the GI tract to the more vital organs such as the heart, brain, and lungs. If the SNS response is not able to meet the demands of the situation, the DMNX is activated to conserve resources, resulting in disengagement, behavioral distress, hypotonia, apnea, and bradycardia (Porges, 2003). This theoretical framework was used to identify outcome variables as behavioral and physiologic indicators of stress related to feeding.

Methods

This was a single-case experimental design study where a single infant with HLHS was studied under two flow conditions: slow-flow and standard-flow. The infant was studied for three feedings, which allowed for replication of one of the conditions. Replication reduces threats to internal validity of study findings due to maturation and history (Tervo, Estrem, Bryson-Brockmann, & Symons, 2003). There were six permutations of the order in which the two conditions could occur over three feedings, with at least one of each flow condition (Barlow & Hayes, 1979; Lander, 1998). The order in which feedings occurred was randomized, which also aimed to support internal validity (Kratochwill & Levin, 2010). Institutional Review Board approval was obtained prior to the study.

Sample and Setting

This study was conducted at a pediatric tertiary care center in the Northeastern United States. The first available infant to meet inclusion criteria and whose parents consented to participation was included in the study. To be included in the study, the infant had to have been born full-term (≥ 37 weeks post-menstrual age (PMA)) with HLHS and to have survived stage 1 palliation. Infants were excluded if they were not orally feeding or if they had another major congenital anomaly that interfered with their

ability to feed orally (e.g., cleft palate). Additionally, infants were excluded from the study if there was not a parent able to consent who was over 18 years of age, because of concerns about their capacity for understanding the informed consent process.

Flow Conditions

Variability in milk flow within and among bottle nipples has been documented (Mathew, 1988; Pados, 2014). In order to maintain consistency between bottle-feedings of the same flow condition, Dr. Brown's (Handi-Craft Co., St. Louis, MO) nipples were used because they were found to have the most consistency in flow rates between nipples of the same type (Pados, 2014). The Dr. Brown's Preemie nipple was used for the slow-flow condition because this was the slowest Dr. Brown's nipple available at the time. Dr. Brown's Level 2 was chosen for the standard-flow condition because it was comparable in flow rate to the Enfamil Slow-Flow (Pados, 2014), which was typically used for feeding infants with HLHS in the unit where the study was conducted. It should be noted that the flow rate of the "standard-flow" nipple used in this study was slower than many standard-flow nipples used for feeding infants in the hospital (Pados, 2014). The infant's nurse was responsible for preparing the milk for each of the study feedings according to clinical orders and unit standards. The investigator chose the appropriate nipple according to the randomized flow assignment, assembled the bottle, and handed it to the feeder, who was blinded to the flow condition of the feeding.

Study Feeding Protocol

Feeders for the study feedings were informed of the Study Feeding Protocol (Appendix 3.1), which guided them to swaddle the infant and hold in a supported, flexed position, facing the feeder, and at a 45 degree angle. Feeders were asked to speak softly, minimize movement of the infant's body during feeding, and avoid manipulation of the nipple to encourage sucking. The infant was to be given a break if he or she displayed signs of physiologic distress, including tachycardia (HR > 200 bpm), bradycardia (HR < 80 bpm), a decrease in Sp02 more than 10% below baseline and to reinitiate only when the infant was physiologically stable and displayed readiness cues. A maximum time limit for study feedings was set at 30 minutes.

Variables & Measures

Four types of variables were collected: 1) infant characteristics, 2) feeding description, 3) behavioral outcomes, including feeder actions and infant behaviors, and 4) physiologic outcomes. Specific variables within each category are described in Table 3.1. The protocol for data collection is in Appendix 3.2.

Infant Characteristics. Descriptive data was collected on the infant to describe the pregnancy, birth history, and medical course, including primary and secondary diagnoses, surgical history, respiratory support requirements, echocardiogram results, lab work, and medications received. Data on the infant's feeding experience since birth was also collected, including type of feeding (bottle or breast), type and caloric density of milk, amount consumed orally, amount of milk administered via feeding tube, type of feeding tube, prescribed type and amount of milk, and any feeding complications.

Feeding Description. Data was collected during feeding to describe the three study feedings. The total *feeding duration* was defined as the duration of time from the first time the nipple was placed in the mouth until the last time the nipple was removed. Nipple-in duration was defined as the duration of time during the feeding period that the nipple was in the infant's mouth. Milk consumed (in milliliters (mL)) was defined as the amount of milk removed from the bottle by the infant as measured by the change in mL using a graduated bottle. Milk flow (mL/minute(min)) was defined as the amount of milk the infant consumed per minute of nipple-in (milk flow=milk consumed (mL)/nipple-in duration (mins)). In order for a study feeding to be included in the analysis, the milk flow had to exceed 0.3 mL/min. The presence of a nasogastric tube and the type of milk offered to the infant was recorded.

Behavioral Outcomes. A Panasonic Digital Video Camera HDC-TM700 was used for video recording. Starting approximately 30 minutes prior to the study feeding and continuing throughout the feeding period, a single camera was used to capture a close-up angle of the infant's face and upper trunk. An observational coding scheme (Appendix 3.3) was used to code the videos using the observational coding program, The Observer XT 11 (Noldus Information Technology Inc., Asheville, NC). Coders were trained in the observational coding scheme until they achieved ≥ 80% reliability using the kappa statistic.

***Feeder Actions*.** Actions of the feeder were evaluated during feeding to confirm that the feedings were performed in a similar way. Study feedings were video-taped and coded continuously for the

frequency (number of events) of stimulating the infant to suck, decreasing milk flow, and repositioning during the nipple-in period. The duration (in seconds (secs)) of burping and pacifier use was coded for the entire feeding period. At the end of the observation, the predominant positioning of the infants arms and shoulders, containment of the limbs in the swaddle, trunk positioning, angle of trunk, alignment of head, and anterior/posterior neck alignment was noted.

***Infant Behavior*.** Of the 30 minute observation period prior to feeding, 6 minutes was selected to represent baseline where the infant was quietly resting with minimal movement. The observational coding scheme was used to code for activity level (continuous coding; no movement or movement) and behavioral state (interval coding every 10 secs; categories defined in Table 3.2). The baseline period was described in terms of percent of time with no movement and percent of time in each behavioral state.

Videos during feeding were coded continuously for infant distress behaviors, including coughing (number of events and duration in secs) and gagging (number of events and duration in secs); infant engagement (proportion of the feeding in each level of engagement); and behavioral organization (proportion of the feeding in each level of organization). Definitions of the levels of engagement and organization are provided in Table 3.2.

Physiologic Outcomes. Physiologic variables included HR, Sp02, and RR. Physiologic data was analyzed during the 6 minute baseline period, throughout the feeding period, and for 30 minutes after feeding. The recovery period was defined as starting at the time the infant was settled into her bed after feeding and caregiving activities were completed and continuing for 30 minutes.

HR data were collected by a three lead electrocardiogram (ECG) using the BioNex Bio-Potential Amplifier (MindWare Technology, Gahanna, OH) and sampled at 1,000 samples per second. The data were digitized by an amplifier, converted to an analog waveform, and stored using BioLab Data Acquisition Software (MindWare Technology, Gahanna, OH). ECG wave complexes were imported into MindWare HRV 3.0.20 (MindWare Technology, Gahanna, OH) and R peaks were marked by the program. The investigator confirmed each R peak of the QRS complex and cleaned the data of artifact. The cleaned data were used to calculate indices of HR, including HR mean, minimum, maximum, and coefficient of variation (CV; calculated as SD divided by the mean), for every 1 minute during the baseline and feeding periods and every 2 minutes during recovery. A change from baseline score was calculated

for feeding as the mean HR during the entire feeding period minus the mean HR during the baseline period. A change from baseline score was also calculated for the recovery period. Additionally, number of bradycardic events, defined as a heart rate < 100 bpm (Dawson, et al., 2013) were calculated. Bradycardic events had to be separated by at least 5 seconds of HR data above 100 bpm to be considered two events.

Sp02 was collected using the Radical-7 Pulse Co-Oximeter (Masimo Corporation, Irvine, CA) at a sampling rate of 1,000 samples per second and an averaging window set at two samples per second. This co-oximeter is reported by the manufacturer to provide Sp02 data with an accuracy of ± 2% in the event of low perfusion, which is critical for the population of infants with HLHS who frequently experience poor perfusion (Masimo, 2012). As with the HR data, Sp02 data was digitized by the amplifier, converted to analog waveform, and stored using BioLab Data Acquisition Software (MindWare Technology, Gahanna, OH). The data was cleaned of artifact by the investigator and the mean, minimum, maximum, SD, and CV of Sp02 were calculated for every 1 minute during baseline and feeding and every 2 minutes during recovery. A change from baseline score was calculated for feeding as the mean Sp02 for the entire feeding period minus the mean Sp02 during baseline. Change from baseline Sp02 was calculated for the recovery period as well.

Mean Sp02 during all three baselines was calculated to create categories of fluctuations in Sp02 data that represent an increase in Sp02 5-10% above baseline, increase in Sp02 > 10% above baseline, a decrease in Sp02 5-10% below baseline, and a decrease in Sp02 > 10% below baseline. The Sp02 data was imported to The Observer XT 11 observational coding program and coded using the categories described. The frequency of events of Sp02 within each of these categories was calculated as well as the percentage of the feeding that the infant's Sp02 was in each category.

RR was collected using the Ambu® Sleepmate RIPmate™ Inductance Belts system (Ambu Inc., Glen Burnie, MD) sampled at 1,000 samples per second. This monitoring system uses respiratory inductance plethysmography (RIP) to measure movement of the thoracic cavity related to respiratory effort via an elastic band placed around the chest. Respiratory data was digitized by the amplifier and stored as an analog waveform using BioLab Data Acquisition Software (MindWare Technology, Gahanna, OH). AcqKnowledge software (BIOPAC Systems, Inc., Goleta, CA) was used to mark the peak, or point of

maximal chest circumference, during each breath. In order to consistently apply a rule to the waveform data regarding whether a chest movement qualified as an adequate respiratory cycle, a period of stable, regular breathing was identified during each baseline period. The mean voltage change for a breath during this regular breathing period was calculated and a chest movement had to be associated with a voltage change of at least 20% of this mean in order to be marked as a peak (Bamford, Taciak, & Gewolb, 1992) (Appendix 3.4). The waveform data was used to calculate RR in breaths per minute. The mean, minimum, and maximum RR were calculated for every 1 minute during the baseline period, during each nipple-in period of the feeding, and every 2 minutes during recovery. Nipple-out periods during feeding are typically when burping and other movement of the infant occurs and therefore, more artifact; his data was not included in the analysis. Change from baseline RR was calculated during feeding as well as during recovery. Additionally, frequency of apneic events**,** defined as greater than three seconds between consecutive respiratory waveform peaks (Nixon, Charbonneau, Kermack, Brouillette, & McFarland, 2008), was calculated.

Statistical Analysis

The analysis of this single-case experimental study occurred in two phases. In Phase 1, the outcome variables were described per feeding using descriptive analyses with the goal of elucidating the ways in which physiology and behavior changed in relation to flow condition. Graphic analysis of data is an accepted method of descriptive analysis of single-subject experimental data (Kratochwill, Hitchcock, Horner, Levin, Odom, Rindskopf, & Shadish, 2010; Tervo, et al., 2003). Meaned values for measures during the baseline, feeding, and recovery periods were tabled. Repeated measures physiologic data were graphed to illustrate change over time. A quadratic regression line was fit for each set of physiologic data to evaluate the direction of change and steepness (i.e., slope) of change over time.

In Phase 2, the repeated measures physiologic data were analyzed using linear mixed modeling (LMM) to evaluate the effect of flow condition on each physiologic outcome. For this analysis, data were grouped by flow condition. Correlation between measurements within the same feeding, and within the same flow condition were taken into account. Additionally, baseline values of the outcome variable being tested were included as covariates in the model. The procedures for the LMM analyses are available in Appendix 3.5.

Results

Infant Characteristics

Baby G was a full-term (39 3/7 weeks PMA) female born via vaginal delivery following labor induction to a 30 year-old Caucasian primagravida. The pregnancy was complicated by a prenatal diagnosis of HLHS found during the anatomical ultrasound between 18-19 weeks PMA. At 21 weeks, a fetal surgical intervention was performed with balloon dilation of the aortic valve. The post-surgical course was uncomplicated. At birth, Baby G was appropriately grown with a birth weight of 3240 grams (50^{th} percentile), head circumference of 36 cm (96^{th} percentile), and length of 48.5 cm (36^{th} percentile). She did not require resuscitation at birth. Apgar scores were 8 at 1 min and 9 at 5 mins.

An echocardiogram after delivery confirmed HLHS with aortic stenosis. The infant was intubated and taken to the catheterization laboratory where balloon dilation of the aortic valve was repeated and a stent was placed across the atrial septum under fluoroscopy. Following the catheterization, the echocardiogram reported a small left ventricle (LV) with endocardial fibroelastosis, aortic stenosis with a gradient of 70 mm Hg, and no aortic insufficiency.

Despite the efforts to dilate the aortic valve and support growth of the LV, her cardiac function remained poor and on day of life (DOL) 10, the infant underwent a stage 1 Sano Damus-Kaye-Stansel procedure. Her procedure was altered from the typical Norwood procedure for HLHS in the hope to continue to grow the LV for an eventual two ventricle heart. The procedure involved: 1) transection of the main pulmonary artery (MPA), patch closure of the distal MPA, and placement of a right ventricle to pulmonary artery (RV-PA) conduit for pulmonary circulation 2) division of the proximal MPA and anastomosis to the ascending aorta (both the MPA and aortic arch were patch augmented), 3) aortic valvulotomy and commiseration of aortic valve fusion, 4) patent ductus arteriosis ligation, and 5) removal of atrial stent and resection of the atrial septum. An illustration of her post-surgical cardiac anatomy is provided in Figure 3.1. Total cardiopulmonary bypass time was 3 hours and 11 mins. She did not experience any major complications during the procedure. Sternal closure was delayed per standard care at this institution due to typical swelling of the intrathoracic structures. She was extubated on DOL 24 to continuous positive airway pressure. On DOL 25 she was transitioned to nasal cannula and then transitioned to room air on DOL 27. Her most recent echocardiogram at the time of the study revealed a

mild to moderate hypoplastic LV with endocardial fibroelastosis, hypoplastic aortic valve annulus, mild aortic regurgitation, patent aortic arch, mild tricuspid valve regurgitation, right ventricular dilation and hypertrophy with good RV function, and mild to moderate LV dysfunction. Her course was also complicated by postoperative chylothorax, hypothyroid, gastroesophageal reflux, and neonatal abstinence syndrome following prolonged perioperative narcotic exposure.

Oral feeding was initiated on DOL 26 while on nasal cannula and for the first 10 days of oral feeding, she took 0-15 mL by mouth per feeding. On DOL 38, the infant was evaluated by the feeding team; they noted that the infant did not readily open her mouth for the feeding, showed no rooting response, used a munching motion when the nipple was placed on the hard palate, had pooling in the oral cavity, and gagged three times, but did not show overt signs or symptoms of aspiration. She continued to be offered oral feedings. Study feeding 1 occurred on DOL 41 after the infant was consistently taking ≥ 20 mL per feeding for two days. Feeding 2 occurred on DOL 42, and Feeding 3 occurred on DOL 46. Feeding experience data at the time of each study feeding is presented in table 3.3. All of her oral feeding experience was with bottle-feeding and a variety of bottle nipples had been used. The lapse of time between study feeding 2 and study feeding 3 was a result of the infant having a gastrostomy tube surgically placed. During this time, the infant was reintubated for the procedure and was not given any fluids enterally for 24 hours.

At the time of the study feedings, the infant was regularly receiving the following medications: Captopril for afterload reduction, Lasix for diuresis, Levothyroxine for treatment of hypothyroid, Omeprazole for treatment of gastroesophageal reflux, Aspirin for anticoagulation given the artificial RV-PA conduit, Simethicone for treatment of gastrointestinal discomfort, and Acetaminophen as needed for pain management. She had received her last dose of a methadone taper for treatment of neonatal abstinence syndrome on DOL 39. In the evening of DOL 41, approximately 12 hours after study feeding 1 and 12 hours prior to study feeding 2, her Cardiac Children's Hospital Early Warning Score (C-CHEWS) (McLellan & Connor, 2013) was rated a 3 and she was given a rescue dose of methadone. Otherwise, in the 24 hours prior to and following each study feeding, her C-CHEWS score was rated 0-1 by the infant's nurse.

Laboratory values for electrolytes, hematology, and thyroid function were within normal limits at

the time of the study, with the exception of elevated bicarbonate and decreased chloride levels. This mild electrolyte imbalance is a physiologic response to normalize pH in the presence of abnormal oxygen/carbon dioxide balance as a result of her cardiac anatomy (Adams, Swan, & Hall, 1970). Her most recent venous blood gas was acceptable given her cardiac disease.

Feeding Description

The infant was randomized to the following order of flow conditions- Feeding 1: Slow-flow; Feeding 2: Standard-flow; and Feeding 3: Slow-flow. The flow conditions are referred to as Slow and Standard. Feeding description data is available in Table 3.4. The nipple-in duration of feeding 1 (Slow) was nearly twice as long as feedings 2 (Standard) or 3 (Slow), however the infant consumed approximately the same amount of milk for all three of the feedings. As a result, the milk flow rate for feeding 1 (Slow) was slower than for feedings 2 (Standard) or 3 (Slow). A nasogastric (NG) tube was present in her left nare for feeding 1(Slow), but during feeding 2 (Standard) it had been removed to trial her ability to meet nutritional requirements by oral intake. At the time of feeding 3 (Slow), she had a gastrostomy tube in place and therefore no longer required a NG tube. Skimmed breastmilk 30 calories/ounce was offered for all three study feedings. Breast milk was skimmed of long-chain fatty acids, which can reaccumulate as chyle in the pleural space in the presence of chylothorax, and replaced with medium-chain triglycerides (Chan & Lechtenberg, 2007).

Behavioral Outcomes

Feeder Actions. Feedings 1 (Slow) and 2 (Standard) were performed by the same speech-language pathologist and member of the feeding team. Feeding 3 (Slow) was performed by the infant's mother, who had been present at the infant's bedside throughout her hospitalization and had fed her more often than anyone else. All three feedings were performed with the infant in a supine position at approximately a 45 degree angle. The infant was swaddled with the shoulders and arms supported throughout feedings 1 (Slow) and 2 (Standard). During feeding 3 (Slow), the infant was more loosely swaddled and over the course of the feeding, the swaddle became less supportive. Throughout all three feedings, her head was in a midline, neutral position appropriate for feeding. During feeding 3 (Slow), the feeder spent more time burping the infant than during either feeding 1 (Slow) or 2 (Standard) and the infant was given a pacifier for a longer period of time during feeding 3 (Slow) (Table 3.5). Otherwise, the

number of events of stimulating the infant to suck, decreasing milk flow, and repositioning were not considerably different between the feedings.

Infant Behavior. During the 6 minute baseline period prior to feeding 1 (Slow), the infant was in active sleep 100% of the time and had no movement 97.3% of the time. During the baseline period prior to feeding 2 (Standard), the infant was in active sleep 100% of the time and had no movement 75.9% of the time. In the baseline period prior to feeding 3 (Slow), the infant was in active sleep 50% of the time, was alert 3%, and in a drowsy daze 47% of the time; she had no movement 71.5% of the time.

Baby G had seven coughing events during feeding 1 (Slow), with a total duration of coughing of 50.5 secs, compared to two coughing events during feeding 2 (Standard; duration 15.2 secs), and one very brief event (1.13 secs) during feeding 3 (Slow) (Table 3.6). She gagged more during feeding 2 (Standard; four events; duration 12.7 secs) compared to one event of gagging (2.2 secs) during feeding 1 (Slow) and none during feeding 3.

In terms of her engagement in the feeding, she was most fully engaged during feeding 2 (Standard) (96%) (Table 3.6). During feeding 1 (Slow), she was fully engaged for 91.6% of the nipple-in time. She was least fully engaged during feeding 3 (Slow; 44.8%). In all three feedings, she was either fully engaged or disengaged; there was no low engagement in any of the feedings. During feeding 3 (Slow), she was disengaged and avoiding the feeding during 55.2% of the nipple-in time.

Behavioral organization during nipple-in periods followed a similar pattern as engagement. During feeding 2 (Standard), she was fully organized for 29.3% of the feeding, while she was only fully organized for 4.8% of feeding 1 (Slow). She was never fully organized during feeding 3 (Slow). She showed signs of compelling disorganization during nearly half (47.9%) of feeding 3 (Slow), while only 5.5% of feeding 1 (Slow) and 1.9% of feeding 2 (Standard).

Physiologic Outcomes

HR. Mean HR increased from baseline to feeding during all three feedings and then decreased to below baseline values during all recovery periods (Table 3.7). During feedings 2 (Standard) and 3 (Slow), mean HR increased similarly from baseline to feeding (10.2 vs. 9.8 bpm, respectively). Mean HR increased the least during feeding 1 (Slow; 4.9 bpm). In Figure 3.2, mean HR was plotted over time and a quadratic regression line was fit to the data, which revealed a concave curve for all three feedings (i.e.,

mean HR increased from baseline to feeding and decreased during recovery). Consistent with the mean HR values, Feeding 3 (Slow) had the steepest curve, indicating the greatest change from baseline to feeding, and feeding 1 (Slow) had the flattest curve, indicating the least change per unit of time (Figure 3.2). The infant did not experience bradycardia during any of the analyzed periods, but did have transient elevations in HR above 190 bpm during both feedings 1 (Slow; maximum HR 194.2 bpm) and 2 (Standard; maximum HR 191.1 bpm).The CV increased from baseline to feeding during all feedings as well, indicating greater variation in HR during feeding. The CV of HR remained elevated above baseline during the recovery periods of all three feedings and were similar in value (Table 3.7). In the Phase 2 analysis, LMM was used to evaluate the effect of flow condition on HR during feeding, covaried on baseline HR. The mean HR during feeding was significantly higher during standard-flow feeding than during slow-flow feeding (p=0.01) (Table 3.8).

Sp02. Mean Sp02 during feeding was highest during feeding 1 (Slow; 87.7%) compared to feeding 2 (Standard; 83.5%) and feeding 3 (Slow; 84.4%) (Table 3.9). During feeding 1 (Slow), the Sp02 increased from baseline by 3.76%, while during feeding 2 (Standard), Sp02 decreased by 3.28%, and it remained essentially unchanged during feeding 3 (Slow; 0.03%). Minimum Sp02 was the same (70.7%) for both feedings 1 (Slow) and 2 (Standard), while Sp02 dropped to a minimum of 62.8% during feeding 3 (Slow). Maximum Sp02 was higher during feeding 1 (Slow; 98.6%) compared to feedings 2 (Standard) and 3 (Slow) (91.6% for both). The CV of Sp02 was similar across all feedings, but in all cases, increased from baseline to feeding and decreased during recovery. Mean Sp02 was plotted over time in Figure 3.3 and a quadratic regression line fit to the data revealed a concave curve for feeding 1 (Slow) (i.e., Sp02 increased from baseline to feeding and decreased during recovery) and a convex curve for feeding 2 (Standard) (i.e., Sp02 decreased from baseline to feeding and increased during recovery). The regression line for feeding 3 (Slow) was also convex, but was nearly flat as it decreased from baseline to feeding and then continued to decrease during recovery.

The grand mean for baseline Sp02 for all feedings was calculated to be 84.7% (range 83.8 – 86%) with 81 – 88.9% being equivalent to +/- 5% of baseline. During feeding 1 (Slow), the infant had 3 events with the Sp02 rising greater than 10% above baseline (i.e., >93%), which accounted for 8% of the feeding period (Table 3.9). There were desaturation events greater than 10% below baseline (<76%) in all

feedings, accounting for 2.3% of each feeding 1 (Slow) and 2 (Standard) and 4.7% of feeding 3 (Slow). In Phase 2, the LMM analysis revealed a trend toward a significant effect of flow on Sp02 (p=0.1), with the Sp02 being higher during slow-flow feedings (Table 3.8).

RR. Mean RR was high during all three feedings, ranging from 68.4 breaths/min during feeding 1 (Slow) to 75.8 breaths/min during feeding 3 (Slow) (Table 3.9). The infant increased her RR during feeding compared to baseline for all three feedings. The change from baseline to feeding was greatest for feeding 2 (Standard; 18.5 breaths/min) and similar for feedings 1 (Slow; 4.4 breaths/min) and 3 (Slow; 3.6 breaths/min). Mean RR returned to near or below baseline during the recovery periods of feedings 2 (Standard) and 3 (Slow), but remained elevated above baseline during the recovery period after feeding 1 (Slow). In Figure 3.4, mean RR was plotted over time and revealed a nearly flat regression line for feeding 1 (Slow) with a slightly positive slope, although a closer look at the repeated measures reveals considerable variation in the mean RR values at each time point. The infant had one apnea during the first nipple-in episode of feeding 1 (Slow), where mean RR for this episode was 31 breaths/min. Otherwise, she did not have any apneic episodes during feeding. Consistent with the greatest changes in mean RR during feeding 2 (Standard), the regression line for feeding 2 had the steepest curve and was concave (i.e., increased from baseline to feeding, then decreased during recovery) (Figure 3.4). Feeding 3 (Slow) also had a concave curve, but was less steep (Figure 3.4).

Although apneic episodes were rare during feeding, this infant did have 2 apneic episodes during the baseline period prior to feeding 1 (Slow) and three episodes during the baseline period prior to feeding 2 (Standard). She had no apneic episodes prior to feeding 3 (Slow). The frequency of apneic episodes during the baseline period of feeding 2 (Standard) may explain the relatively low mean RR of 53 breaths/min, and resultant large increase from baseline to feeding. During the 30 minute recovery period, she had five apneic episodes after feeding 1 (Slow), eight after feeding 2 (Standard), and only one after feeding 3 (Slow). In the Phase 2 analysis, LMM revealed that there was a statistically significant effect of flow condition on RR during feeding (p=0.09); she had a higher RR during slow-flow feeding than during standard-flow, when covaried on baseline RR.

Discussion

Feeding was a challenging event for this infant and was associated with adverse events, such as

coughing and gagging, and evidence of physiologic expense and behavioral disorganization regardless of whether she was fed with a slow-flow or standard-flow nipple. There is evidence throughout the literature that infants with HLHS have difficulty feeding (Davis, et al., 2008; Jadcherla, et al., 2009), but this is the first study that we know of that has looked closely at the physiologic and behavioral effects that occur as an infant with HLHS faces the challenge of oral feeding.

The medical course of this infant with HLHS was not atypical for infants with similar cardiac disease. Her intubation time was somewhat lengthy as a result of her slightly delayed stage 1 procedure and the presence of chylothorax postoperatively, but she did not experience any life-threatening complications and had the benefit of some left ventricular function.

In comparing the feedings to one another, the feeders' actions were not considerably different. During feeding 3 (Slow), the infant's mother offered her a pacifier and burped her more often than during feedings 1 (Slow) and 2 (Standard). It is speculated that this was done in an attempt to help the infant to organize her behavior for the feeding. The behavioral outcomes of feeding 3 (Slow) show that the infant was never fully organized. Between feedings 2 (Standard) and 3 (Slow), the infant was reintubated for placement of a gastrostomy tube and feeding 3 was particularly difficult for her. Her behavioral state prior to feeding 3 (Slow) was also different with her being in active sleep only 50% of the time, compared to 100% of both baseline periods prior to feedings 1 (Slow) and 2 (Standard). This change in behavioral state during rest may be indicative of pain related to her abdominal incision, since she was given acetaminophen immediately prior to the feeding and it may not have had the time to take effect. When a pacifier was offered, she was able to calm down and appeared ready for feeding, but as soon as flow started when the bottle nipple was placed in her mouth, she would become disorganized again.

The behavioral outcomes provided important information about this infant's response to feeding. Feeding was behaviorally distressing for Baby G. She coughed with concerning frequency during feeding 1 (7 events accounting for 50.5 seconds), despite the use of a slow-flow nipple. Given her surgical history, she is at high risk for aspiration due to vocal cord and/or swallowing dysfunction (Carpes, et al., 2011; Sachdeva, et al., 2007; Skinner, et al., 2005). Although the feeding team evaluation prior to the study did not find evidence of aspiration, at the time of the study, she had not had an evaluation of her vocal cord movement or a swallow study to evaluate the safety of her swallow during feeding. One

difference between feeding 1 (Slow) and the other two study feedings was the presence of a nasogastric tube during feeding. Theoretically, the presence of a nasogastric tube in the posterior oropharynx may alter the anatomy during swallowing and increase the risk of aspiration, which may have contributed to the increased coughing seen in feeding 1 (Slow).

Clinicians need to be vigilant in assessing for evidence of aspiration events, which have the potential to not only be life-threatening in these very high-risk infants, but also be experienced negatively by the infant, which has ramifications for future engagement in oral feeding. Some institutions have moved away from the use of videofluroscopic swallow studies because of radiation exposure. If fluoroscopy is to be avoided, new techniques for evaluating the safety of swallowing during feeding in infants need to be developed. For example, pharyngo-esophageal micromanometry may be a useful method for evaluating swallowing and peristalsis (Barlow, 2009).

Baby G had 4 gagging episodes during feeding 2 (Standard), which was also concerning. The gag reflex is a protective mechanism to prevent aspiration and choking and occurs when food or fluid remains in the posterior oropharynx after swallowing (Morris & Klein, 2000). The higher flow rate of the standard-flow condition may have resulted in pooling of milk in the posterior oropharynx, especially if she was having difficulty swallowing completely, which initiated the gag reflex. Aside from being an indication that this infant was at high risk for aspiration, gagging is a negative experience during feeding (Byars, et al., 2003). This infant had multiple factors that put her at risk for aversion to oral feeding, including prolonged intubation, multiple intubations, gastroesophageal reflux, prolonged nasogastric tube use, and prolonged period without oral feeding (Dodrill, et al., 2004; Einarson & Arthur, 2003; Hyman, 1994; Jadcherla, Wang, Vijayapal, & Leuthner, 2010). Infants with HLHS are frequently found to experience long-term feeding difficulty (Hill, et al., 2014; Maurer, et al., 2011), and negative experiences like gagging during feeding should be actively avoided to prevent the development of long-term feeding disorders.

As discussed previously, feeding 3 (Slow) was particularly difficult for this infant and she displayed indicators of disengagement, avoiding feeding and compelling disorganization during a significant proportion of this feeding, despite being slow-flow. This study feeding occurred about 48 hours after placement of the gastrostomy tube, which required a reintubation. A number of factors associated with the procedure, including an additional negative oral experience, pain at the incision site, and swelling

of the pharyngeal structures, may have contributed to the difficulty that she experienced during feeding 3. Feedings 1 (Slow) and 2 (Standard) were relatively similar with regards to her level of engagement in the feeding and, although she was organized for more of feeding 2 than feeding 1 (29.3% vs. 4.8%), she was compellingly disorganized during very little of either of those two feedings.

Physiologically, feeding was a challenge for this infant and, consistent with Polyvagal Theory (Porges, 1995), her SNS response to this challenge was to increase HR in order to meet physiologic demands. Normal HR range for young infants is considered 110-150 bpm (Park, 2010) and her HR at baseline was at the high end to above normal (range 137.3 – 172.9 bpm). Her HR increased during feeding to 141.8 – 194.2 bpm. In this case, an increase in HR much above baseline was potentially problematic because when the HR rises above approximately 180 bpm, ventricular filling time and end-diastolic volume are diminished and myocardial oxygen consumption increases (Gupta, 2014). Since myocardial perfusion occurs during the diastolic phase, increases in HR above 180 bpm have the potential to cause cardiac ischemia and ventricular dysfunction (Gupta, 2014). In an infant recovering from open heart surgery and with pre-existing diminished ventricular function, cardiac protection is critical. The results of the HR data suggest that feeding 1 (Slow) allowed this infant to maintain her HR closest to baseline and the LMM analysis also revealed that slow-flow bottle-feeding allowed this infant to maintain a lower HR during feeding than standard-flow feeding (p=0.01), indicating that slow-flow feeding may have been less physiologically taxing.

The results of the respiratory indices were less clearly supportive of either flow condition. The unique cardiac physiology of infants after stage 1 palliation for HLHS requires a careful balance of pulmonary to systemic blood flow ratio (Q_p:Q_s). In this infant, since the RV was essentially providing blood flow to both the pulmonary and systemic circulations, the vascular resistance in each of these systems helped to determine the ratio of blood that flowed in either direction. Management of infants after stage 1 palliation aims to maintain Q_p:Q_s = 1, which is generally achieved with a systemic arterial oxygen saturation of approximately 75-80% (Photiadis, et al., 2006).

Sp02 as measured in this study is a measure of arterial oxygen saturation. During baseline, this infant's mean Sp02 was 83.8 – 86%, which was already higher than the target range (Photiadis, et al., 2006). Given the fragile balance of Q_p:Q_s, the goal for Sp02 during feeding should be to remain as close

to baseline as possible. Sp02 outside of the range of 75-90% is considered concerning in this population (Feinstein, et al., 2012). These infants are already hypoxic and further significant decreases in oxygenation are potentially detrimental to oxygen delivery to the systemic and coronary perfusion. Similarly, increases in Sp02 above 90% are also problematic, as they suggest preferential shunting of blood to the pulmonary circulation, which may result in excessive pulmonary blood flow and reduced systemic perfusion. In addition to the obvious issues with reduced systemic perfusion and/or oxygen delivery, such as perfusion of the brain and heart, adequate perfusion and oxygen delivery to the gastrointestinal tract is critical for the infant to utilize the nutrients that are ingested during feeding.

Given the constraints of the physiology in this patient, the LMM results of the analysis of Sp02 indicating a trend toward higher Sp02 during slow-flow feedings (p=0.1) was not necessarily better. During feeding 1 (Slow), the infant had frequent increases in Sp02 greater than 5% above baseline (>89%). This was surprising given the number and duration of coughing events. Coughing can be a sign of aspiration, which is typically associated with decreases in Sp02. In this infant, coughing events resulted in a rise in Sp02. The mechanism behind this is unclear. It may be that the infant had small residual pleural effusions and coughing resulted in increased lung volume and therefore a temporary fall in pulmonary vascular resistance, resulting in increased Sp02 (Gabrielli, et al., 2009). During feeding 3 (Slow), the infant only had 1 brief coughing event, but still had several increases in Sp02 greater than 5% above baseline. The maximum Sp02 during feeding 3 (Slow), however, was 91.6% compared to 98.6% in feeding 1 (Slow). During feeding 2 (Standard), the infant had rare events above 89% or below 76%.

The results of RR during feeding also suggested that both feeding conditions caused significant physiologic distress. The LMM analysis revealed that RR was higher during slow-flow feedings (72.1 vs. 71.5 breaths/min; p=0.09), however the change from baseline to feeding was greatest for feeding 2 (Standard) (18.5 breaths/min). Normal RR for infants is considered to be approximately 24-50 breaths/min (Bardella, 1999). On one hand, since Baby G's RR was above normal, the higher RR during slow-flow feeding could be interpreted as being an indicator of this feeding condition being more physiologically-demanding and requiring the infant to therefore increase RR accordingly. On the other hand, this could be interpreted as being supportive of physiologic stability because the infant is able to breathe during feeding. Some medically fragile infants are unable to coordinate breathing with sucking and swallowing

during feeding and experience a decrease in RR (Mathew, 1991a).

This infant's mean RR across all three feedings was approximately 72 breaths/min, which is equivalent to one breath every 0.83 seconds. In normal infants, swallowing behavior is dominant to respiration in order to protect the airway from aspiration, and respiration is paused for about 0.5-1.5 seconds during swallowing (Barlow, 2009). At this rate of respiration, it would have been very difficult, if not impossible, for her to fit a safe swallow in between breaths. Since she was hypoxic at rest, she may have been less able to tolerate a decrease in respiration during feeding than infants who are normally oxygenated at the start of feeding. RR does not give any indication of how safely the infant is breathing during feeding and it may have been that her need to breathe was so great that she did so during feeding despite the risk for aspiration. The coordination of sucking, swallowing, and breathing is controlled by a complex system of interacting nervous system networks that are dependent on sensory experiences to facilitate pattern development (Barlow, 2009). Given that this infant was intubated for the first 24 days of life, she had been deprived of early oral sensory input that would have supported the development of these patterns for safe swallowing and breathing.

There were limitations to the respiration data, however, and these results should be interpreted with caution. Respiratory rate was evaluated by chest movement using respiratory inductance plethysmography. Although a minimum change in chest movement was used to identify a respiratory cycle, this method did not identify a change in tidal volume. There may have been considerable alterations in minute ventilation due to interrupted or shallow breaths during feeding that were not identified. The reliability of RIP for measuring chest wall movements in infants who have undergone a median sternotomy and have the potential for increased pliability of the chest wall may warrant further investigation.

Another limitation of the study was the time between study feedings, particularly between feedings 2 and 3. The effect of maturation and history could have been limited by performing all three study feedings in one day, but the research team and clinical staff were concerned that this would unnecessarily stress an already vulnerable baby. As a single case experiment, there are natural limitations to the study data, but this design allowed for close evaluation of this infant's response to the different feeding conditions while considering the variable context of each feeding.

Conclusion

Although this was a single-subject experiment and the results cannot be assumed to reflect the feeding responses of all infants with HLHS, this study provides data that reflects what many clinicians have long observed- infants with HLHS can experience significant distress during feeding. This is the first extensive examination of the physiologic and behavioral responses of an infant with HLHS to the challenge of oral feeding. Despite the use of a very slow-flow nipple (Dr. Brown's Preemie) for the slow-flow feeding condition, this infant experienced significant distress during feeding. Feeding typically occurs every three hours in young infants and this degree of distress eight times a day is highly problematic for this fragile infant. Feeding interventions aimed at reducing physiologic and behavioral stress, preserving metabolic resources, and encouraging positive oral experiences are needed to support infants with HLHS, especially during the tumultuous time between stage 1 and stage 2 palliative procedures.

The results of this study suggest that future studies should consider testing an even slower flow nipple combined with additional supportive feeding interventions. A semi-elevated side-lying position has been found to support physiologic stability in infants born premature by slowing the movement of the bolus to the back of the oral cavity and reducing the work of breathing (Park, Thoyre, Knafl, Hodges, & Nix, 2014). In the population of infants with HLHS, a right side-lying position with the left vocal cord positioned up may be supportive for these same reasons as well as minimizing the risk of aspiration resulting from any malfunction of the left vocal cord; positioning the functioning right vocal cord down utilizes it for primary protection of the airway.

A right side-lying position is easily attained during breastfeeding with the infant positioned in a cross-body/cradle hold while feeding from the left breast or a football hold when feeding from the right breast. Although there is evidence that breastfeeding may reduce the stress of feeding in preterm infants (Chen, Wang, Chang, & Chi, 2000; Meier, 1988) and infants with other types of congenital heart disease (Marino, O'Brien, & LoRe, 1995), clinicians continue to express concern about allowing infants with HLHS to feed at breast. Research needs to be done to evaluate whether these concerns are valid for this population. With the use of test weighing (Meier, Lysakowski, Engstrom, Kavanaugh, & Mangurten, 1990), there should be no concern about ability to assess intake and there is the potential for highly significant benefits to both mother and baby with breastfeeding (Ip, et al., 2007).

Future studies also need to evaluate the coordination of sucking, swallowing, and breathing in this population of infants that have been deprived of early oral feeding experience. These infants may benefit from a co-regulatory approach to feeding with close evaluation by the feeder of sucking, swallowing, and breathing behavior (Thoyre, Holditch-Davis, Schwartz, Melendez Roman, & Nix, 2012). Using this approach, the feeder could cue the infant to rest (i.e., pacing) and recover before he or she becomes overtly distressed.

The results of this study support the need for further investigation of the responses of infants with HLHS to oral feeding and the development of feeding methods to support these extremely fragile infants as they learn to feed orally and await further surgical intervention. Identifying methods to reduce physiologic and behavioral distress during feeding in these babies has the potential to facilitate recovery and growth, as well as prevent long-term neurodevelopmental and feeding difficulties. Feeding has been identified by parents of infants with CHD to be a source of stress (Medoff-Cooper, Naim, Torowicz, & Mott, 2010; Svavarsdottir & McCubbin, 1996). Emphasis should be placed on identifying feeding strategies that are simple, easily implemented by parents, and will reduce, not increase, parenting stress.

Table 3.1.

Specific Measures

Variable	Measure
Infant Characteristics	Pregnancy and Birth History, Medical Course, Feeding Experience
Feeding Description	Feeding Duration, Nipple-In Duration, Milk Consumed, Milk Flow, Nasogastric Tube Presence, and Milk Type
Behavioral	Feeder Actions: Stimulating Sucking, Decreasing Milk Flow, Repositioning, Burping, and Pacifier Infant Behavior: Baseline: Movement and Behavioral State; Feeding: Coughing, Gagging, Engagement, and Behavioral Organization
Physiologic	HR, Sp02, and RR

Note. HR = heart rate; Sp02 = oxygen saturation; RR = respiratory rate.

Table 3.2.

Coding Scheme Descriptions of Behavioral State, Engagement, and Organization

Category	Description
Behavioral State	
Cry/Fuss	Infant is crying wholeheartedly or fussing (emits at least three brief fuss sounds during epoch). Infant is usually active. Eyes are usually closed.
Alert	Eyes are open and bright, and may be scanning. Motor activity is typically low, but the infant may be active.
Drowse/Daze	Eyes are “heavy-lidded” or “slit-like”, and occasionally opening and closing slowly or open but dazed. Motor activity is typically low, respiration is even.
Sleep	Eyes closed. Sporadic motor movements, but tone low between movements.
Engagement	
Fully engaged	Ready, participating in feeding; directing energy toward feeding; flexed arms/hands with observable tone; cues of readiness to continue feeding.
Low engagement	Low or no energy, as evidenced by loss of energy or low muscle tone; may still be sucking but passively/reflexively.
Disengaged, avoiding	Infant is directing energy away from feeding, using energy to move away from the nipple, pushing away, pulling away, turning away, extending arms
Organization	
Organized behaviorally	No indicators of disorganized behavior.
Mild disorganization	Mild indicators of disorganized behavior (e.g., slight eyebrow raise or eyelid flutter, splayed fingers or furrowed brow).
Compelling disorganization	Compelling indicators that the infant is actively trying to pull away from nipple, or extending fingers or arms, pushing nipple away, or flaccid. Behaviors may be isolated or occur along with eyebrow raise or eyelid flutter, furrowed brow.

Table 3.3.

Feeding Experience

	Feeding 1 (Slow)	Feeding 2 (Standard)	Feeding 3 (Slow)
Number of Oral Feeding Experiences	117	124	149
Oral Intake in Previous 24 hours	63%	61%	91%

Note. Oral intake in previous 24 hours = percent of prescribed nutrition consumed orally in prior 24 hours.

Table 3.4.

Feeding Description

	Feeding 1 (Slow)	Feeding 2 (Standard)	Feeding 3 (Slow)
Feeding Duration (mins)	21.2	13.7	15.5
Nipple-In Duration (mins)	14.3	6.6	7.1
Milk Consumed (mL)	40	40	35
Milk Flow (mL/min)	2.8	6.1	4.9
Nasogastric Tube Present	Yes	No	No

Note. Feeding duration = time from first nipple-in to last nipple-out. Nipple-in duration = time the nipple was in the infant's mouth. Milk Flow = Milk Consumed/Nipple-In duration. Mins = minutes. mL = milliliters.

Table 3.5.

Feeder Actions

	Feeding 1 (Slow)	Feeding 2 (Standard)	Feeding 3 (Slow)
Stimulating Sucking (No.)	4	3	2
Decreasing Milk Flow (No.)	1	0	3
Repositioning (No.)	4	6	1
Burping (secs)	118.2	104.5	241.3
Pacifier (secs)	25.8	50.9	144.5

Note. The variables stimulating sucking, decreasing milk flow, and repositioning were calculated during the nipple-in period and are given in number of events. Burping and pacifier durations were calculated during the entire feeding period and are given in seconds. No. = Number. Secs = seconds.

Table 3.6.

Behavioral Outcomes During Feeding

	Feeding 1 (Slow)	Feeding 2 (Standard)	Feeding 3 (Slow)
Distress Behaviors			
Coughing			
Number of Events	7	2	1
Duration (secs)	50.5	15.2	1.13
Gagging			
Number of Events	1	4	0
Duration (secs)	2.2	12.7	0
Engagement			
Full Engagement	91.6 %	96.0%	44.8%
Low Engagement	0%	0%	0%
Disengaged, Avoiding	8.4%	4%	55.2%
Organization			
Organized	4.8%	29.3%	0%
Mild Disorganization	89.7%	68.8%	52.1%
Compelling Disorganization	5.5%	1.9%	47.9%

Note. Distress behaviors were evaluated during the entire feeding period, including time when the nipple was not in the infant's mouth. Engagement and organization was evaluated only during nipple-in periods. Secs = seconds.

Table 3.7.

Heart Rate Indices

	Feeding 1 (Slow)			Feeding 2 (Standard)			Feeding 3 (Slow)		
	Base	Feed	Recover	Base	Feed	Recover	Base	Feed	Recover
HR (bpm)									
Mean	167.9	172.9	162.6	156.6	166.6	152.8	146.6	156.8	142.8
Minimum	161.3	149.6	127.7	150.8	152.3	127.1	137.3	141.8	127.9
Maximum	172.9	194.2	176.5	162.6	191.1	170.5	152.7	172.9	156.3
Change from Baseline		4.9	-5.3		9.8	-3.9		10.2	-3.8
CV	0.010	0.023	0.026	0.013	0.018	0.025	0.019	0.027	0.024

Note. Mean values for HR were calculated as a true mean of the entire baseline, feeding, and recovery periods. HR = heart rate. Bpm = beats per minute. Base = baseline. Feed = feeding. Recover = recovery. CV = coefficient of variation (standard deviation/mean).

Table 3.8.

Physiologic Changes During Feeding by Flow Condition

	Slow	Standard	*p*
HR (bpm)	166.2	166.6	0.01*
Sp02 (%)	86.3	83.5	0.1**
RR (breaths/min)	72.1	71.5	0.09*

Note. Linear Mixed Modeling was used to analyze the physiologic variables during feeding, covaried on baseline values. Results were grouped by flow condition (slow vs. standard). HR = heart rate; bpm = beats per minute. Sp02 = oxygen saturation; RR = respiratory rate; Min = minute.
* $p < 0.1$. ** $p < 0.2$.

Table 3.9.

Respiratory Indices

	Feeding 1 (Slow)			Feeding 2 (Standard)			Feeding 3 (Slow)		
	Base	Feed	Recover	Base	Feed	Recover	Base	Feed	Recover
Sp02 (%)									
Mean	83.8	87.7	84.8	86.0	83.5	82.8	84.4	84.4	79.9
Minimum	81.8	70.7	71.0	77.8	70.7	70.9	79	62.8	71.8
Maximum	87.7	98.6	92.7	94.6	91.6	89.8	88.5	91.6	87.2
Change from Baseline		3.76	0.96		-3.28	-3.18		0.03	-4.53
CV	0.014	0.057	0.041	0.030	0.058	0.028	0.018	0.059	0.030
Sp02 Events (No.(% of feeding))									
Sp02 > 93%		3 (8.0%)			0 (0%)			0 (0%)	
Sp02 89-92.9%		11 (28.7%)			2 (3.6%)			11 (11.2%)	
Sp02 76-80.9%		7 (5.8%)			7 (7.1%)			8 (10.8%)	
Sp02 <76%		3 (2.3%)			1 (2.3%)			3 (4.7%)	
RR (breaths/min)									
Mean	64.0	68.4	69.1	53.0	71.5	53.7	72.2	75.8	71.9
Minimum	54	31	56	47	62	47	68	66	65.5
Maximum	74	82	76	64	83	67	76	76	82
Change from Baseline		4.4	5.1		18.5	0.7		3.6	-0.2
Apnea (Frequency)	2	1	5	3	0	8	0	0	1

Note. Sp02 events are given in number of events and percent of the feeding. Two events had to be separated by at least 5 seconds of data within the range of +/- 5% (81-88.9%) to be considered separate. The most extreme value for Sp02 during the event was used for calculating the number of events. Apnea was defined as ≥ 3 seconds between breaths. Sp02 = oxygen saturation given in percent. No. = number. Base = baseline. Feed = feeding. Recover = recovery. Min = minute. CV = coefficient of variation (standard deviation/mean).

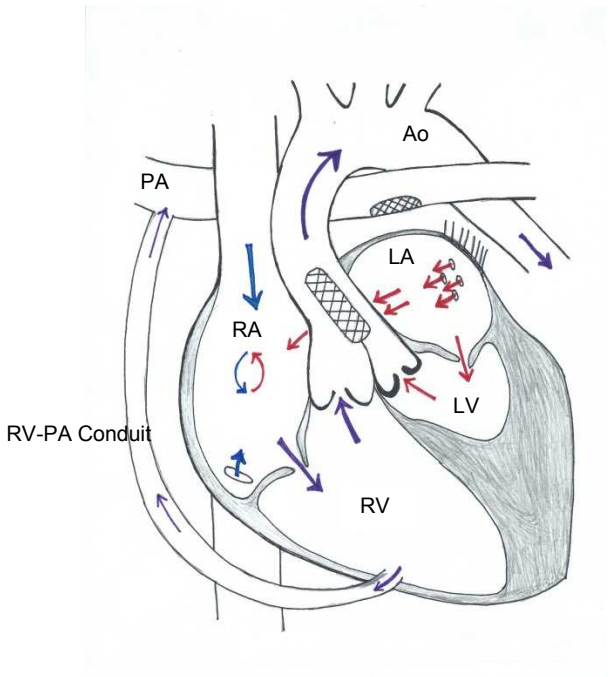


Figure 3.1. Illustration of post-surgical cardiac anatomy of Baby G. Deoxygenated blood returning from the body to the right atrium (RA) is indicated in blue. Oxygenated blood returning from the lungs to the left atrium (LA) is indicated in red and moves in two directions: 1) across the atrial septum and 2) to the hypoplastic left ventricle (LV) where it exits the aortic valve. There is mixing of blood in the RA. Mixed blood is indicated in purple and can be seen exiting the right ventricle (RV) in two directions: 1) via the RV-pulmonary artery (RV-PA) conduit to the lungs and 2) via the proximal pulmonary artery, which has been surgically connected to the aorta (Ao), to the body. Drawing courtesy of Britt Pados.

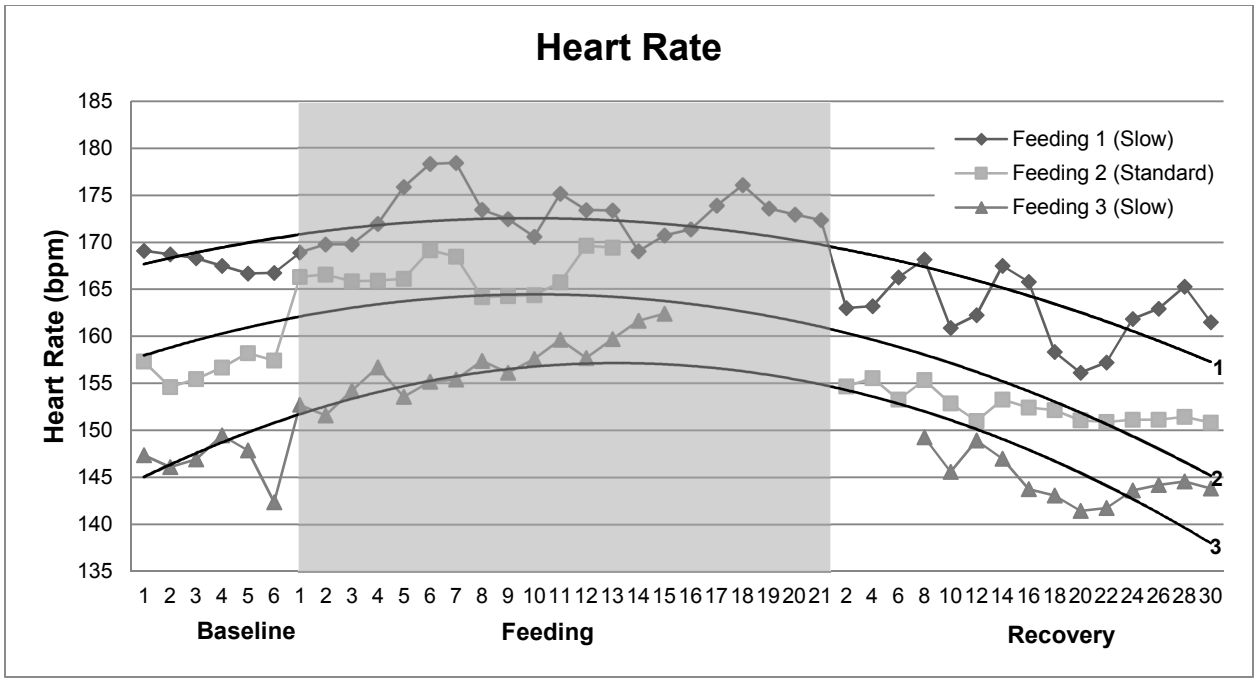


Figure 3.2. Heart Rate (bpm) plotted every 1 minute during baseline (6 minutes) and feeding (indicated in grey), and every 2 minutes during recovery (30 minutes). Quadratic regression equations- Feeding 1 (Slow): $y= -0.02x^2 + 0.70x + 167.01$ ($R^2=0.60$); Feeding 2 (Standard): $y= -0.03x^2 + 0.92x +157.07$ ($R^2=0.71$); Feeding 3 (Slow): $y= -0.04x^2 + 1.41x + 143.67$ ($R^2=0.75$). bpm = beats per minute.

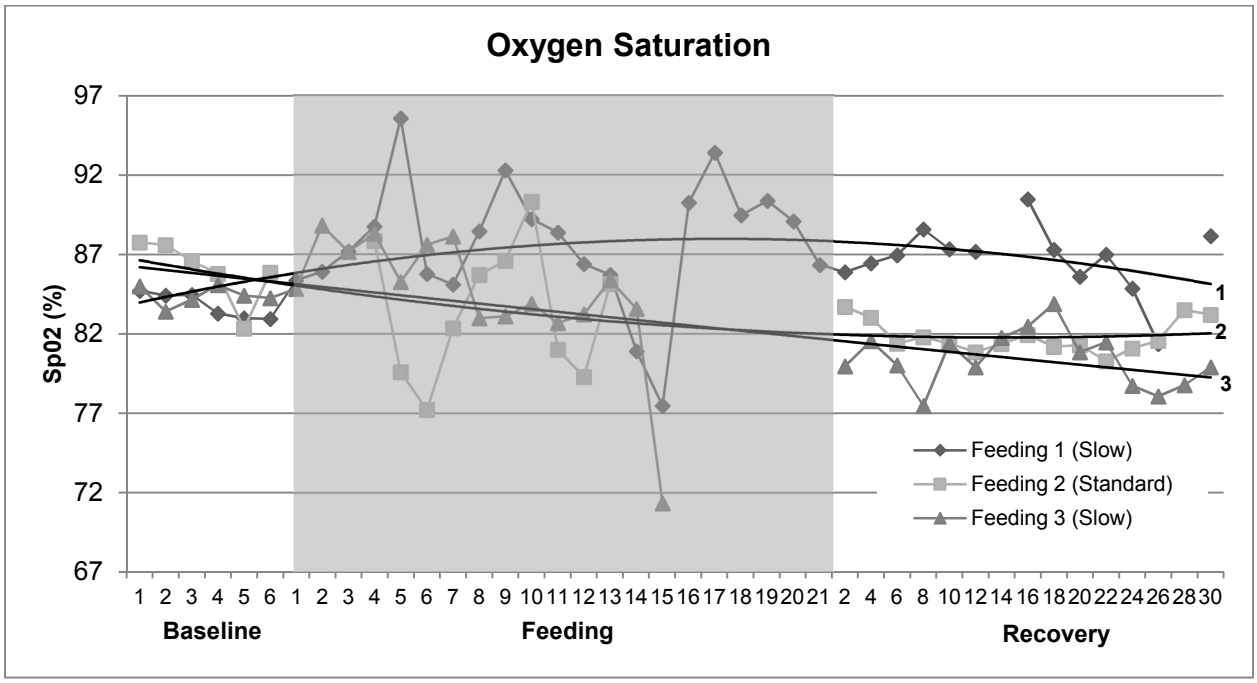


Figure 3.3. Sp02 plotted every 1 minute during baseline (6 minutes) and feeding (in grey), and every 2 minutes during recovery (30 minutes). Quadratic regression equations: Feeding 1 (Slow): $y= -0.01x^2 + 0.38x + 83.61$ ($R^2=0.11$); Feeding 2 (Standard): $y= 0.004x^2 + 0.30x + 86.94$ ($R^2=0.28$); Feeding 3 (Slow): $y=0.0003x^2 – 0.18x +86.38$ ($R^2=0.40$). Sp02 = oxygen saturation.

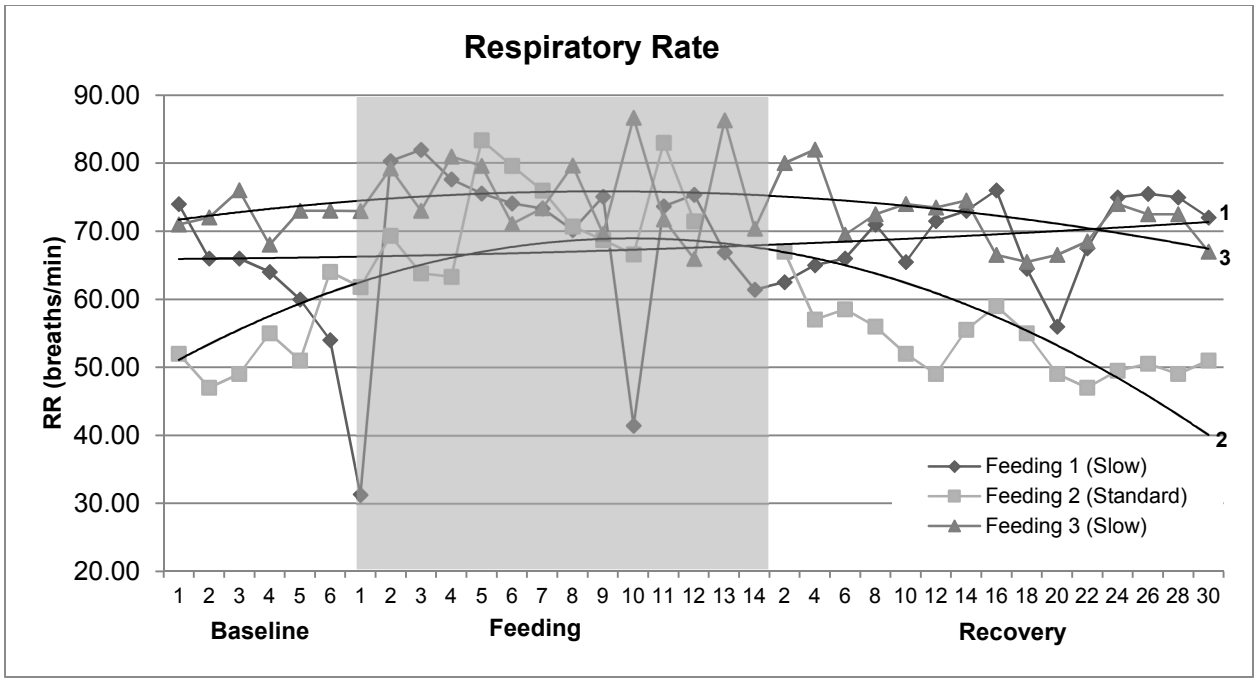


Figure 3.4. RR (breaths/min) plotted every 1 minute during baseline (6 minutes) and nipple-in periods of feeding (in grey), and every 2 minutes during recovery (30 minutes). Quadratic regression equations- Feeding 1 (Slow): $y= 0.004x^2 + 0.03x + 65.89$ ($R^2= 0.03$); Feeding 2 (Standard): $y= -0.08x^2 + 2.55x + 48.61$ ($R^2=0.57$); Feeding 3 (Slow): $y= -0.02x^2 + 0.63x + 71.08$ ($R^2=0.19$). RR = respiratory rate.

APPENDIX 3.1: STUDY FEEDING PROTOCOL

Pre-Feeding Preparation:

1. Swaddle infant with hips and shoulder girdles supported in flexed position and lower arms free to move.
2. Hold infant en face supported at shoulders with blanket and feeders' hands.
3. Hold infant in a flexed body position (shoulders adducted, hips and knees flexed).
4. Provide minimal movement of the infant's body.
5. Speak softly to the infant to bring infant to an alert state.

Feeding Strategies:

1. Hold infant in left arm supported at the shoulders.
2. Hold infant in a flexed body position.
3. Hold infant in a head elevated position at 45 degrees.
4. Provide minimal movement of the infant's body.
5. Avoid prodding of the nipple to encourage sucking.
6. Avoid increasing milk flow from the nipple by manipulating the nipple in any way.
7. Place nipple in infant's mouth when he/she displays cues of readiness to feed, including opening mouth and lowering tongue on presentation of nipple.
8. If infant displays signs of physiologic distress, defined as tachycardia (HR>200 beats/min), bradycardia (HR < 80 beats/min) or a decrease in Sa02 by more than 10% from baseline, remove the bottle and allow recovery period. Reinitiate feeding when the infant is physiologically stable and displays cues of readiness to feed.
9. If infant is no longer engaged in the feeding, remove the bottle and burp if needed. Reinitiate feeding when the infant displays cues of readiness to feed.
10. Discontinue feeding if the infant has taken the prescribed amount or if 30 minutes have elapsed from the first time the bottle was placed in the infant's mouth.

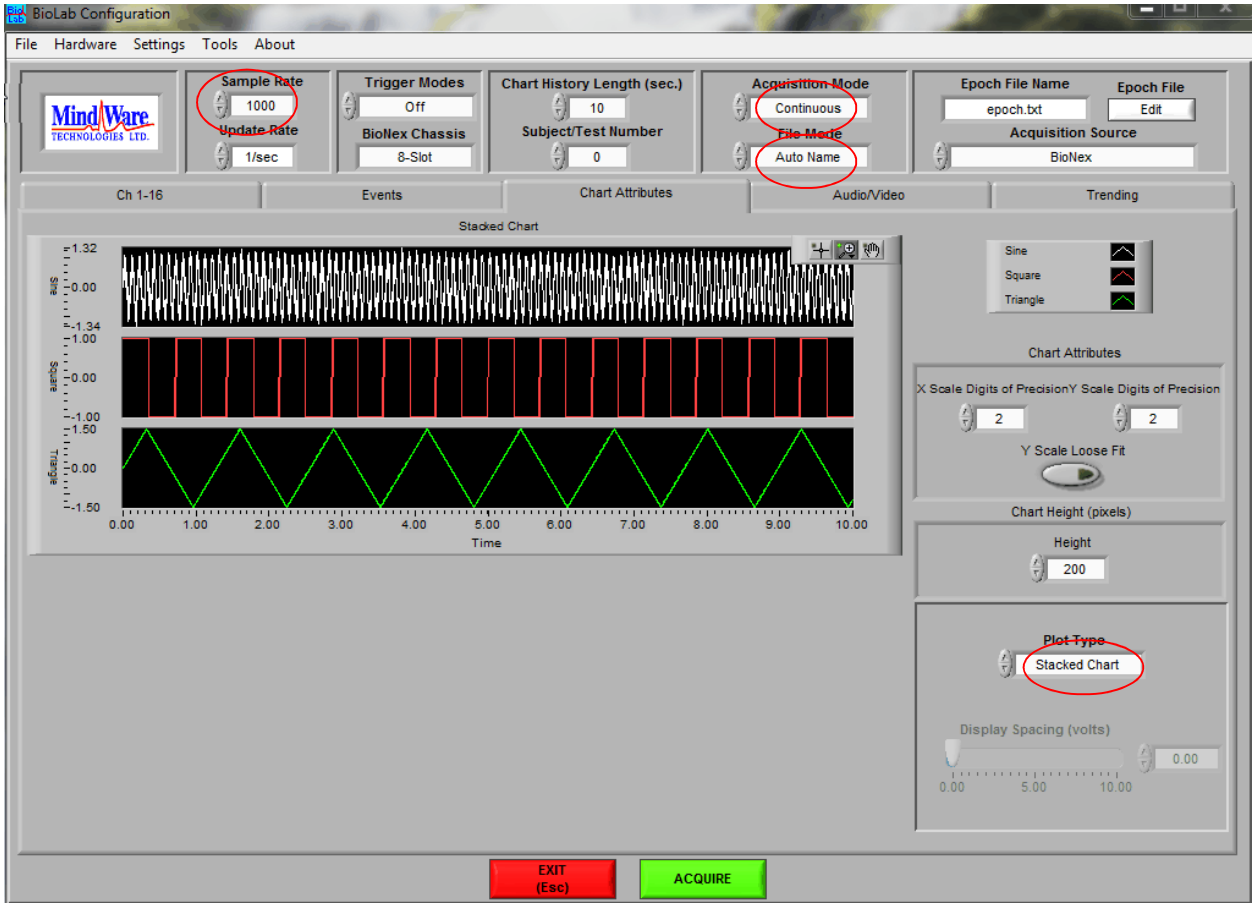
APPENDIX 3.2: PROTOCOL FOR DATA COLLECTION

This protocol was created by Jinhee Park on 6/27/2011 and modified for the purposes of this study by Britt Pados on 10/10/2012.

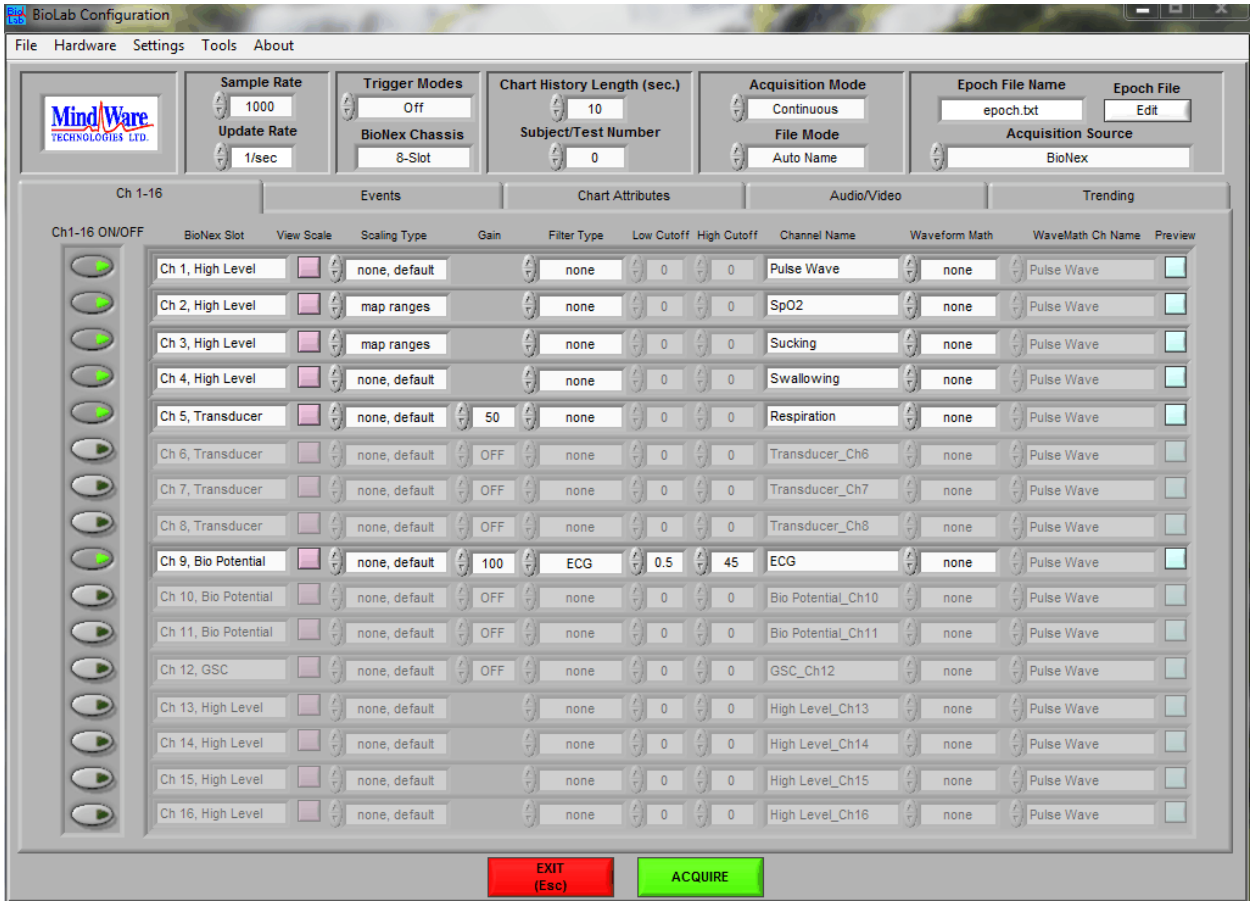
Prior to Arriving at Infant's Bedside:

- Check everything that belongs on the feeding cart (use the checklist).
- Turn on the Lenovo computer and select 'computer only log on' (enter ID and password).
- Turn on the BioNex Chassis and all the equipment and open the BioLab 3.0.10 icon.
- Open the BioLab 3.0.10.
- Open the configuration file (Diss_config_file.mwcfg) that was previously set up and saved in the D issertation folder on the desktop.
- Check the settings of the equipment according to the Protocol for Preparation of the Data Collecti on Cart.
- Check the settings of the configuration window:

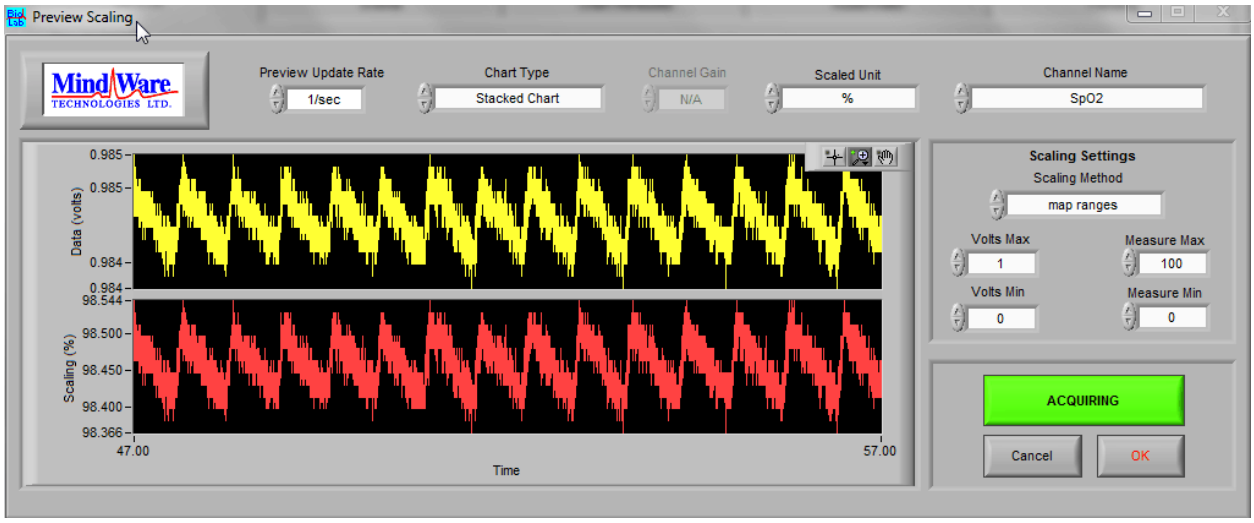
General settings



Channel setting

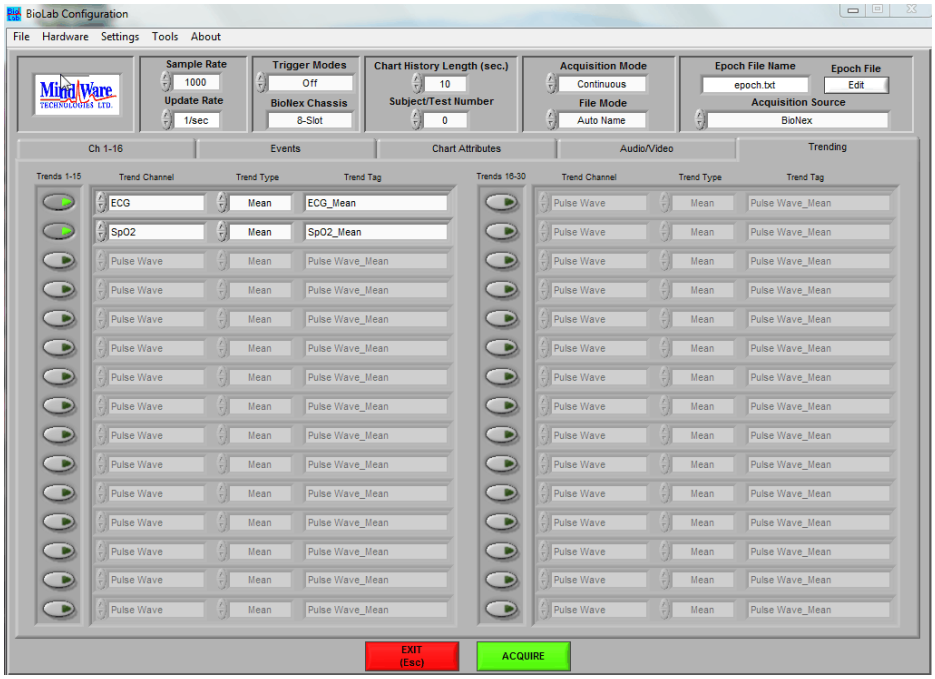


Scale setting for Sp02 channel



Audio/Video Setting

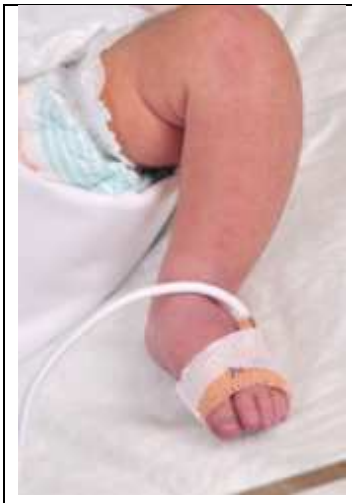
Trending setting

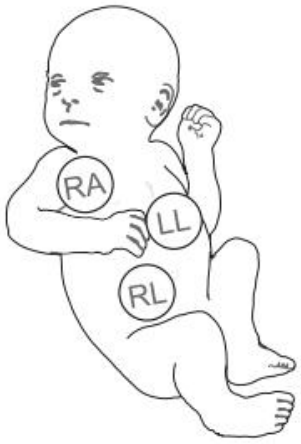


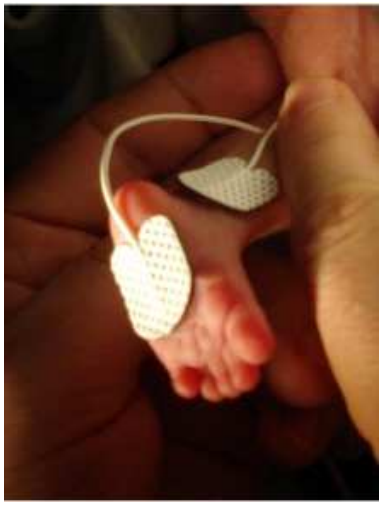
- Exit the BioLab program.
- Shut down the all equipment and computer.
- Disconnect the pulse oximeter sensor, transducer, respiration band, ECG leads, and microphone from the equipment and put them back into the first drawer.
- Fold the camera up inside the room under the top of the cart.

At the Infant's Bedside:

- Make environment is as quiet as possible (Pull the curtain down, Dim the lights, etc.)
- Connect the cables to the BioNex Chassis and to the equipment
- Turn the computer on (*The receiver of wireless mouse must be unplugged from the USB port bef ore you turn on the computer*).
- Turn on the power of the BioNex Chassis and all equipment (Pulse oximeter, Microphone, ECG, Ambu Sleepmate, and camera)
- Open BioLab 3.0.6.
- Open the configuration file (Diss_config_file.mwcfg) that previously set up and saved in the Disse rtation folder on the desktop.
- Put sensors on the baby
  - ✓ *Pulse oximeter: Place pulse oximeter sensor on the infant's left foot and secure with additional placement wrap.*
  - ✓ *ECG: place electrodes on the infant's chest as below. Note. Do not remove the infant's own electrodes if he/she has them, but you can move them if needed.*
  - ✓ *Skin conductance ECG: Place electrodes on the infant's foot and ankle as shown below.*







  - ✓ *Respiration: Place respiratory band around the infant's chest at nipple level on the top of the infant's clothes.*
  - ✓ *Microphone: Place the mic on the infant's mid neck at the suprasternal notch with double-side d tape and secure with a hydrogel tape.*
- Make sure the signals are all good before the infant is settled.
- Let all instruments warm up 30 min

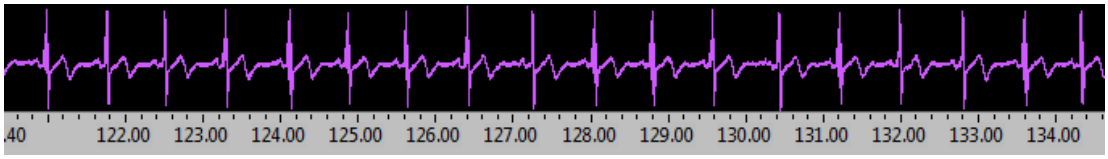
Baseline data collection:

- Begin to record after completion of the feeding prior to the first study feeding (When recording, you can see green light on the right bottom)
  - ✓ *Click AQUIRE on the bottom of the configuration window*
- Enter the random, 5-digit subject ID in Britt's dissertation folder
- Hit START/STOP button on the upper left of the BioLab acquisition screen to start recording.
- Video camera should be collecting a picture of the infant's entire body. Hit record button on the video camera.

- Send the analog out calibration signals <u>from the Masimo to the BioNex Chassis.</u>
  - ✓ *Pulse wave: Hit menu , select 'output', and set 'Analog 1' to '0V Signal'. Verify that the Bio Lab shows a voltage of approximately 0V on pulse wave channel. Set 'Analog 1' to '1V Signal ' and then verify that the BioLab shows a voltage of approximately 1V on pulse wave channel.*
  - ✓ *Hit menu , select 'output', and set 'Analog 2' to '0V Signal'. Verify that that the BioLab sho ws an approximately 0% of Sp02 on the second channel. Set 'Analog 2' to '1V Signal' and then verify that the BioLab shows approximately 100% of Sp02 on the second channel.*

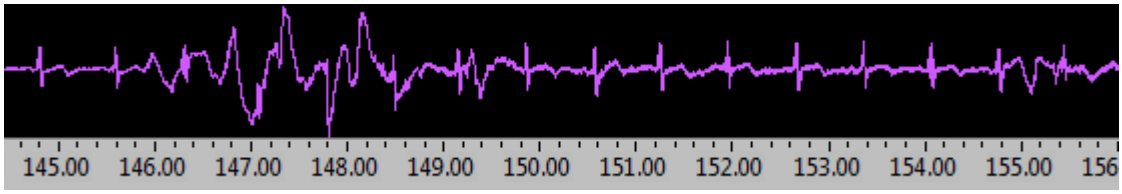
- Continue to record until the infant wakes for the feeding after data collection is complete.
- Monitor the quality of data signals in each channel. If the signal is lost or has too much noise, PI should be informed.

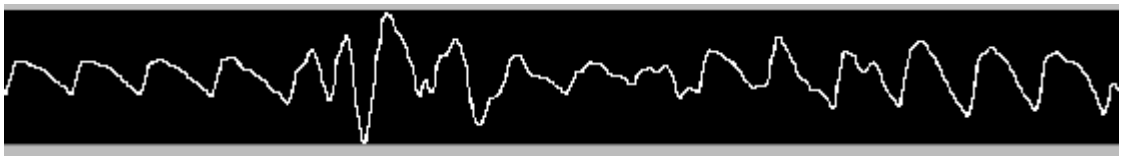
  - ✓ Pulse wave & EKG
    Normal EKG signal: regular R-R interval



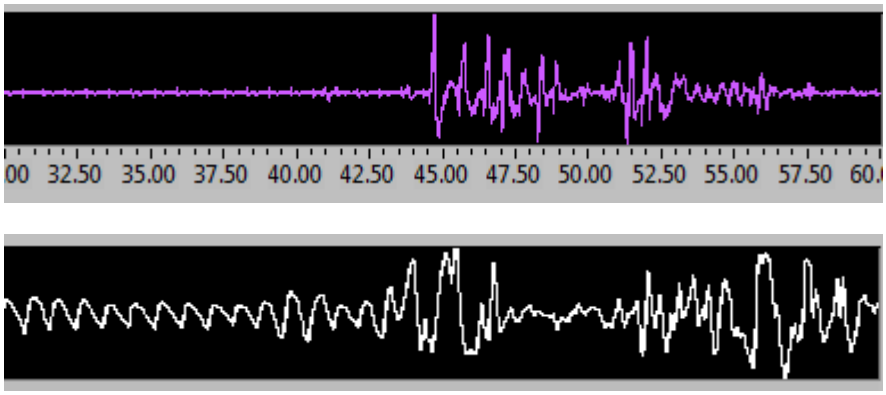
It usually comes with regular pulse wave form.

When the baby is moving, EKG can be disturbed a bit.

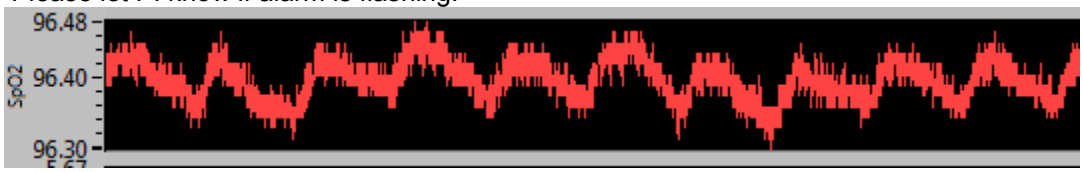




If the signal goes flat or has irregular waveform for few seconds, report this to PI.

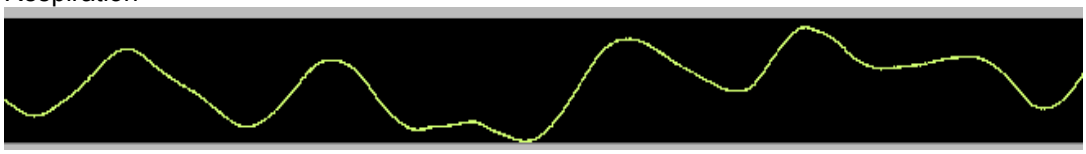


✓ SaO2: The signals on the Masimo and the BioLab need to be corresponding to each othe r. Note: The alarm of the Masimo will be indicated only by the symbol of alarm flashing. Please let PI know if alarm is flashing.



✓ Swallowing

✓ Respiration



Data collection during feeding:

- When the infant wakes up for feeding, routine nursery care will be provided by his/her assigned nurse or parent.
- Weigh infant in clothing and diaper in which he/she will be fed.
- Swaddle the infant.
- Proceed with feeding according to predetermined feeding order. Video camera angle will need to be adjusted for feeding position to capture the infant from the waist up.
- Write a memo when significant events occur using Journal (F12) of the BioLab program.

- When feeding is determined to be finished, record the length of feeding time.
- Weigh infant in same clothing and diaper as prior to feeding.
- Post feeding nursery care will be provided by nurse and/or parent and infant will be placed supine in crib or held by family member. Pacifier may be offered as necessary.

Data collection after feeding

- All equipment is to remain on the infant and the physiologic data collection continues.
- The video camera should be repositioned to capture the infant's entire body in the crib and video recording begun.

After Data collection

- Shut down all equipment, computer, and BioNex Chassis.
- Gently remove all monitoring equipment.
- Wipe down all cables and monitoring equipment as it is put away from the baby and put it back int o the drawer in the physiologic cart. *Note: Put the equipment that requires cleaning into the zip ba g (e.g., microphone, resp. band).*
- Wash bottle and nipple used for study according to unit standard of practice.
- Clean up all the equipment or throw away if it is disposable.

  <Pulse oximeter sensor and cable>

  - ✓ *Wash the light blue band.*
  - ✓ *Wipe down the black band and the crystal sensor with alcohol swab or disinfectant wipes.*

  <Respiration band>

  - ✓ *Wash the light blue band.*
  - ✓ *Wipe down the black band and the crystal sensor with alcohol swab or disinfectant wipes.*

  <Others>

  - ✓ *Wipe down other equipment with alcohol swab or disinfectant wipes.*

- Back up the data on an encrypted, password-protected external hard drive and lock in filing cabi net.

- Download the trend data from the Masimo Radical-7 pulse co-oximeter.
  - ✓ *Assure that the Radical-7 serial output mode is set to "ASCII2" (MENU <OUPUT)*
  - ✓ *Open Trendcom software on the computer.*
  - ✓ *Under the 'instrument' menu in Trendcom, select 'Radical-7 (V7619 or greater)'.*
  - ✓ *Under the 'COM Port' menu, select 'COM5'.*
  - ✓ *Click on 'Retrieve Trend'. The following message may appear: "please make sure the Radical is set to ASCII2 output mode" and click OK.*
  - ✓ *Name the file.*
  - ✓ *Save the file to the appropriate folder.*
  - ✓ *You will have date, time, Sp02, pulse rate and perfusion index (PI) every 2 seconds that is s ame as averaging rate you set.*

- Download the video files from the Panasonic HDC-TM700 if you need a high resolution AVCHD vi deo file.
  - ✓ *Assure that HD Writer AE 2.1 is installed in the computer.*
  - ✓ *Connect the video camera to the computer with the USB cable.*
  - ✓ *Turn the video camera on.*
  - ✓ *Open HD Writer AE 2.1.*
  - ✓ *Click 'Copy to PC'< 'Video Camera (E)'< 'Next'.*
  - ✓ *Select the video file what you want to download.*
  - ✓ *Assign the folder and name the file.*
  - ✓ *Click 'Execute'.*

- After downloading the video files, format built-in memory on the Panasonic camera
  - ✓ *MENU<SETUP<FORMAT MEDIA<BUILT-IN MEMORY.*
  - ✓ *When formatting is complete, touch 'EXIT' to exit the message screen.*

APPENDIX 3.3: OBSERVATIONAL CODING SCHEME

Code Class and Codes	*Description*
1.0 Structure of the Feeding *Mutually exclusive/continuous codes:*	
1.1 Nipple in	Fully seated nipple
1.2 Nipple out	Code when entire nipple is visible as outside the infant's lips/outside the mouth (visualize nipple tip); can be resting on outside of lips.
1.3 Non-feeding	Default code at start of coding session. Nipple is not in, no feeding.
2.0 Caregiver Feeding Actions *Mutually exclusive/continuous codes:*	
2.1 No nipple in	Default code at start of coding session.
2.2 Nipple held still	Nipple in neutral position; no or minimal or brief movement of the nipple in the infant's mouth; other than the very first code when nipple in, if nipple held still is 1 second or less it is considered increase intraoral stimulus to suck (e.g., decrease milk flow – up for less than one second and then decrease again, the up is considered stim)
2.3 Increase intraoral stimulus to suck	Movement of the nipple that may stimulate a suck; intention is not assumed; the feeder may be adjusting the nipple to hold the bottle better or to seat the nipple on the tongue better, may occur in the process of checking to see if the infant is drooling, or trying to arouse the infant or to stimulate the infant to engage in sucking by moving the nipple; from the infant's perspective, the nipple has moved in the mouth and this reflexively may stimulate sucking; it may also cause fluid to drip into the mouth placing a demand on the infant to manage the fluid; has the effect of increasing the stimulus of the nipple; if less than 5 seconds between increase oral stimulus events in the absence of decrease milk flow, increase intraoral stimulus stays on. For breast feeding, this could include mom moving her breast tissue sufficient to have the potential of stimulating the suck, making an airspace for the infant's nose may or may not be sufficient to stimulate the suck – i.e., it can be done without stimulating the suck (e.g., moving the nipple shield away from the infant's nose); mom positioning infant for the latch is not stimulating suck
2.4 Decrease milk flow by moving nipple	Moving nipple down, back, or to the side – has the effect of decreasing the milk flow or of milk in the nipple; code nipple still when nipple is moved back to the neutral position; may include stimulation but flow is not available so decrease milk flow is the correct code
2.5 Unsure/unable to determine	

3.0 Vestibular Stimulation *Mutually exclusive/continuous codes:*	* The video was focused on the infant; this may be difficult to code.
3.1 Non-feeding vestibular	Default code at start of coding session.
3.2 No vestibular stimulation	
3.3 Infant moved in space, rhythmically	Infant rocked or bounced; moved in space; has more than an event quality; has some repetitive, rhythmical quality; turned off if stimulus from reposition occurs.
3.4 Repositioned	Pillow placed under baby; position adjusted; maternal or infant movement that is time limited and brings infant essentially back to same position (not more upright, not more flat/back). Goes back to no vestibular right away after adjustment.
3.5 Unsure/unable to determine vestibular	
4.0 Infant Engagement: *Mutually exclusive/continuous codes:*	
4.1 Non-feeding engagement	Default code at start of coding session.
4.2 Fully engaged	Ready, participating in the feeding; engaged, directing energy toward feeding, bringing energy to the feeding; flexed arms/hands with observable motor tone; observable cues of readiness to continue feeding; may bring body toward feeding position – midline, flexed
4.3 Low engagement	Low or no energy, as evidenced by loss of energy or low muscle tone; may still be sucking but passively/reflexively
4.4 Disengaged, avoiding	Observable indicators that the infant is directing energy away from feeding, using energy to move away from the nipple, pushing away, pulling away, turning away, extending arms
4.5 Unsure/unable to determine	
	As all categories, more than 5 seconds apart from each other to count as a new state.

5.0 Infant Behavioral Organization *Mutually exclusive/continuous codes*:*	
5.1 Non-feeding organization	Default code at start of coding session.
5.2 Organized behaviorally	No indicators of disorganized behavior
5.3 Mild cues of disorganization	Mild indicators of disorganized behavior, e.g., slight eyebrow raise or eyelid flutter (not due to social initiative), splayed fingers or furrowed brow.
5.4 Compelling cues of disorganization	Compelling Indicators that the infant is actively trying to pull away from nipple, or extending fingers or arms, pushing nipple away. These behaviors may be isolated or may occur along with eyebrow raise or eyelid flutter, furrowed brow. May be flaccid. More than 5 seconds apart from each other to count as a new state.
5.5 Unsure/unable to determine	
6.0 Activity Level *Mutually exclusive/continuous codes*:*	
6.1 No movement	No movement of any part of infant except for eyes and small, slow movements of face.
6.2 Movement	Movement of any part of body except eyes and face.
6.3 Unsure	Unable to determine activity level.
7.0 Behavioral state *Interval codes every 10 seconds*	
7.1 Cry/Fuss	The infant is crying wholeheartedly or fussing (emits at least three brief fuss sounds during epoch). The infant is usually active. The eyes are usually closed during crying.
7.2 Active Waking	The infant's eyes are usually open, dull and unfocused. Motor activity varies but is typically high. During periods of high-level activity the eyes may close.
7.3 Alert	The infant's eyes are open and bright, and may be scanning. Motor activity is typically low, but the infant may be active.
7.4 Drowse/Daze	The infant's eyes are "heavy-lidded" or "slit-like", and occasionally opening and closing slowly OR open but dazed in appearance. The level of motor activity is typically low and respiration is fairly even.

7.5 Sleep-Wake Transition	The infant shows behaviors of both wakefulness and sleep. There is generalized motor activity (usually this movement involves trunk), and although the eyes are typically closed, there may be rapid opening and closing of the eyes. Brief fussy vocalizations may occur. *Note.* Generally 4 epochs (40 seconds) of continuous activity without REMs in the middle of sleep are scored sleep-wake transition. *Exception of 40 seconds rule.* • If the infant is disturbed by the caregiver while the camera angle is blocked and the infant was moving after the camera was back on, then S-W transition could be coded for less than 40 seconds by counting the period when the camera angle was blocked (we assume during that period, the infant was in movement). • In the middle of waking, S-W transition could occur for less than 40 seconds.
7.6 Active Sleep	The infant's eyes are closed (may not be fully closed with REM observed). Respiration is uneven and primarily costal in nature. Sporadic motor movements occur, but muscle tone is low between these movements. REMs occur intermittently in this state.
7.7 Quiet Sleep	The infant's eyes are closed. Respiration is relatively slow and is abdominal in nature. A tonic level of motor tone (i.e., has some tone, not lethargic) is maintained, and motor activity is limited to occasional startles, sigh sobs, or other brief discharges. *To code as Quiet sleep, this state should stay at least for 1 minute (60 seconds) *Note.* Generally 2 epochs (20 seconds) of continuous movement when transitioning from quiet sleep are scored active sleep.
7.8 Unclassified Sleep	The infant is asleep but the video is not clear enough to distinguish specific sleep states (active vs. quite sleep).
7.9 Unscorable States	Scored whenever the video is not clear enough to score states, e.g., the caregiver is blocking the camera angle.
8.0 Caregiver Activity *Mutually exclusive/continuous codes**	
8.1 No burping	Default code at start of coding session. Caregiver is not burping infant.
8.2 Burping	Caregiver is burping infant. Code from beginning of patting to end of patting.

9.0 Infant Behavior *Mutually exclusive/continuous codes**	
9.1 None	Default code at start of coding session. Infant is not coughing, gagging, or stooling.
9.2 Coughing	The infant is coughing. Code from first sound of cough to last sound of cough.
9.3 Gagging	The infant is gagging, retching, or heaving. There may or may not be sound associated with the gag. Infant may thrust tongue, move the body or head as if to vomit, but without obvious expulsion of fluid.
9.4 Stooling	The infant shows behaviors related to stooling, including bearing down.
10.0 Pacifier Use *Mutually exclusive/continuous codes**	
10.1 No pacifier	No pacifier is in the infant's mouth. When pacifier is removed, code "no pacifier" when the entire nipple of the pacifier can be seen.
10.2 Pacifier	A pacifier is in the infant's mouth. Code from when the pacifier is fully seated in the infant's mouth.
11.0 Sa02 *Mutually exclusive/continuous codes**	
11.1 >93%	Oxygen saturation greater than 93% (>10 increase above baseline).
11.2 89-93%	Oxygen saturation 89-93% (5-10% increase above baseline).
11.3 81-88.9%	Oxygen saturation 81-88.9% (within +/- 5% of baseline).
11.4 76-80.9%	Oxygen saturation 76-80.9% (5-10% decrease below baseline).
11.5 <76%	Oxygen saturation < 76% (> 10% decrease below baseline).
11.6 Artifact	Oxygen saturation reading is not reliable.

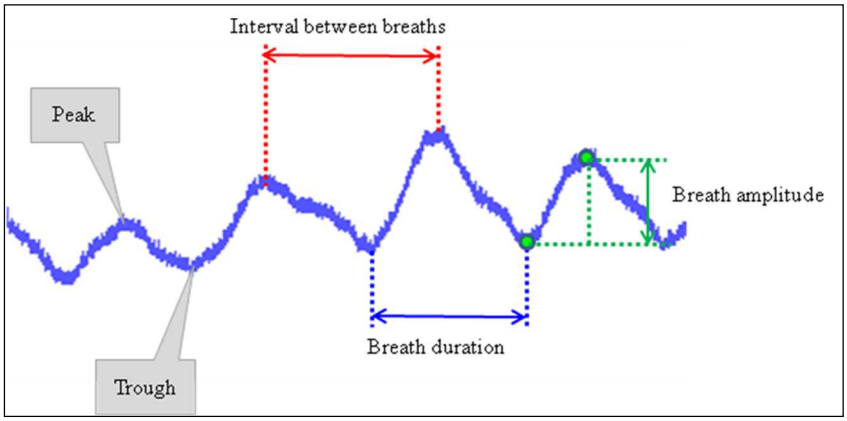
Independent variables selected at the end of the observation:		
Shoulder Position	1) Most of the time 2) Some of the time 3) Rare or none of the time 4) Unable to determine	Shoulders flexed toward midline (either due to how the infant is able to position self, how infant is being held, or by a blanket swaddle)
Arm Position	1) Most of the time 2) Some of the time 3) Rare or none of the time 4) Unable to determine	Arms flexed toward midline (either due to how the infant is able to position self, how infant is being held, or by a blanket swaddle)
Containment (predominantly)	1) No blanket 2) Blanket supporting lower body 3) Blanket supporting arms in midline flexed position but shoulders extended 4) Blanket supporting arms and shoulders in midline flexed position	
Trunk Position (predominantly)	1) Supine 2) Side-lying - full side-lying has ear, shoulder and hip toward ceiling 3) In-between supine and side-lying 4) Cradled – infant is held in the crook/elbow area of the mom's arm 5) Unable to determine	
Upright Versus Flat Trunk Position (predominantly)	1) Upright/inclined (approximately 90 degrees) 2) Semi-upright/inclined (approximately 45 degrees) 3) Completely or nearly flat 4) Unable to determine	
Midline Alignment of Head and Neck (predominantly)	1) Most of the time 2) Some of the time 3) Rare or none of the time 4) Unable to determine	Chin and sternum in straight line/aligned; head and neck in midline alignment; head not tilted or rotated toward one of the shoulders
Anterior/Posterior Neck Alignment (predominantly)	1) Neutral neck - slight chin tuck; neck flexion, so that the chin is directed slightly downward and inward 2) Extension backward (sniffing position) 3) Flexion – moderate to excessive chin tuck 4) Unable to determine	

APPENDIX 3.4: RESPIRATORY DATA MANAGEMENT PROTOCOL

This protocol was created by Dr. Jinhee Park and modified for the purposes of this study by Britt Pados.

Definitions of Respiratory Variables

- *Respiratory rate*: Number of peaks per minute.
- *Apnea*: Pause in respiration lasting ≥ 3 seconds.



A. Converting Biolab file (.mw) to an AcqKnowledge file (.acq)

1. Open the file collected with the BioLab 3.1.0K.
2. In the configuration window, cancel the channels except for respiration by clicking ON/OFF button on the left side of each channel.
3. Click VIEW and you can see the respiration channel only.
4. Click SAVE ALL TEXT to save the respiration channel as a text file. Save this with the same name plus _resp (i.e., xxxxx_resp.txt).
5. Open the AcqKnowledge 4.2.
6. Click FILE > OPEN and select the text file you saved from the BioLab program
7. Put wave data start on the line (**3**), sample rate interval (**1 millisecs**), column delimiter (**tab**) when the window pops up to ask these. **Make sure the line data start by opening the text file with word pad*
8. Two channels will be opened: Channel 0 (Time) and Channel 1 (Respiration)
9. Save this waveform file as xxxxx_resp.acq

B. Mark the peaks on the respiratory waveform

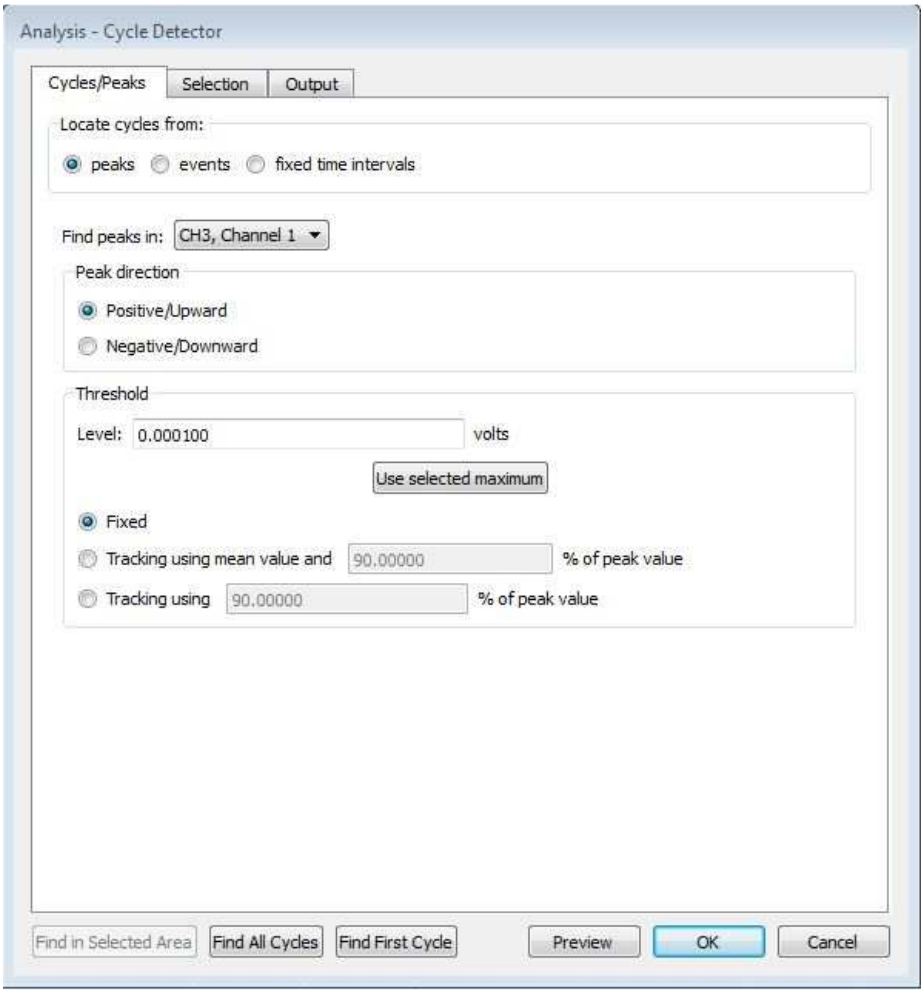
1. File preparation

a) Open the acqknowledge file (xxxxx_resp.acq) that converted from the BioLab.
b) Take off the visibility of channel 0 on the screen by clicking channel icon with holding down ALT key in the upper part of graph display.
c) When you see only channel 1(Respiration), duplicate the respiration channel on channel 2 to work on (EDIT<DUPLICATE WAVEFORM)
d) Take off the visibility of original waveform and then you can only see the duplicated respiration channel on the channel 2.
e) Filter the waveform to adjust the file adequately to mark peaks and troughs (TRANSFORM<DIGITAL FILTER<FIR<HIGH PASS) and fix the frequency cutoff at “0.5” Hz then click OK. *Make sure if the frequency cutoff is too high, the peak will be flattened.*
f) Smooth the waveform to remove noisy points on the waveform (TRANSFORM<SMOOTHING)

g) In the field for smoothing factor, put the number that multiply 0.1 by the sample rate of the waveform (i.e., sampling rate * 0.1=1000*0.1=100) and choose mean value and transform entire waveform.
h) Resample the waveform to appropriate rate for respiration. It would be recommended to use the number that multiply maximum signal of respiration of infant with CHD by 4 (i.e., 80 per minute * 4 = 320). So, resample to **500**. (TRANSFORM<RESAMPLE WAVEFORM)
i) Duplicate channel 2 to now mark peaks in channel 3. Take off visibility of channel 2.

2. Mark the peaks

a. Run cycle detector (ANALYSIS<FIND CYCLE or Click  on the toolbar). Set up the dialog box as the below. *Make sure the cursor need to be at the beginning of the waveform before running cycle detector. Do not hit OK or Enter. Proceed to the Selection Tab.*



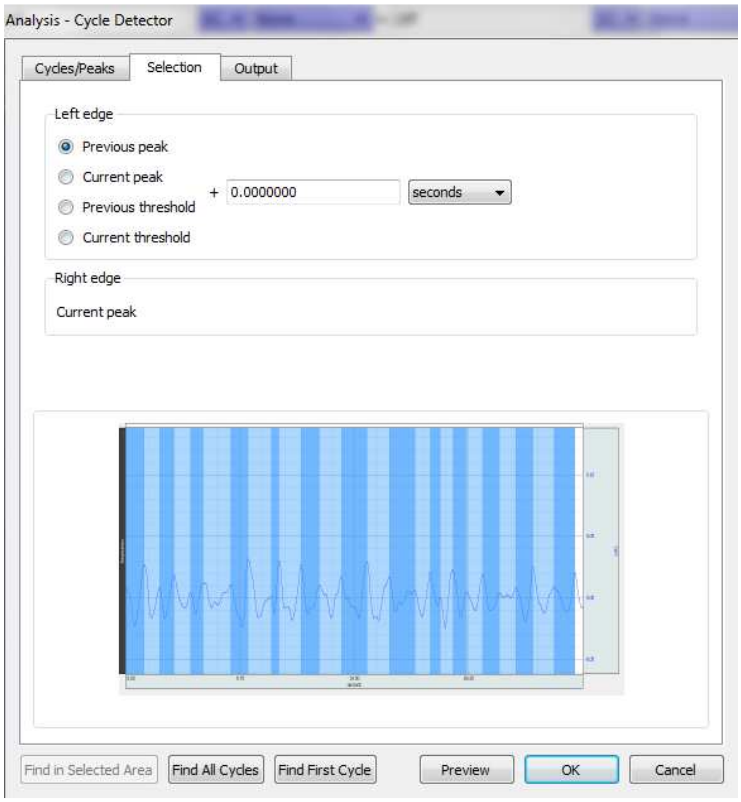
Note.

You can move cursor or make selection on the waveform using I-beam tool  *on the toolbar.*

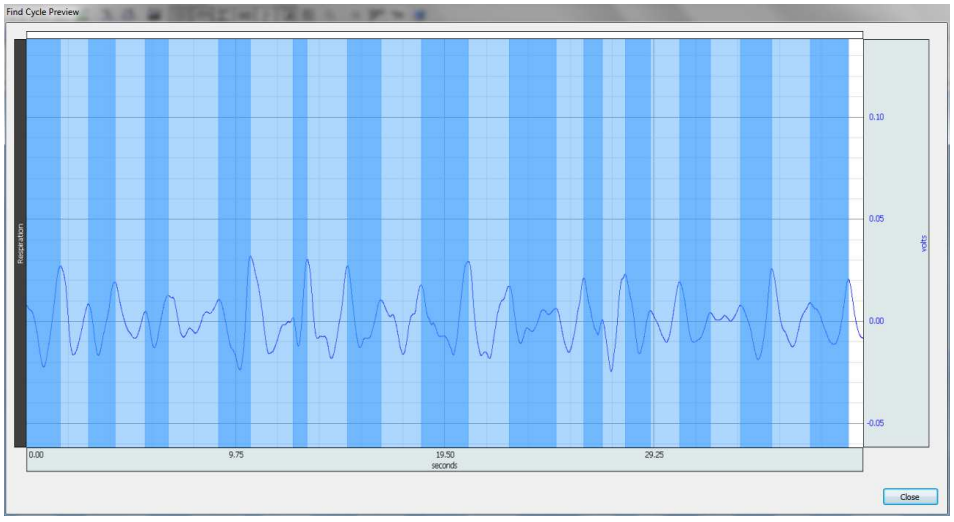
You can autoscale the waveform vertically using  *on the toolbar.*

You can autoscale the waveform vertically using  *on the toolbar.*

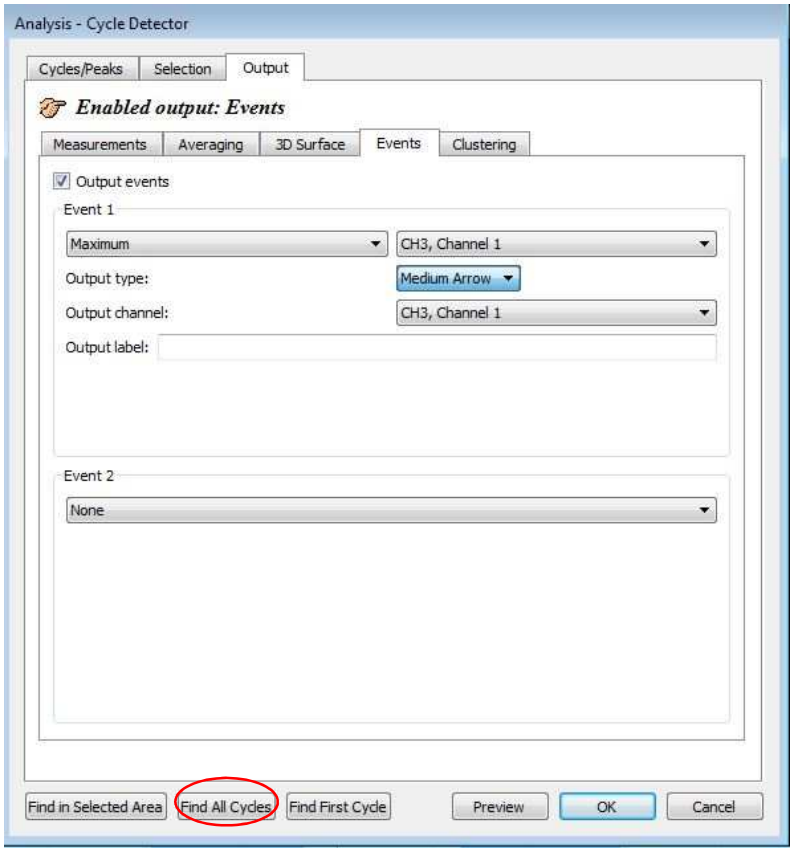
b. Next, choose the selection tab and set it up as below.



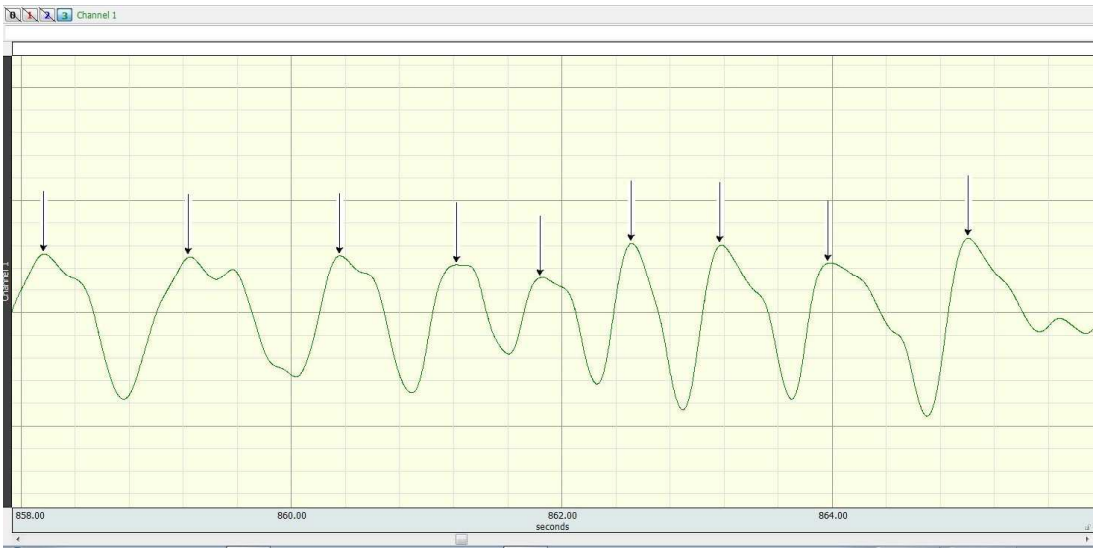
c. Then, click “PREVIEW” to see if the peaks are captured adequately. Play with the data until a majority of peaks are captured by adjusting threshold.



d. When you are satisfied with the peaks captured, select the output tab and set it up as below.



e. Select “Find All Cycles.” Then, you can see the arrow marked on the waveform.



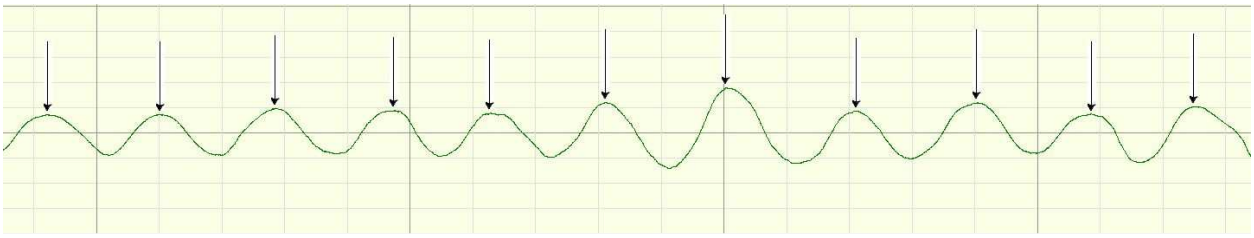
3. **Clean Artifact:** Clean artifacts by removing and adding marks manually.

   a) To remove a mark, click event zap tool  and put the cursor on the mark you want to remove and click. To add a mark, the first time you insert a mark, you need to move the cursor onto the waveform and right click. Go to Assign Current Event Type → Notes → Medium Arrow. To insert a mark click 

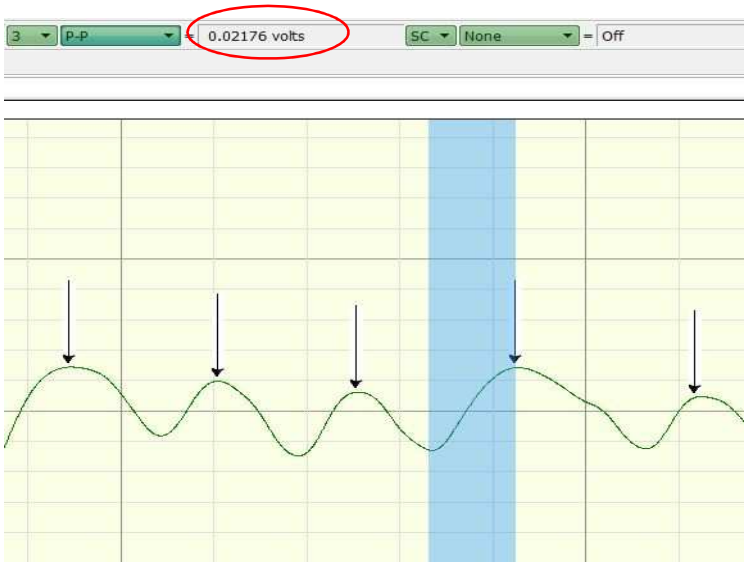
   b) Rules for Marking Peaks on Respiratory Waveforms:

   Definition of Peak = The point of maximum chest circumference, between inspiration and expiration.

   i. **Determine minimum voltage change of an adequate breath:** Identify period of stable, regular breathing within the baseline period prior to the feeding. The waveform should look similar to this:



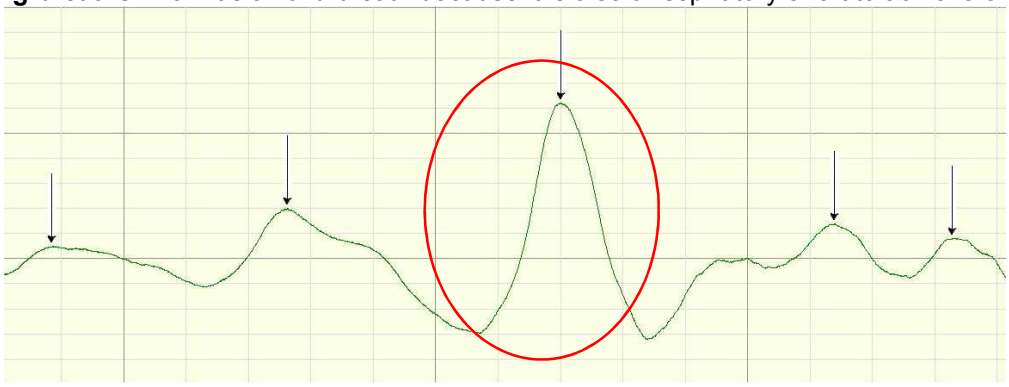
   ii. Calculate the mean voltage change associated with each breath. To calculate voltage change, use the I-beam tool  and select the area from the trough to the peak. Use the calculate features at the top to select channel 3, P-P (peak to peak) and the voltage change will appear in the box.



   iii. Calculate the mean voltage from 5 regular breaths. Calculate 20% of this mean and this will be the minimum voltage change necessary for a peak to be considered an adequate breath. The breath must meet this minimum voltage change prior to *and* after the peak in order to be considered as having met an adequate respiratory cycle of inhalation and exhalation.

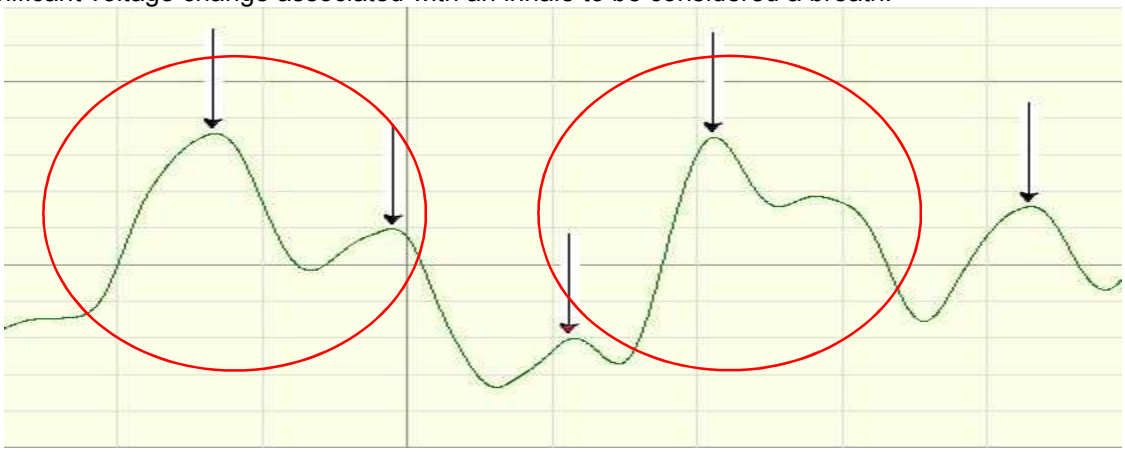
   iv. Note these common variations in the respiratory waveform:

Gasp or big breaths: mark as a valid breath because it is also a respiratory effort to achieve oxygen.

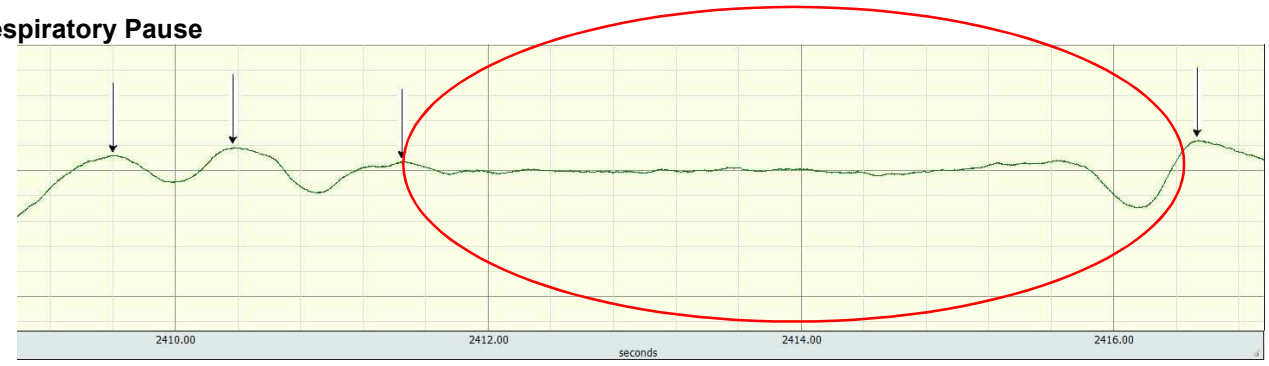


Breaths with double peaks

If both peaks meet the minimum required voltage change prior to and after the peak, then mark both breaths. If not, mark only the highest peak. Below, the is an example on the left of a breath where there is not a complete exhale before the next inhale (the trough does not return all the way down to where the prior inhale started), but there was adequate voltage change before and after the peak to be considered a breath. On the right, this would be considered an interrupted exhale; there is not a significant voltage change associated with an inhale to be considered a breath.

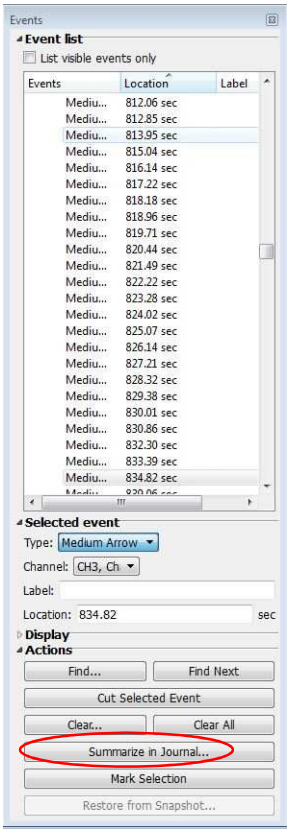


Respiratory Pause

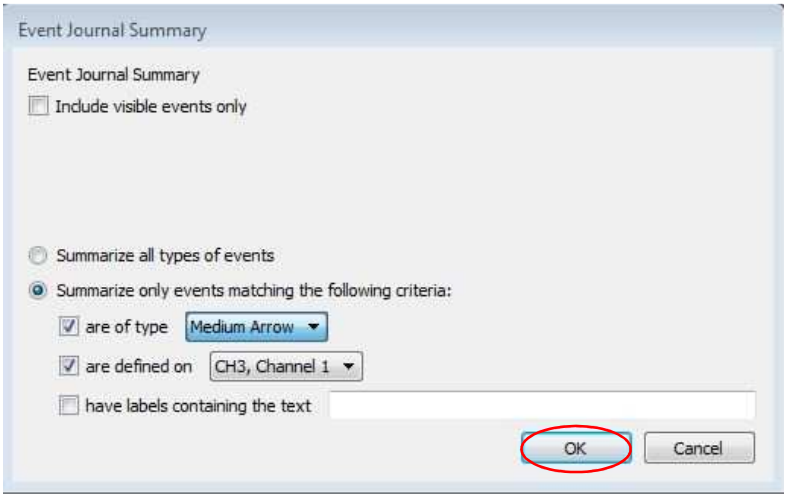


C. Create Excel File:

1. Next, click the event palette tool  in the top right corner or go DISPLAY<SHOW<EVENT PALETTE. Set up the event palette as below. Next, select “Summarize in Journal…” and select “yes” when the program asks if you want to create a journal.



2. An Event Journal Summary box will pop up. Set it up as below and choose OK.



3. The Event Summary Journal will appear under the waveform. Click on the “Save” Icon.

4. Select the location you want to save the file to. Name the file (e.g., F1_B_resp_eventsummary) and save as type “Excel Spreadsheet.”

5. Copy all data from sheet 1 into sheet 2. Name sheet 2 “MW Time.”

6. In sheet 2, delete the first row that contains “Event Summary” and delete columns A (Index), C (Type), D (Channel), and E (Label).

7. Rename what is now column A “Time” to “ACQ Time”. Name column B “ACQ Time Number Only” and “column C “MW Time.”

8. Copy the data from column A into column B.

9. Select the data in column B and click “Find & Select” → Replace → Find what: sec / Replace with: (leave blank). Select “Replace All.”

10. Repeat this last step, but now Replace → Find what: min / Replace with: (leave blank). Select “Replace All.” Close this box.

11. In Column C (MW Time) create a formula so that the data in column C is equal to the data from column B if the data was in seconds (refer to column A). Starting with the row of data that is in mins in column A, create a formula in column C = column B * 60. This will convert the minute data into seconds.

12. The database should now look like this in sheet 2:

	A	B	C	D
1	ACQ Time	ACQ Time Number Only	MW Time	
2	1.70 sec	1.7	1.7	
3	1.70 sec	1.7	1.7	
4	2.91 sec	2.91	2.91	
5	4.42 sec	4.42	4.42	
6	4.42 sec	4.42	4.42	
7	5.51 sec	5.51	5.51	
8	7.06 sec	7.06	7.06	
9	7.06 sec	7.06	7.06	
10	9.03 sec	9.03	9.03	
11	9.03 sec	9.03	9.03	
12	10.13 sec	10.13	10.13	
13	11.42 sec	11.42	11.42	
14	11.42 sec	11.42	11.42	

13. Now, select the data segment(s) that you are interested in and copy to a new sheet. The analysis for baseline and recovery periods is slightly different than the feeding periods.

For feeding periods:

Name sheet 3 “Nipple Ins Cleaned.” In sheet 3, name column A “MW Time”, name column B “Peak Times” and name column C “Interval.” Copy the data from column A into column B so that column B “Count” contains each event’s MW Time. The data in column B will be used to calculate RR as the number of events per minute.

After the event times are copied to column B, then add to column A:
1) the MW time that corresponds to the either the start of baseline/recovery or the nipple-in time.
2) the MW time that corresponds to the end of the baseline/recovery period or the nipple-out time.

Create a formula in column C to calculate the interval between peaks. The interval data is not specific enough to report interval data, but will be used to identify pauses > 3 seconds.

For baseline & recovery periods:

Name sheet 3 to describe the data segment that is contained in the sheet (e.g., 510-870 Cleaned).

D. CLEAN DATA & CALCULATE RR & APNEAS

1. In sheet 3, select data in column C (Interval) → Conditional Formatting → Highlight Cells → Equal to → enter “0”

2. Delete the rows of data that accompany an interval of “0.” Your data is now clean.

    a. For baseline and recovery periods, create columns for Epoch, MW Start Time, count, MeanRR, MinRR, MaxRR, and apnea. The database should look like this:

F1_B_resp_eventsummary

	A	B	C	D	E	F	G	H	I	J	K
1	MW Time	Peak Times	Interval		Epoch	MW Start Time	Count	MeanRR	MinRR	MaxRR	Apnea
2	510.00	510.00			1	510	74.00	74.00			
3	511.20	511.20	1.20		2	570	66.00	66.00			
4	511.80	511.80	0.60		3	630	66.00	66.00			
5	513.00	513.00	1.20		4	690	64.00	64.00			
6	514.20	514.20	1.20		5	750	60.00	60.00			
7	514.80	514.80	0.60		6	810	54.00	54.00			
8	516.00	516.00	1.20								
9	516.60	516.60	0.60		Entire baseline		385	64.16667	54	74	2

    b. For feeding periods, create columns for epoch, MW Time, duration, behavior, count, MeanRR, MinRR, MaxRR, and apnea. The database should look like this:

F1_F_resp_eventsummary

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	MW Time	Peak Time	Interval		Epoch	MW Time	Duration_	Behavior	Count	RR	Apnea			MeanRR	MinRR	MaxRR	Apnea
2	15.2149				1	15.2149	9.57604	Nipple in	5.00	31.32819	1		Entire Feeding Period	68.44881	31.32819	81.9514	1
3	15.94	15.94	0.73			24.7909		Nipple out									
4	16.33	16.33	0.39		2	38.0706	62.0274	Nipple in	83.00	80.2871							
5	17.34	17.34	1.01			100.098		Nipple out									
6	19.04	19.04	1.70		3	119.917	191.821	Nipple in	262.00	81.9514							
7	19.57	19.57	0.53			311.739		Nipple out									
8	24.7909		5.22		4	375.901	190.153	Nipple in	246.00	77.6217							

c. Set up formulas to calculate the variables:
Count – use the “count” function and select an epoch of data (1 min for baseline, nipple-in epoch for feeding, or 2 min for baseline)

MeanRR – divide the Count column by the Duration of the epoch (use the duration column for feeding periods)

MinRR- calculate min value for the RR value of all feeding epochs.

MaxRR-calculate the max value for RR for all feeding epochs.

Apnea – identify the number of pauses > 3 seconds. For any apnea identified, review the acq file to confirm.

APPENDIX 3.5: LINEAR MIXED MODELING ANALYSIS OF CHAPTER 3 DATA

Linear mixed modeling (LMM) was used to analyze these repeated-measures physiologic data. The variable name is denoted in square brackets. The effects of flow (slow-flow vs. standard-flow) [FLOW] on the outcome measures described below were evaluated using appropriate covariance structures to account for correlation within the same feeding [FDG] and within the same flow category [FLOW]. When appropriate, baseline values were used as a covariate in the model.

For each dependent variable, a step-wise LMM analysis was done. The model tested, model problems, results, and AIC/BIC scores are tabled below. Decisions about variables included in the random effects models and fixed effects models are also described within each table. The results of the final model selected are presented and interpreted at the end of each table.

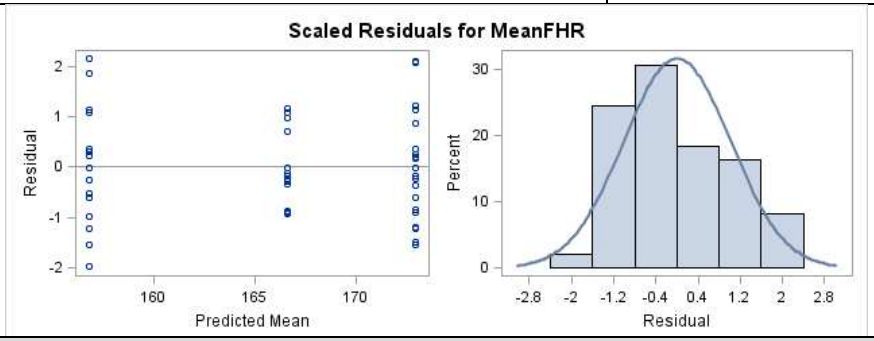
A. **Mean Heart Rate During Feeding [MeanFHR]:**

Model	Model Problems	Results	AIC BIC
The purpose of this analysis was to evaluate whether there was a difference between slow-flow and standard-flow feedings for the outcome variable mean heart rate during feeding [MeanFHR], taking into account correlation within the same feeding [FDG] and within flow category [FLOW], as well as covarying on baseline heart rate [MeanBHR]. MeanFHR was calculated every 1 minute during feeding. MeanBHR was calculated as a single measure for the entire 6 minute baseline period.			
The fixed effects model was determined first, starting with a full factorial model and then reducing in a step-wise fashion.			
*Model A1; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFHR=flow|fdg/ solution; random intercept fdg meanBHR / subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		
*Model A2; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFHR=flow fdg/ solution; random intercept fdg meanBHR / subject=id v=**1** vcorr=**1**;	Overparameterized		

run;			
*Model A3; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFHR=flow/ solution; random intercept fdg meanBHR / subject=id v=**1** vcorr=**1**; **run**;	The model was able to estimate the fixed effect of flow, but was still overparameterized and not able to estimate covariance parameters.		
The simple fixed effects model with flow is most appropriate. Next, the random effects model was simplified.			
*Model A4; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFHR=flow/ solution; random intercept fdg / subject=id v=**1** vcorr=**1**; **run**;	G matrix not positive definite.	FLOW p=0.8294	257.9 249.9
*Model A5; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFHR=flow/ solution; random intercept meanBHR / subject=id v=**1** vcorr=**1**; **run**;	G matrix not positive definite.	FLOW p=0.0139	253.6 245.6
Model A5 with meanBHR as a covariate improved the AIC/BIC scores. Next, the random effects model with just intercept tested.			
*Model A6; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFHR=flow/ solution; random intercept / subject=id v=**1** vcorr=**1**; **run**;	G matrix not positive definite.	FLOW p=0.8599	339.8 333.8
Model A5 continues to have better AIC/BIC scores. MeanBHR contributes significantly to the model. Next, the model was tested without the random intercept.			
*Model A7; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFHR=flow/ solution; random meanBHR / subject=id v=**1** vcorr=**1**; **run**;	G matrix not positive definite.	FLOW p=0.8599	339.8 333.8
The most appropriate random effects model was with random intercept and meanBHR. Finally, the fixed effects model was retested with this			

random effects model.			
*Model A8; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFHR=flow|fdg/ solution; random intercept meanBHR / subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		
*Model A9; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFHR=flow fdg/ solution; random intercept meanBHR / subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		
Model A9 was overparameterized. The model was then tested with fdg in the fixed effects model with a random intercept only.			
*Model A10; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFHR=flow fdg/ solution; random intercept/ subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		
*Model A11; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFHR=flow meanBHR/ solution; random intercept meanBHR / subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		
Model A11 was overparameterized. The model was then tested with only a random intercept.			
*Model A12; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFHR=flow meanBHR/ solution; random intercept / subject=id v=**1** vcorr=**1**; **run**;	G matrix not positive definite.	FLOW p=0.0128 MeanBHR p<0.0001	241.1 233.1
Model A12 had the best AIC/BIC scores. Next, a residual analysis was run to test for outliers.			
*A residual analysis was then run with Model A12; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFHR=flow meanBHR/ solution outpm=residls vciry;; random intercept / subject=id v=**1** vcorr=**1**; **run**;		No outliers outside of +/- 3.	

```
data residls;
set residls;
label pred="Predicted Value"
scaledresid="Scaled Residual";
format pred scaledresid 7.2;
run;
```



The mean procedure was run to get group estimates for MeanFHR for the two flow groups.

FLOW=0

Analysis Variable : MeanFHR				
N	Mean	Std Dev	Minimum	Maximum
36	166.1846942	8.5589935	151.6216490	178.4073630

FLOW=1

Analysis Variable : MeanFHR				
N	Mean	Std Dev	Minimum	Maximum
13	166.6037602	1.9479823	164.1655760	169.6276220

Proc means was also used to estimate values per feeding.

FDG=1

Variable	N	Mean	Std Dev	Minimum	Maximum
MeanFHR	21	172.9042224	2.7391000	168.9036340	178.4073630

MeanBHR	21	167.9021414	0	167.9021414	167.9021414

FDG=2

Variable	N	Mean	Std Dev	Minimum	Maximum
MeanFHR	13	166.6037602	1.9479823	164.1655760	169.6276220
MeanBHR	13	156.6497913	0	156.6497913	156.6497913

FDG=4

Variable	N	Mean	Std Dev	Minimum	Maximum
MeanFHR	15	156.7773549	3.1425173	151.6216490	162.3923536
MeanBHR	15	146.5621806	0	146.5621806	146.5621806

Interpretation of results: Taking into account correlation between measurements within the same feeding and within the same flow group (slow or standard) and covarying on baseline heart rate, mean heart rate was significantly lower when the infant was fed with slow-flow than with standard flow (p=0.01; 166.2 vs. 166.6).

B. **Mean Oxygen Saturation During Feeding (MeanFSat):**

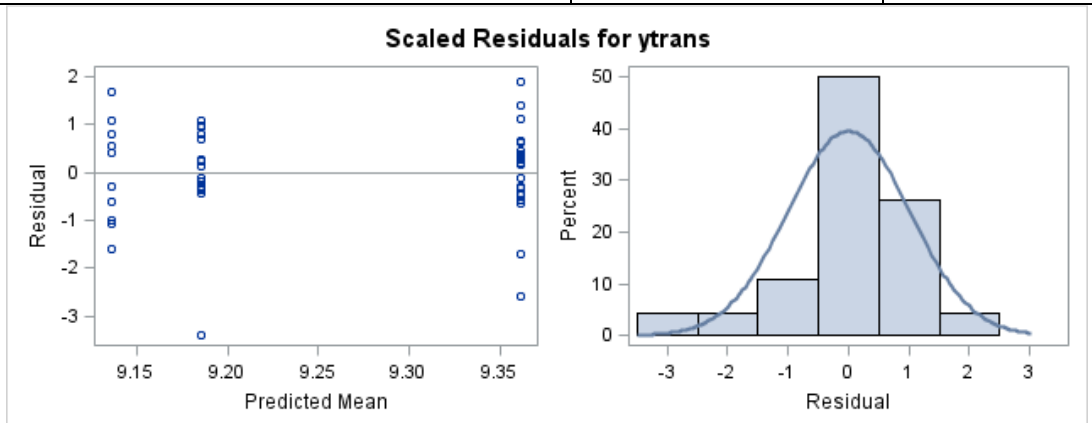
Model	Model Problems	Results	AIC BIC
The purpose of this analysis was to evaluate whether there was a difference between slow-flow and standard-flow feedings for the outcome variable mean oxygen saturation during feeding, taking into account correlation within the same feeding [FDG] and within flow category [FLOW], as well as covarying on baseline oxygen saturation [MeanBSat]. MeanFSat was calculated every 1 minute during feeding. MeanBSat was calculated as a single measure for the entire 6 minute baseline period.			
Starting with a maximal fixed effects model, the random effects model was determined. First, a maximal random effects model was tested and then one variable was removed at a time.			
*Model B1; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFSat=flow fdg meanBSat / solution; random intercept flow fdg meanBSat / subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		

*Model B2; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFSat=flow fdg meanBSat / solution; random intercept flow fdg / subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		
*Model B3; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFSat=flow fdg meanBSat / solution; random intercept flow meanBSat / subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		
*Model B4; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFSat=flow fdg meanBSat / solution; random intercept fdg meanBSat / subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		
*Model B5; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFSat=flow fdg meanBSat / solution; random intercept flow/ subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		
*Model B6; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFSat=flow fdg meanBSat / solution; random intercept fdg / subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		
*Model B7; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFSat=flow fdg meanBSat / solution; random intercept meanBSat / subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		
*Model B8; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFSat=flow fdg meanBSat / solution;	Overparameterized		

random intercept / subject=id v=**1** vcorr=**1**; **run**;			
All of the models tested thus far were overparameterized. Next, the fixed effects model was determined with only a random intercept.			
*Model B9; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFSat=flow fdg / solution; random intercept / subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		
*Model B10; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFSat=flow meanBSat / solution; random intercept / subject=id v=**1** vcorr=**1**; **run**;	Estimated G matrix not positive definite.	FLOW p=0.0935 MeanBSat=0.0194	265.4 257.4
Model B10 is the only model thus far that has not been overparametized. Using this fixed effects model, variables were then added back into the random effects model.			
*Model B11; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFSat=flow meanBSat / solution; random intercept fdg / subject=id v=**1** vcorr=**1**; **run**;	Estimated G matrix not positive definite.	FLOW p=0.0935 MeanBSat=0.0194	265.4 257.4
*Model B12; **proc mixed** data=sv covtest method=ML; class id flow fdg; model meanFSat=flow meanBSat / solution; random intercept meanBSat / subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		
Model B10 and B11 had the same results and AIC/BIC scores, but B10 is more parsimonious, so this is the better model. B12 was overparameterized. A residual analysis was then run using model B10.			
proc mixed data=sv covtest method=ML; class id flow fdg; model meanFSat=flow meanBSat / solution outpm=residls vciry; random intercept / subject=id v=**1** vcorr=**1**; **run**; **data** residls;		Outliers outside of -3.	

set residls; label pred="Predicted Value" scaledresid="Scaled Residual"; format pred scaledresid **7.2**; **run**;			
Since there were outliers outside of -3, a square root transformation was applied.			

%let ypower=0.5;
data adjusted;
set sv;
ytrans=meanFSat**&ypower;
label ytrans="meanFSat to Power &ypower";
run;
 	
proc mixed data=adjusted covtest method=ML;
class id flow fdg;
model ytrans=flow meanBSat / solution outpm=residls vciry;
random intercept / subject=id v=**1** vcorr=**1**;
run;
		No longer ouliers.
		FLOW p=0.0976
		MeanBSat p=0.0207	

			
Proc means was used to estimate group values for meanFSat.			
proc sort data=sv; by flow; **Proc means** data=sv; by flow; var meanFSat; **run**;			

FLOW=0

Analysis Variable : MeanFSat				
N	Mean	Std Dev	Minimum	Maximum
36	86.3247774	4.3283691	71.3568713	95.5532011

FLOW=1

Analysis Variable : MeanFSat				
N	Mean	Std Dev	Minimum	Maximum
10	83.5143588	4.2417796	77.2311341	90.3147783

Proc means was also used to estimate values per feeding.

FDG=1

Variable	N	Mean	Std Dev	Minimum	Maximum
MeanFSat	21	87.6830048	3.9699620	77.4790997	95.5532011
MeanBSat	21	83.8056300	0	83.8056300	83.8056300

FDG=2

Variable	N	Mean	Std Dev	Minimum	Maximum
MeanFSat	10	83.5143588	4.2417796	77.2311341	90.3147783
MeanBSat	10	85.9930982	0	85.9930982	85.9930982

FDG=4

Variable	N	Mean	Std Dev	Minimum	Maximum
MeanFSat	15	84.4232591	4.2048298	71.3568713	88.8088242
MeanBSat	15	84.3901546	0	84.3901546	84.3901546

Note. Do not use the min and max values from these results. Actual min and max values were calculated from the data that was analyzed at 1 sample/sec.

Interpretation of results: Taking into account correlation between measurements within the same feeding and within the same flow group (slow or standard) and covarying on baseline oxygen saturation, there was a trend toward a difference in mean oxygen saturation between the flow groups (p=0.0976; 86.3 vs. 83.5%).

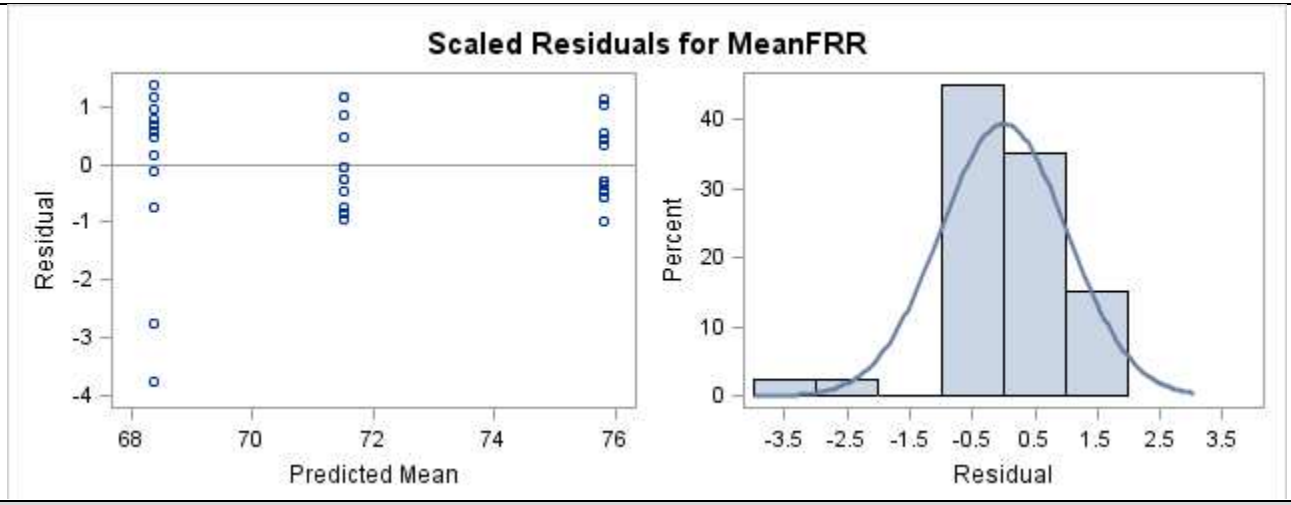
C. **Respiratory Rate (RR)**

Model	Model Problems	Results	AIC BIC
The purpose of this analysis was to evaluate whether there was a difference between slow-flow and standard-flow feedings for the outcome variable RR during feeding [MeanFRR], taking into account correlation within the same feeding [FDG] and within flow category [FLOW], as well as covarying on baseline RR [MeanBRR]. MeanFRR was calculated every 1 minute during feeding. MeanBRR was calculated as a single measure for the entire 6 minute baseline period.			
Starting with a maximal fixed effects model, the random effects model was determined. First, a maximal random effects model was tested and then one variable was removed at a time.			
*Model C1; **proc mixed** data=rr covtest method=ML; class id flow fdg; model meanFRR=flow fdg meanBRR/ solution; random intercept fdg meanBRR / subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		
*Model C2; **proc mixed** data=rr covtest method=ML; class id flow fdg; model meanFRR=flow fdg meanBRR/ solution; random intercept fdg / subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		
*Model C3; **proc mixed** data=rr covtest method=ML; class id flow fdg; model meanFRR=flow fdg meanBRR/ solution; random intercept meanBRR / subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		
*Model C4; **proc mixed** data=rr covtest method=ML; class id flow fdg; model meanFRR=flow fdg meanBRR/ solution; random intercept / subject=id v=**1** vcorr=**1**; **run**;	Overparameterized		

<table>
<tr><td colspan="4">All of the above models were overparameterized. Next, the fixed effects model was reduced one variable at a time.</td></tr>
<tr><td>*Model C5;
proc mixed data=rr covtest method=ML;
class id flow fdg;
model meanFRR=flow fdg/ solution;
random intercept / subject=id v=1 vcorr=1;
run;</td><td>Overparameterized</td><td></td><td></td></tr>
<tr><td>*Model C6;
proc mixed data=rr covtest method=ML;
class id flow fdg;
model meanFRR=flow meanBRR/ solution;
random intercept / subject=id v=1 vcorr=1;
run;</td><td>Estimated G matrix not positive definite.</td><td>Type 3 Tests of Fixed Effects
Effect | Num DF | Den DF | F Value | Pr > F
FLOW | 1 | 37 | 2.88 | 0.0979
MeanBRR | 1 | 37 | 3.88 | 0.0565</td><td>305.5
297.6</td></tr>
<tr><td colspan="4">Model C6 was the first model that was not overparameterized. Next, the model was tested without meanBRR in the fixed effects.</td></tr>
<tr><td>*Model C7;
proc mixed data=rr covtest method=ML;
class id flow fdg;
model meanFRR=flow/ solution;
random intercept / subject=id v=1 vcorr=1;
run;</td><td>Estimated G matrix not positive definite.</td><td>Flow p=0.875</td><td>307.3
301.3</td></tr>
<tr><td colspan="4">MeanBRR contributes significantly to the fixed effects model. Using the fixed effects model in model C6, the random effects model was then confirmed by adding the variables back in, one at a time.</td></tr>
<tr><td>*Model C8;
proc mixed data=rr covtest method=ML;
class id flow fdg;
model meanFRR=flow meanBRR/ solution;
random intercept fdg / subject=id v=1 vcorr=1;
run;</td><td>Not able to estimate covariance parameters.</td><td></td><td></td></tr>
<tr><td>*Model C9;
proc mixed data=rr covtest method=ML;
class id flow fdg;
model meanFRR=flow meanBRR/ solution;
random intercept meanBRR / subject=id v=1 vcorr=1;
run;</td><td>Overparameterized</td><td></td><td></td></tr>
<tr><td colspan="4">The above models were not improved by adding variables back to the random effects model. Next, the random effects model was tested without intercept in the model.</td></tr>
<tr><td>*Model C10;
proc mixed data=rr covtest method=ML;</td><td>Overparameterized</td><td></td><td></td></tr>
</table>

<table>
<tr><td>class id flow fdg;
model meanFRR=flow meanBRR/ solution;
random meanBRR / subject=id v=1 vcorr=1;
run;</td><td></td><td></td><td></td></tr>
<tr><td>*Model C11;
proc mixed data=rr covtest method=ML;
class id flow fdg;
model meanFRR=flow meanBRR/ solution;
random fdg / subject=id v=1 vcorr=1;
run;</td><td>Estimated G matrix not positive definite.</td><td>Type 3 Tests of Fixed Effects
<table><tr><th>Effect</th><th>Num DF</th><th>Den DF</th><th>F Value</th><th>Pr > F</th></tr><tr><td>FLOW</td><td>1</td><td>37</td><td>2.88</td><td>0.0979</td></tr><tr><td>MeanBRR</td><td>1</td><td>37</td><td>3.88</td><td>0.0565</td></tr></table></td><td>305.6
302.0</td></tr>
<tr><td colspan="4">Model C11 had a better AIC score than model C6, but model C6 had a better BIC score.</td></tr>
<tr><td>*Model C11 with residual analysis;
proc mixed data=rr covtest method=ML;
class id flow fdg;
model meanFRR=flow meanBRR/ solution outpm=residls vciry;
random fdg / subject=id v=1 vcorr=1;
run;

data residls;
set residls;
label pred="Predicted Value"
scaledresid="Scaled Residual";
format pred scaledresid 7.2;
run;</td><td>Estimated G matrix not positive definite.</td><td>Type 3 Tests of Fixed Effects
<table><tr><th>Effect</th><th>Num DF</th><th>Den DF</th><th>F Value</th><th>Pr > F</th></tr><tr><td>FLOW</td><td>1</td><td>37</td><td>2.88</td><td>0.0979</td></tr><tr><td>MeanBRR</td><td>1</td><td>37</td><td>3.88</td><td>0.0565</td></tr></table>
There are outliers outside of -3.</td><td>305.6
302.0</td></tr>
</table>



Since there were outliers, the data was transformed. First, a square root transformation was applied.

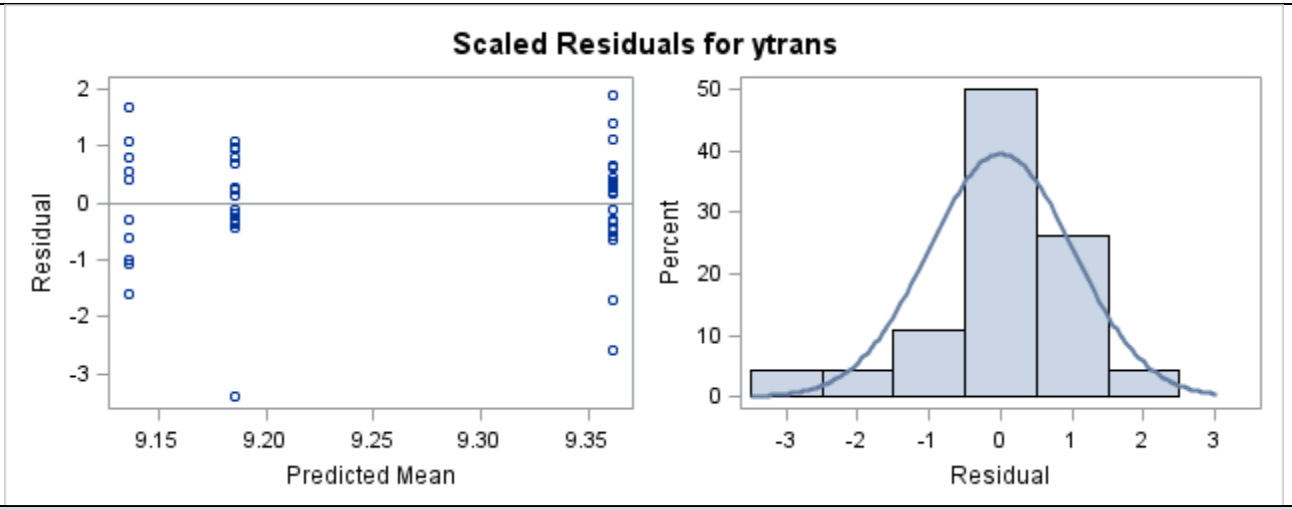
```
%let ypower=0.5;
data adjusted;
set rr;
ytrans=meanFRR**&ypower;
label ytrans="meanFRR to Power &ypower";
run;

*Model C11 with square root transformation;
proc mixed data=adjusted covtest method=ML;
class id flow fdg;
model ytrans=flow meanBRR/ solution outpm=residls vciry;
random fdg / subject=id v=1 vcorr=1;
run;
```

Estimated G matrix not positive definite.

Type 3 Tests of Fixed Effects

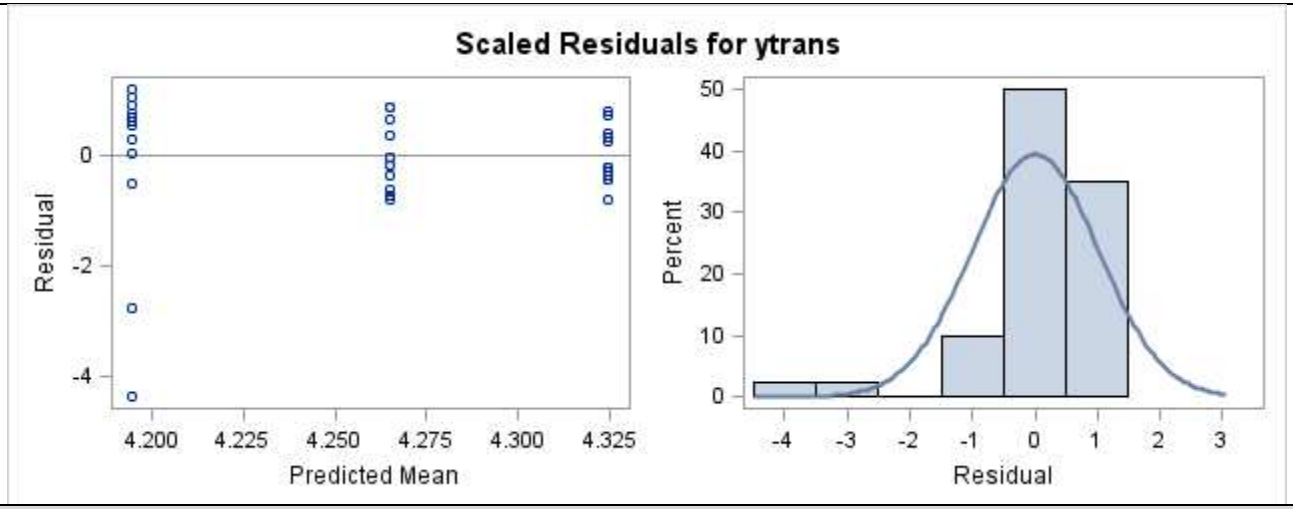
Effect	Num DF	Den DF	F Value	Pr > F
FLOW	1	37	3.11	0.0861
MeanBRR	1	37	3.92	0.0551



This square root transformation improved the data, but there was still an outlier outside of -3. Next, a log transformation was applied.

```
*Log transformation;
data adjusted;
set rr;
ytrans=log(meanFRR);
label ytrans="MeanFRR to Log";
run;

proc mixed data=adjusted covtest method=ML;
class id flow fdg;
model ytrans=flow meanBRR/ solution outpm=residls vciry;
random fdg / subject=id v=1 vcorr=1;
run;
```



Log transformation did not improve the data. Model D11 with square root transformation was used for the analysis. In this case, it does not make sense to remove outliers because the outliers are true data points and important for the analysis of these results. Proc means was used to estimate group values.

The MEANS Procedure
FLOW=0

Analysis Variable : MeanFRR				
N	Mean	Std Dev	Minimum	Maximum
28	72.0714286	11.8037300	31.0000000	87.0000000

FLOW=1

Analysis Variable : MeanFRR				
N	Mean	Std Dev	Minimum	Maximum
12	71.5000000	7.4406745	62.0000000	83.0000000

Proc means was used to estimate values per feeding.

The MEANS Procedure
FDG=1

Variable	N	Mean	Std Dev	Minimum	Maximum
MeanFRR	14	68.3571429	14.8044024	31.0000000	82.0000000

MeanBRR	14	64.0000000	0	64.0000000	64.0000000

FDG=2

Variable	N	Mean	Std Dev	Minimum	Maximum
MeanFRR	12	71.5000000	7.4406745	62.0000000	83.0000000
MeanBRR	12	53.0000000	0	53.0000000	53.0000000

FDG=4

Variable	N	Mean	Std Dev	Minimum	Maximum
MeanFRR	14	75.7857143	6.3630976	66.0000000	87.0000000
MeanBRR	14	72.0000000	0	72.0000000	72.0000000

Interpretation: When the infant was fed with slow-flow, RR was significantly higher than when fed with standard-flow (p=0.09; 72.1 vs. 71.5).

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CHAPTER 4: HEART RATE VARIABILITY AS A FEEDING INTERVENTION OUTCOME MEASURE IN THE PRETERM INFANT

Overview

Feeding interventions for preterm infants aim to reduce the stress of feeding to promote growth. One challenge to evaluating efficacy of feeding interventions is identifying non-invasive, sensitive outcome measures. Heart rate variability (HRV) is a potential measure of stress that may be useful. This study tested whether HRV was a sensitive measure of stress when usual care was compared to a gentle, co-regulated approach (CoReg) to feeding preterm infants (n=14) born < 35 weeks post-menstrual age using a secondary analysis of data from a within-subjects cross-over design study. HRV indices were calculated from electrocardiogram data and compared to standard physiologic outcomes, including oxygen saturation (Sp02), respiratory rate (RR), apneic events, heart rate (HR), and seconds of bradycardia. Data were analyzed using linear mixed modeling. Infants were positioned side-lying more (p<0.01) and stimulated to suck less (p=0.03) during CoReg feedings. Infants fed using the CoReg approach had fewer apneic events (p=0.04) and higher RR (p=0.03), suggesting they were able to breathe more during feeding. No statistically significant differences were found in Sp02, HR, bradycardia, LF Power, HF Power, or LF/HF ratio, but infants fed using the usual care approach had significantly higher SD12 (p=0.04), a non-linear measure of HRV based on the Poincaré plot. Higher SD12 in infants fed with usual care suggests increased randomness in HR of non-respiratory origin. Further exploration of HRV as an intervention outcome measure is needed, particularly evaluating non-linear indices such as SD12.

Keywords: feeding, preterm infant, heart rate variability, stress

Introduction

Feeding is a physiologically challenging event for the preterm infant. Much research is being done to evaluate interventions aimed at reducing the work of feeding for these fragile infants in order to conserve energy for growth and prevent the development of long-term feeding problems. The typical physiologic measures used to study the effect of interventions in this population are heart rate (HR), respiratory rate (RR), and oxygen saturation (Sp02). These measures are non-invasive and provide important information about the infant's physiologic response to the work of feeding. While these measures are generally accepted, they may not be the most sensitive measures, or alterations in their values may be late indicators of distress. Heart rate variability (HRV) is an additional non-invasive measure that can be used to evaluate the adaptability of the autonomic nervous system (ANS) to respond to the physiologic stress of feeding. Feeding has been shown to produce changes in HRV (Brown, 2007; McCain, Fuller, & Gartside, 2005; McCain, Knupp, Fontaine, Pino, & Vasquez, 2010). It is theorized that HRV may be a more sensitive measure of physiologic stress or provide important information about infant response to feeding in addition to these traditional measures. It remains unknown whether HRV is a useful outcome measure of physiologic stress in intervention studies where the intervention aims to change the level of stress experienced by the infant.

Heart Rate Variability

HRV is the rhythmic variation in HR that results from the dynamic influences of the ANS (Kleiger, Stein, & Bigger, 2005). Contraction of the heart is initiated by an electrical impulse in the sinoatrial (SA) node located in the right atrium (Powers & Howley, 2009). The SA node is innervated by both the sympathetic and parasympathetic divisions of the ANS via the vagus nerve (Pumprla, Howorka, Groves, Chester, & Nolan, 2002). The sympathetic and parasympathetic divisions have opposing effects on the HR. Sympathetic input results in release of noradrenaline, which increases HR, while parasympathetic input results in release of acetylcholine, which slows HR (Pumprla, et al., 2002). The balance of sympathetic and parasympathetic input is determined by the ANS to maintain physiologic homeostasis in response to internal and external demands (Pumprla, et al., 2002).

The variation that occurs in HR to produce HRV results from the balance of sympathetic and parasympathetic input and is measured by variations in the time period between normal heartbeat

complexes, specifically R waves (Kleiger, et al., 2005). The variation in intervals between R waves (R to R interval (rr_i)) has a cyclical pattern with some cyclical changes occurring infrequently or at low frequencies (LF) and some occurring frequently or at high frequencies (HF).

Since there are connections within the vagus nerve between the heart and other physiologic systems, input from the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) is mediated by other cyclical rhythms in the body. Fluctuations in blood pressure and thermoregulation contribute to LF rhythms in HRV (Pumprla, et al., 2002). Rhythmic alterations in HR that coincide with respiration contribute to HF rhythms in HRV (Pumprla, et al., 2002). It is generally accepted that HF components of HRV may be interpreted as a measure of PNS influences, but the interpretation of the LF component remains controversial (Malik, et al., 1996). While some interpret the LF component as reflecting SNS input, others believe that it reflects some combination of SNS and PNS input (Malik, et al., 1996).

The HF rhythm associated with respiration, whereby the HR accelerates during inspiration and decelerates during expiration, is often referred to as respiratory sinus arrhythmia (RSA) (Grossman & Taylor, 2007). The frequency range of HRV that RSA occupies is dependent on the RR of the individual. In human neonates, the RR ranges generally from 20 to 80 breaths per minute. Frequency is measured in Hertz (Hz). One Hz is equal to 60 cycles per second. Therefore, a RR of 20 to 80 breaths per minute is equal to a frequency range of 0.3-1.33 Hz (DiPietro & Porges, 1991).

There are three methods of HRV analysis: time domain, frequency domain, and non-linear analyses. Time domain methods are preferred for long-term studies of HRV (e.g., 24 hours) while frequency domain methods are preferred for short-term evaluation of HRV (e.g., less than 5 minutes) (Malik, et al., 1996). The frequency domain measures of HRV include LF Power (milliseconds squared (ms^2)), HF Power (ms^2), and LF/HF ratio. While the interpretation of the LF component remains under debate, a consensus statement suggests it is worth measuring, but must be done in accordance with standards that limit the lowest frequency measured by the length of the recording (Malik, et al., 1996). For recordings of two minutes, the lowest appropriate LF band is 0.04 Hz and the upper bound is the lower bound of the HF band or 0.3 Hz (Malik, et al., 1996). The LF/HF ratio is a measure of the balance of SNS to PNS (or some combination of SNS and PNS) activity and the ability of the infant to maintain

physiologic stability (McCain, et al., 2010). When evaluating HRV indices, it is necessary to compare epochs of the same length because epoch length affects the accuracy with which the frequency components are estimated (Malik, et al., 1996).

Non-linear analysis of HRV is a relatively new technique, which is in the early stages of development, but may contribute to the interpretation of frequency and time domain analysis by distinguishing between sinus arrhythmia of respiratory and non-respiratory origin (Stein, Domitrovich, Hui, Rautaharju, & Gottdiener, 2005). One method of non-linear analysis is the creation of a Poincaré plot (Figure 4.1), where the interval between a pair of heart beats (e.g., R wave 1 and R wave 2) is plotted against the interval between the next two heart beats (e.g., R wave 2 and R wave 3), such that the x,y coordinates are rr_i, rr_{i+1} (Stein & Reddy, 2005). Poincaré plots may be qualitatively analyzed for the shape and dispersion of the intervals or quantitatively analyzed for the degree of randomness in the heart rate pattern (Stein & Reddy, 2005). Quantitatively, the index SD12 provides information about the shape of the Poincaré plot by giving the ratio of the length of the transverse axis (SD1) and longitudinal axis (SD2) of the ellipse (Stein & Reddy, 2005). SD1 is an indicator of short-term variability of the HR and SD2 is an indicator of intermediate-term variability of the HR (Stein & Reddy, 2005). Low SD12 indicates high correlation between interbeat intervals while high SD12 indicates low correlation or increased randomness in interbeat intervals (Figure 4.2). SD12 has been found to be the strongest non-linear predictor of mortality in patients with cardiovascular disease by identifying patients with increased randomness in the heart rate from non-respiratory sinus arrhythmia (high SD12) (Stein & Reddy, 2005).

Theoretical Framework

Polyvagal Theory (Porges, 1995) was used in this study to conceptualize the infant response to the challenge of feeding and aid in the selection of outcome measures. Polyvagal Theory (Porges, 1995) describes the mammalian physiologic response to varying levels of stress as a function of the two pathways of the vagus nerve: the myelinated nucleus ambiguous (NA) and the unmyelinated dorsal motor nucleus (DMNX). Polyvagal Theory suggests that during times of low stress, there is primarily input from the highly-evolved NA, which results in conservation of metabolic resources for growth and restoration, as evidenced by low HR and high HF HRV. Additionally, increased vagal tone via the NA results in increased tone of the muscles of the head and face for social interaction and for coordination of sucking,

swallowing, and breathing during feeding (Porges, 2007). At the same time, minimal input from the DMNX during times of low stress encourages digestion and absorption of nutrients from the gastrointestinal (GI) tract (Porges, 2001).

During times of increased stress, there is upregulation first of the SNS, which responds by increasing HR, decreasing HF HRV, activating the stress response system of the hypothalamic pituitary axis (e.g., release of cortisol), stimulating the immune system (e.g., release of cytokines), and diverting blood away from the GI tract to the more vital organs such as the heart, brain, and lungs (Porges, 1992, 2009). If these responses still do not meet the demands of the situation, the secondary response system controlled by the DMNX is activated to conserve resources resulting in disengagement, hypotonia, apnea, and bradycardia (Porges, 2003). Given the high oxygen needs of humans, the apnea and bradycardia associated with activation of the DMNX response can lead to detrimental oxygen deprivation (Porges, 2007).

Although feeding is inherently a physiologically challenging event, the feeding encounter may be altered to either increase or decrease the degree of stress the infant experiences. A feeding with lower stress would theoretically support input from the NA and result in lower HR, higher HF HRV, and improved coordination of sucking, swallowing, and breathing. Improved coordination of sucking, swallowing, and breathing may allow the infant to maintain Sp02 and RR closer to that during non-feeding times. Alternately, a feeding with a higher degree of stress would require activation of the stress response systems and result in higher HR, lower HF HRV, release of cortisol and cytokines, disengagement, hypotonia, apnea, and bradycardia.

State of the Literature

Nine published studies have measured HRV during active oral feeding of infants (Brown, 2007; Cohen, Brown, & Myers, 2009; Harrison, 2011; Harrison & Brown, 2012; Lappi, et al., 2007; McCain, et al., 2005; McCain, et al., 2010; Portales, et al., 1997; Suess, et al., 2000). The majority of these have been descriptive studies of the changes that occur in HRV measures as infants develop over time (Brown, 2007; Harrison, 2011; Harrison & Brown, 2012; Lappi, et al., 2007) or how birth characteristics, such as birth weight, gestational age, or congenital heart disease, affect HRV responses to feeding (Cohen, et al., 2009; Harrison, 2011; Harrison & Brown, 2012; Suess, et al., 2000). Consistent with

Polyvagal Theory, Portales and colleagues (1997) found that vagal tone decreased from baseline to feeding and returned towards baseline during the post-feeding period in very low birth weight infants. McCain (2010) described the HRV responses of three preterm infants with bronchopulmonary dysplasia to oral feeding and also found that HF HRV decreased during feeding, but in this sample, HF HRV did not recover to pre-feeding levels by 10 minutes post-feeding. Suess and colleagues (2000) also found that HF HRV decreased during feeding for both a group of earlier-born (≤ 30 weeks gestational age) and a group of later-born (≥31 weeks gestational age) preterm infants, but that in the 10 minutes after feeding, only the later-born preterm infants began to return to pre-feeding levels. Lappi (2007) examined the effects of nutritive versus non-nutritive sucking on HRV measures and found that nutritive sucking resulted in a decrease in HRV at all ages studied (newborn, 6, 12, and 24 weeks of age) while non-nutritive sucking did not have any effect on HRV at any age.

The only feeding intervention study that measured HRV as an outcome was McCain's study (2005) of a semi-demand protocol for healthy preterm infants, which included a protocol that used infant cues to discontinue feeding and did not "encourage" the infant to consume the prescribed volume of feeing. Theoretically, this protocol could decrease the amount of physiologic stress experienced by the infant by not pushing the infant to continue feeding beyond the point they become fatigued or stressed. This study found that during feeding, the experimental group had higher HF power and lower LF/HF ratio, suggesting greater PNS input and lower physiologic stress (McCain, et al., 2005). The two groups were similar in terms of mean HR and feeding bradycardia episodes, suggesting that these outcome measures may be less sensitive than HRV (McCain, et al., 2005). McCain (2002) previously presented behavioral state outcomes of this same data set and found that infants fed with the semi-demand approach did not exhibit significantly different behavioral states during feeding than control infants. No other measures of physiologic stress have been published from this study to determine whether the experimental protocol should be considered as having decreased physiologic stress with regards to the interpretation of HRV findings, but the findings are supportive of further exploration of the use of HRV in this way.

Different methods of feeding (breast vs. bottle) or other differences in feeding (e.g., milk flow, position, etc.) may affect the physiologic stress of feeding (Chen, Wang, Chang, & Chi, 2000; Clark, 2007; DiPietro, Larson, & Porges, 1987; Mathew, 1991; Park, Thoyre, Knafl, Hodges, & Nix, 2014) and

therefore HRV measures. Additionally, Harrison (2011) found that in a group of infants with transposition of the great arteries, factors such as feeding skill and maternal sensitivity may affect the degree of autonomic responsiveness to feeding. Infants with low feeding skill, but high maternal sensitivity demonstrated increases in HF HRV over the course of feeding, suggesting that maternal sensitivity may aid in the infant maintaining homeostasis despite their low skill level (Harrison, 2011). In all studies except those by McCain and colleagues (2005; 2010), feeding was studied as a naturally occurring phenomenon and the feeding experience was not controlled. Although Harrison (2012) did not control the feeding experience, she did monitor it and reported that variations in position and sleep states were similar between groups. McCain and colleagues (2005; 2010) did standardize the study feedings with regards to bottles used, milk flow, and body positioning.

The research done to date utilizing HRV during feeding has been supportive of the theoretical changes that occur within the ANS during a physiologically-stressful challenge such as oral feeding and supports the use of this measure during feeding. Only McCain's (2005) study has used HRV as a measure to evaluate differences between feedings where an intervention was used to decrease the degree of stress encountered by the infant. The purpose of this study was to test whether HRV is a sensitive measure of physiologic stress when standard care feeding is compared to a gentle, co-regulated approach to feeding preterm infants.

Methods

Setting and Sample

This secondary analysis of de-identified data was deemed exempt by the Institutional Review Board. The setting of the original data collection was a Level III Neonatal Intensive Care Unit in North Carolina.

The original sample was 20 preterm infants who were born at less than 35 weeks gestation weighing less than 1500 grams and were less than or equal to 37 weeks post-menstrual age (PMA) at the time of the study, remained hospitalized, and had been orally feeding at least once per day for 3 consecutive days (Thoyre, Holditch-Davis, Schwartz, Melendez Roman, & Nix, 2012). Infants may have been receiving supplemental oxygen. Infants were excluded from the study if they had a history of Grade IV intraventricular hemorrhage (IVH), congenital disorders that may have interfered with sucking (e.g.,

cleft palate, Down Syndrome, congenital hydrocephalus, or microcephaly), symptoms from substance exposure, or a mother less than 15 years of age. Infant feeding data were excluded from this secondary analysis if active feeding episodes with the bottle in the infant's mouth were ≤ 2 minutes or if the HR data were of poor quality and not analyzable for HRV.

Procedure

The data collection procedures that are described refer to the procedures carried out in the original study (Thoyre, et al., 2012). The methods of HRV analysis described refer to the procedures carried out in this secondary analysis. The original study was a within-subjects, cross-over design study of a gentle, co-regulated approach to feeding preterm infants. The intervention (CoReg) included a head elevated, side-lying position; minimal oral and tactile stimulation; and enhanced auditory assessment of sucking, swallowing, and breathing with the use of a microphone placed on the infant's neck to allow for feeder co-regulation of swallowing and breathing. The CoReg approach was compared to usual care feeding by a bedside nurse who was unaware of the intervention protocol. Infants were studied during two feedings per day for two days (one CoReg and one usual care feeding each day) and the order of the conditions was randomized without replacement.

Feedings were video recorded and The Observer XT (Noldus Information Technology, Asheville, NC) was used to code the videos for the following feeder actions: positioning of the infant (side-lying or other), number of times the feeder stimulated the infant to suck, number of times the feeder gave the infant a pause by tipping the bottle back or stopping milk flow, and number of times the feeder gave the infant a rest period by removing the nipple. Positioning of the infant was calculated as the percent of the feeding the infant was in side-lying. A co-regulation score was created and defined as the number of times the feeder provided a rest period for the infant plus the number of times the feeder cued the infant to pause feeding by tipping the bottle back or stopping milk flow. To account for differences in the length of feedings, frequency of feeder action events were divided by the number of minutes the bottle was in the infant's mouth. The observational data was used to evaluate fidelity to the CoReg protocol and to describe the feeding method used during usual care.

In order to compare HRV outcomes to traditional physiologic outcomes used in evaluating feeding interventions, the following measures were assessed during feeding: mean Sp02, mean RR, number of

apneic events, HR, and number of seconds of bradycardia. These measures were chosen based on the expected infant responses to the challenge of feeding as described by Polyvagal Theory (Porges, 1995).

Two methods were used to assess respiratory function during feeding. Sp02 was collected using a pulse oximeter (Ohmeda, Boulder, CO) placed on the infant's foot. Sp02 data was sampled using a 2 second averaging window and cleaned of artifact. Mean Sp02 during feeding was calculated. Respiration data was collected using respiratory inductance plethysmography bands placed around the infant's chest and abdomen (Respitrace, Ambulatory Monitoring Ind., Ardsley, NY). Electrocardiogram (ECG) data was collected from a three-lead ECG monitor (Gould Electronics, Valley View, Ohio). Physiologic data (Sp02, RR, and HR) was collected at a sampling rate of 1,000 samples per second, digitized by an A-D converter, and stored on a computer using Windaq Data Acquisition software (Dataq Instruments Inc., Akron, OH).

Respiratory waveform data was imported into Windaq Waveform Browser (Dataq Instruments Inc., Akron, OH), marked using an algorithm within the program, and confirmed for accuracy by the investigators. Respiratory waveform data was used (to calculate mean RR and number of episodes of apnea (defined as absence of breath for more than 4 seconds (sec)) (Hanlon, et al., 1997). Number of episodes of apnea were divided by the total length of feeding to account for variation in length of feedings.

Digitized ECG data was imported into MindWare HRV (MindWare Technologies LTD, Gahanna, OH) for review and analysis. MindWare HRV uses an algorithm to mark each R wave peak of the QRS heartbeat complex and to calculate the interbeat interval (IBI). Artifact is identified by IBI that is inconsistent with those intervals before and after. The investigator manually reviewed the data to confirm accuracy of the R wave peak detection. ECG data were used to calculate HR (in beats per minute (bpm)) and number of seconds of bradycardia (defined as HR < 100 bpm) (Dawson, et al., 2013). Mean HR was calculated for each two-minute bottle-in period. ECG data were also used for HRV analyses.

Frequency domain analysis of HRV was performed using two-minute epochs of artifact-free data. Power spectral analysis with Hamming windowing function was executed with the frequency bandwidths set for LF: 0.04-0.3 Hz and HF: 0.3-1.33 Hz. The outcome measures were: LF Power (ms^2), HF (ms^2), and LF/HF ratio. Two minutes was chosen as the epoch for comparison because this is the shortest

period that allows for the measurement of both LF and HF components of HRV (Malik, et al., 1996) and because this is a reasonable amount of time to expect vulnerable preterm infants to be able to feed without having the bottle removed from the mouth. Only data collected when the bottle was in the infant's mouth was analyzed. In some cases, the infant required prolonged breaks with the bottle out of the mouth. Since this study aimed to evaluate HRV during feeding, only data during active feeding was analyzed. Data for active feeding periods that were not two minutes in length were excluded.

Non-linear analysis of HRV was completed by combining all two-minute epochs of artifact-free data during each feeding. Kubios HRV version 2.0 (Kuopio, Finland) was used to create a single Poincaré plot for each feeding and to calculate SD12.

Statistical Analysis

Linear mixed modeling was used to analyze these repeated-measures data for effects of feeding method (Usual care vs. CoReg) using appropriate covariance structures to account for correlation between feedings within the same infant and within the same feeding over time, as well as variances possibly changing over time. The order effect of feeding method was tested and included in the model, when appropriate. Frequency domain measures of HRV have been found to have skewed distributions (Kleiger, et al., 2005). These data were assessed for skewness and transformed, if needed. An alpha of 0.05 was used for all tests of statistical significance. A trend towards statistical significance was defined as an alpha of 0.1. Appendix 4.1 provides details of the linear mixed modeling analyses.

Results

Sample

The sample included fourteen infants and 34 feedings. Of the 75 original feedings, 22 were excluded because the data were collected at a sampling rate of only 50 samples per second, which is not sufficient for HRV analysis; 14 were excluded because there were no active feeding periods of at least 2 minutes; 21 were excluded because of poor ECG data or no artifact-free segments of active oral feeding; and 4 (all from the same infant) were excluded because of abnormal cardiac rhythm, specifically frequent premature ventricular contractions.

All 14 infants included in the study had a usual care feeding that was included in the analysis. Ten of the infants had at least one of each feeding type that was analyzed. There were 22 usual care

feedings and 12 CoReg feedings analyzed. Table 4.1 provides information about the demographic and clinical data of the infants and feedings included in the study. The sample of infants that had a usual care feeding analyzed (n=14) was similar to the sample of infants that had an intervention feeding analyzed (n=10) in terms of birth weight (996 vs. 992 grams), PMA (28.6 vs. 28.8 weeks), and chronic lung disease (79% vs. 80%). The sample of feedings that were included for usual care (n=22) and CoReg (n=12) were also similar in terms of the age of the infant at the time of the feeding (36.4 vs. 36.9 weeks), oxygen use on the day of the study (64% vs. 67%), and number of days of feeding experience the infant had at the time of the study feeding (13 vs. 13).

Results

Feeder Actions. The CoReg and usual care feedings were significantly different from one another in terms of feeder actions (Table 4.2). Taking into account correlation between feedings of the same infant, CoReg feedings were performed with the infant in a side-lying position significantly more than usual care feedings (p<0.01) and the feeder stimulated the infant to suck significantly less during CoReg feedings (p=0.03). The CoReg and usual care feedings were not significantly different in terms of the co-regulation score (p=0.80). Given the differences in feeder actions between the two feeding methods, there was reason to believe that the physiologic stress experienced by the infant during the two methods may be different.

Standard physiologic measures (Table 4.3). Accounting for order of study feedings and correlation within feedings of the same infant, there were no significant differences between the two feeding methods for mean Sp02 (p=0.26) or number of seconds of bradycardia during feeding (p=0.11). Baseline Sp02 was used as a covariate in the analysis to evaluate mean Sp02 during feeding. There was also no difference between the groups for HR (p=0.38), accounting for order of feedings and correlation between feedings of the same infant and between measurements within the same feeding.

Infants fed using the CoReg method had significantly fewer feeding-related apneas (p=0.04). This analysis also took into account order of study feedings and correlation within feedings of the same infant. Infants fed using the CoReg method also had significantly higher RR than infants fed using usual care (p=0.03), accounting for baseline RR, correlation within feedings of the same infant, and order of study feedings.

Heart rate variability measures (Table 4.4). Taking into account correlations between feedings of the same infant and between measurements within the same feeding, as well as taking into account the order of study feedings, there was no statistically significant difference in LF Power (p=0.06) or HF Power (p=0.26) between feeding methods, although there was a trend towards significance with regards to LF Power. There were no significant differences between feeding methods for LF/HF ratio (p=0.86). However, infants fed with the usual care technique had a significantly higher SD12 than infants fed with the CoReg approach (p=0.04).

Discussion

A gentle, co-regulated approach to feeding involving side-lying positioning, decreased stimulation, and enhancement of auditory assessment of sucking, swallowing, and breathing was found to significantly reduce the number of apneic events during feeding and to allow the infants to breathe more during feeding, as evidenced by higher RR. Side-lying positioning as a means of reducing physiologic stress during feeding in preterm infants has been supported by another study by Park and colleagues (2014), who also found that side-lying allowed infants to maintain respiratory rate closer to the pre-feeding state, have briefer feeding-related apneic events, and less severe and fewer decreases in heart rate. The results of the standard physiologic measures suggested that the intervention feeding technique reduced the physiologic stress of feeding for this group of preterm infants, however none of the frequency domain measures of HRV revealed a significant difference between the feeding methods. There was a trend toward significance for the outcome LF Power (p=0.06), with infants fed with the usual care technique having LF Power of 366 ms^2 compared to 75 ms^2 for infants fed with the CoReg technique. These results suggest that infants fed with the CoReg technique may have had a trend toward lower SNS input, and therefore less stress, during feeding. As mentioned previously, however, the interpretation of LF HRV remains controversial.

The non-linear HRV index of SD12 revealed a significant difference between the feeding methods. Infants fed with the usual care technique were found to have significantly higher SD12 than infants fed with the usual care technique (p=0.04). Increased SD12 indicates increased randomness of the heart rate, or sinus arrhythmia of non-respiratory origin as opposed to respiratory sinus arrhythmia (RSA) (Stein, et al., 2005; Stein & Reddy, 2005). Frequency domain analyses are unable to differentiate

variability in the heart rate from these two sources, which is important because increased variability from respiratory origin is considered an indicator of lower risk, while increased variability from non-respiratory origin is an indicator of higher risk (Stein, et al., 2005). Lower SD12 in the infants fed with the CoReg technique suggests that the intervention may have been protective in some way, although it is not clear whether this should be interpreted as decreased stress.

Conclusion

The results of this study support further exploration of HRV as a feeding intervention outcome and highlight the need for consideration of both linear and non-linear HRV measures during this exploration. The significance of nonlinear HRV measures is yet to be fully defined, especially in infants, although it was the only HRV index in this study to find a statistically significant difference between feeding methods. Infants, particularly preterm infants, are more likely to experience bradycardia, or transient decreases in HR, as a result of stress compared to adults. The impact of this physiologic difference between infants and adults on HRV outcomes needs further clarification. Nonlinear HRV may be particularly useful for evaluation of HRV in this population.

Future research may consider exploring the use of HRV for identification of readiness for oral feeding in preterm infants and the usefulness of real-time HRV during feeding for evaluation of stress during feeding. The combination of HRV with other measures, such as cortisol, cytokines, galvanic skin response, and/or near infrared spectroscopy of abdominal tissue oxygenation may provide a more comprehensive evaluation of stress during feeding. This body of literature is in the early stages of development and would benefit from consistency in future studies with regards to the HRV parameters measured and units reported, epoch lengths studied, and definitions of HRV frequency bandwidths so that comparisons can be made between study findings.

Table 4.1.

Demographic and Clinical Data

Parameter	Usual Care (n=14[a])	CoReg (n=10[a])
Birth weight (grams)	996 (713 - 1390)	992 (713 - 1390)
Birth PMA (weeks)	28.6 (24.6 - 32.3)	28.8 (24.6 - 32.3)
Gender (n, %)		
Female	11 (79%)	8 (80%)
Male	3 (21%)	2 (20%)
Race (n, %)		
African-American	4 (29%)	2 (20%)
Euro-American	8 (57%)	6 (60%)
Other	2 (14%)	2 (20%)
NBRS	3 (0 - 10)	3 (0 - 10)
Lung Disease (n, %)		
None	0	0
RDS	3 (21%)	2 (20%)
CLD	11 (79%)	8 (80%)
IVH (n, %)		
None	9 (64%)	7 (70%)
Grade 1	4 (29%)	2 (20%)
Grade 2	0	0
Grade 3	1 (7%)	1 (10%)
	Usual Care (n=22[b])	CoReg (n=12[b])
Study PMA (weeks)	36.4 (33.9 - 40.3)	36.9 (34 – 40.3)
0_2 Day of Study (n, %)		
Yes	14 (64%)	8 (67%)
No	8 (36%)	4 (33%)
0_2 During Feeding		
Yes	15 (68%)	10 (83%)
No	7 (32%)	2 (17%)
Feeding Experience (days)	13 (2 – 39)	13 (2 – 39)

Note. Parameters are presented as mean (minimum - maximum) unless otherwise noted. NBRS = Neurobiologic Risk Score (Brazy, Goldstein, Oehler, Gustafson, & Thompson, 1993); RDS = Respiratory Distress Syndrome; CLD = Chronic Lung Disease; IVH = Intraventricular Hemorrhage; PMA = Post-menstrual age; Birth PMA = PMA at birth; Study PMA = PMA on day of study; 0_2 = Oxygen; [a] =Sample size of infants; [b] = Sample size of feedings.

Table 4.2.

Feeder Actions

	Usual Care	CoReg	*p*
Side-lying	10.14%	100%	<0.01*
Stimulation to Suck	1.71	0.15	0.03*
Co-regulation score	1.88	2.09	0.80

Note. Side-lying = Percent of time during feeding the infant was held in a side-lying position. Stimulation to suck = number of events per minute where feeder stimulated the infant to suck. Co-regulation score = number of events per minute where feeder gave a rest period or cued the infant to pause feeding.
* $p < 0.05$.

Table 4.3.

Standard Physiologic Measures

	Usual Care	CoReg	*p*
Sp02	91.8%	92.4%	0.26
RR	62	70	0.03*
Apnea	0.68	0.17	0.04*
HR	159.4	157.1	0.38
Bradycardia	0.59	0.43	0.11

Note. * Indicates $p<0.05$. Sp02 = mean oxygen saturation during feeding. RR = respiratory rate (breaths/min) during feeding. Apnea = number of apneic events per minute during feeding. HR = mean heart rate during feeding. Bradycardia = number of seconds of bradycardia per minute of feeding.

Table 4.4.

Heart Rate Variability Measures

	Usual Care	CoReg	*p*
LF Power (ms^2)	366.06	75.16	0.06
HF Power (ms^2)	22.92	6.80	0.26
LF/HF Ratio	12.40	12.34	0.86
SD12	0.26	0.19	0.04*

Note. LF = low frequency. HF = high frequency. SD12 = ratio of the length of the transverse axis (SD1) and longitudinal axis (SD2) of the ellipse (Figure 4.1).
* $p < 0.05$.

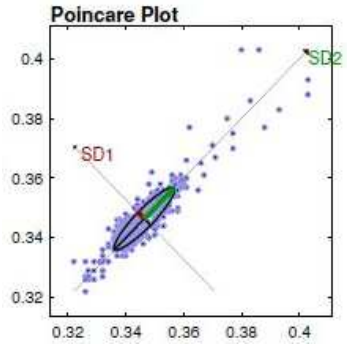


Figure 4.1. Poincaré plot with SD1 (red) and SD2 (green).

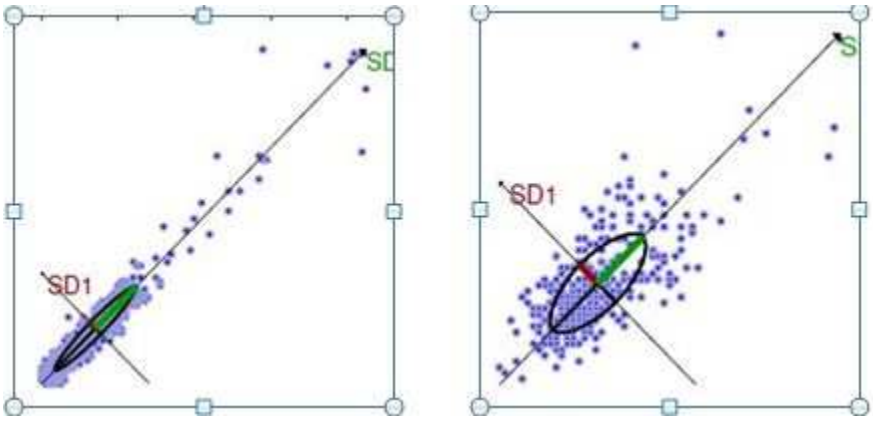


Figure 4.2. Poincaré plot examples of low SD12 (left) and high SD12 (right). Low SD12 indicates high correlation between interbeat intervals. High SD12 indicates low correlation, or increased randomness, in interbeat intervals.

APPENDIX 4.1: LINEAR MIXED MODELING ANALYSIS OF CHAPTER 4 DATA

Linear mixed modeling (LMM) was used to analyze these repeated-measures data. The variable name is denoted in square brackets. The effects of feeding method (intervention vs. standard-care) [GROUP] on the outcome measures described below were evaluated using appropriate covariance structures to account for correlation between feedings of the same infant [ID] and within the same feeding over time [FDGEVENT], as well as variances possibly changing over time [FTIMEINDEX or FHRVINDEX]. The order effect of feeding method was tested and included in the model, if appropriate [STUDYDAY & DAYFDGORDER].

For each dependent variable, a step-wise LMM analysis was done. The model tested, model problems, results, and AIC/BIC scores are tabled below. Decisions about variables included in the random effects models and fixed effects models are also described within each table. The results of the final model selected are presented and interpreted at the end of each table.

Question 1: Is there a difference between the feeding methods used in the intervention and that of standard care?

The intervention included a head elevated, side-lying position; minimal oral and tactile stimulation; and enhanced auditory assessment of sucking, swallowing, and breathing to allow for feeder co-regulation of swallowing and breathing. The intervention (CoReg) was compared to usual care feeding by a bedside nurse who was unaware of the intervention protocol. To evaluate whether the feeding methods used in the CoReg intervention were different from the usual care, feedings were analyzed for differences in the following variables: percent of feeding the infant was in a side-lying position [SIDELYING], the number of events where the feeder stimulated the infant to suck [STIMSUCK], and the co-regulation score (defined as the number of times the feeder provided a rest period for the infant plus number of times the feeder cued the infant to pause feeding by tipping the bottle back or stopping milk flow) [COREG]. These variables had only one measurement per feeding so there was no need to account for correlation within the same feeding event, but a given infant may have had more than one feeding, so correlation within infant [ID] was considered.

A. Side-lying: Percent of feeding the infant was in a side-lying position [SIDELYING].

<table>
<tr><th>Model</th><th>Model Problems</th><th>Results</th><th>AIC
BIC</th></tr>
<tr><td colspan="4">The purpose of this analysis was to evaluate whether there was a difference between groups for the outcome variable SIDELYING, taking into account correlation within infant (ID was included in the class statement as well as group). Since there was only 1 measurement per feeding, there was no need to account for correlation within feeding. Feeder actions do not have a carry-over effect, so there was no need to account for order effect variables. The fixed effects model only has group as a variable, so the random effects model was determined.</td></tr>
<tr><td>*Model A1;
proc mixed data=hrv covtest method=ML;
class id group;
model sidelying=group / solution;
random intercept group / subject=id type=cs v=4 vcorr=4;
run;</td><td>None</td><td>Group <0.0001</td><td>311.4
314.6</td></tr>
<tr><td>*Model A2;
proc mixed data=hrv covtest method=ML;
class id group;
model sidelying=group / solution;
random intercept / subject=id type=cs v=4 vcorr=4;
run;</td><td>Convergence criteria met but final hessian not positive definite.</td><td>Group <0.0001</td><td>313.4
316.6</td></tr>
<tr><td colspan="4">Solution for Fixed Effects
Effect GROUP Estimate Standard Error DF t Value Pr > |t|
Intercept 100.00 6.4808 9 15.43 <.0001
GROUP 0 -89.8615 8.3077 9 -10.82 <.0001
GROUP 1 0 . . . .</td></tr>
</table>

Interpretation of results: Taking into account correlation between feedings of the same infant, infants fed with the intervention method were fed in a side-lying position significantly more than infants fed with standard care (p<0.0001). Estimates for percent of the feeding in a side-lying position were for Group 0 (Usual care) 100 + -89.86 = 10.14% and for Group 1 (CoReg) = 100%.

B. Stimulation to Suck: STIMSUCK was the number of events where the feeder stimulated the infant to suck. A variable was created called STIMSUCKRATE, which was calculated as the number of STIMSUCK events divided by the length of time the bottle was in the mouth. The result is the number of events per second.

Model	Model Problems	Results	AIC BIC
The purpose of this analysis was to evaluate whether there was a difference between groups for the outcome variable STIMSUCKRATE, taking into account correlation within infant (ID was included in the class statement as well as group). Since there was only 1 measurement per feeding, there was no need to account for correlation within feeding. Feeder actions do not have a carry-over effect, so there was no need to account for order effect variables. The fixed effects model only has group as a variable, so the random effects model was determined.			
*Model B1; **proc mixed** data=hrv covtest method=ML; class id group; model stimsuckrate=group / solution; random intercept group / subject=id type=cs v=**4** vcorr=**4**; **run**;	None	Group=0.0284	-145.5 -142.3
*Model B2; **proc mixed** data=hrv covtest method=ML; class id group; model stimsuckrate=group / solution; random intercept / subject=id type=cs v=**4** vcorr=**4**; **run**;	None	Group=0.0044	-143.0 -139.8
Model B1 had the lower AIC/BIC scores, so this model was chosen.			

Solution for Fixed Effects

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		0.002525	0.007868	9	0.32	0.7556
GROUP	0	0.02605	0.009990	9	2.61	0.0284
GROUP	1	0	.	.	.	.

Interpretation of results: Taking into account correlation between feedings of the same infant, infants fed with usual care were stimulated to suck significantly more than infants fed with the CoReg method (p=0.03). Estimates for the stimulation to suck events per second were for Usual

care = 0.02605 + 0.002525 = 0.028575 and for CoReg = 0.002525. Converted to minutes, infants fed with the usual care method were stimulated to suck, on average, 1.71 times per minute. Infants fed with the CoReg method were stimulated to suck 0.15 times per minute.

C. Co-regulation score [COREG]: Co-regulation score was defined as the number of times the feeder provided a rest period for the infant plus number of times the feeder cued the infant to pause feeding by tipping the bottle back or stopping milk flow. A variable was created called COREGRATE, which was calculated as the co-regulation score divided by the length of time the bottle was in the mouth. The results for this outcome are in number of co-regulation events per second.

Model	Model Problems	Results	AIC BIC
The purpose of this analysis was to evaluate whether there was a difference between groups for the outcome variable COREGRATE, taking into account correlation within infant (ID was included in the class statement as well as group). Since there was only 1 measurement per feeding, there was no need to account for correlation within feeding. Feeder actions do not have a carry-over effect, so there was no need to account for order effect variables. The fixed effects model only has group as a variable, so the random effects model was determined.			
*Model C1; **proc mixed** data=hrv covtest method=ML; class id group; model coregrate=group / solution; random intercept group / subject=id type=cs v=**4** vcorr=**4**; **run**;	None	Group = 0.7996	-120.8 -118.2
*Model C2; **proc mixed** data=hrv covtest method=ML; class id group; model coregrate=group / solution; random intercept / subject=id type=cs v=**4** vcorr=**4**; **run**;	Stopped because of too many likelihood evaluations.		
Model C1 was the better model since C2 was stopped because of too many likelihood evaluations.			

Solution for Fixed Effects

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		0.03483	0.01038	9	3.35	0.0085
GROUP	0	-0.00347	0.01328	9	-0.26	0.7996
GROUP	1	0	.	.	.	.

Interpretation of Results: The intervention and usual care feeding methods were not significantly different in the amount of co-regulation provided (p=0.7996). Estimated coregrate (#/sec) for group 0 (usual care) was 0.03483 – 0.00347 = 0.03136 and Group 1 (CoReg) = 0.03483. Converted to number of events per minute, infants fed with the usual care method were co-regulated 1.88 times per minute while infants fed with the CoReg method were co-regulated 2.09 times per minute.

Question 2: Is there a difference between the feeding methods for standard physiologic outcome measures?

An analysis was run to determine if there was a difference in standard physiologic variables between the intervention and usual-care feeding methods [GROUP] in terms of: mean oxygen saturation (Sa02) during feeding, feeding-related apneic and bradycardic events, mean heart rate (HR), and mean respiratory rate (RR). These variables had only one measurement per feeding, so there was no need to account for correlation within feedings, but correlation between feedings of the same infant was evaluated. Order of study feedings was also considered. When baseline values for a variable were available and appropriate, these were considered in the covariance structure.

D. Mean Sa02 during feeding [MEANFDGSAO2] covarying on BASEO2:

Model	Model Problems	Results	AIC BIC
The purpose of this analysis was to evaluate whether there was a difference between groups for the outcome variable MEANFDGSa02, taking into account correlation within infant (ID was included in the class statement as well as group). Since there was only 1 measurement per feeding, there was no need to account for correlation within feeding. If the prior feeding was particularly stressful, there is the possibility for a carry-over effect on Sa02, so order effect variables were included (studyday and dayfdgorder). Mean baseline Sa02 (BASE02) was also included in the analysis as a covariate. First, the fixed effects model was determined, with all variables in the random effects model.			
*Model D1; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group studyday dayfdgorder BASEO2 / solution; Random intercept group studyday dayfdgorder BASEO2 / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.4343 STUDYDAY 0.6350 DAYFDGORDER 0.4351 BASEO2 0.1116	180 185.8
*Model D2; **Proc mixed** data=hrv covtest method=ML; Class id group;	None	GROUP 0.4414 STUDYDAY 0.6655	193.3 197.7

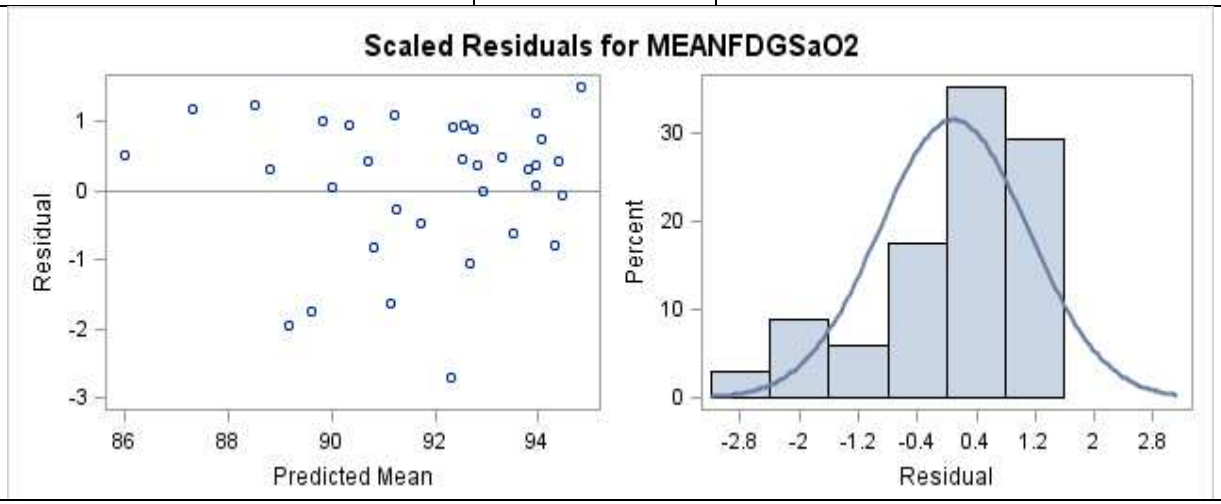
Model MEANFDGSaO2=group studyday dayfdgorder / solution; Random intercept group studyday dayfdgorder BASEO2 / subject=id v=**4** vcorr=**4**; **Run**;		DAYFDGORDER 0.3215	
*Model D3; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group studyday BASEO2 / solution; Random intercept group studyday dayfdgorder BASEO2 / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.4939 STUDYDAY 0.5884 BASEO2 0.1081	180.7 186.5
*Model D4; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder BASEO2 / solution; Random intercept group studyday dayfdgorder BASEO2 / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.4043 DAYFDGORDER 0.4326 BASEO2 0.1137	178.3 183.4
*Model D5; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group studyday / solution; Random intercept group studyday dayfdgorder BASEO2 / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.5181 STUDYDAY 0.6825	192.6 196.4
*Model D6; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder / solution; Random intercept group studyday dayfdgorder BASEO2 / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.4641 DAYFDGORDER 0.3221	191.5 195.3
*Model D7; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group BASEO2 / solution; Random intercept group studyday dayfdgorder BASEO2 /	None	GROUP 0.4697 BASE02 0.1099	179.0 184.1

subject=id v=**4** vcorr=**4**; **Run**;			
*Model D8; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group / solution; Random intercept group studyday dayfdgorder BASEO2 / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.5462	190.7 193.9
Model D4 had the best AIC/BIC scores for the fixed effects model. Using this fixed effects model, now start to remove one random effects variable at a time.			
*Model D9; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder / solution; Random intercept group studyday dayfdgorder BASEO2 / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.4044 DAYFDGORDER 0.4326 BASE02 .	178.3 183.4
*Model D10; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder BASEO2 / solution; Random intercept group studyday BASEO2 / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.4044 DAYFDGORDER BASE02 0.0116	178.3 183.4
*Model D11; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder BASEO2 / solution; Random intercept group dayfdgorder BASEO2 / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.3958 DAYFDGORDER 0.4037 BASE02 0.0317	176.3 180.8
*Model D12; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder BASEO2 / solution; Random intercept studyday dayfdgorder BASEO2 /	None	GROUP . DAYFDGORDER 0.2434 BASE02 0.0464	175.9 179.7

subject=id v=**4** vcorr=**4**; **Run**;			
*Model D13; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder BASEO2 / solution; Random intercept group studyday / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.4044 DAYFDGORDER 0.4614 BASE02 0.0311	178.3 183.4
*Model D14; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder BASEO2 / solution; Random intercept group dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.3958 DAYFDGORDER 0.4038 BASE02 0.1149	176.3 180.8
*Model D15; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder BASEO2 / solution; Random intercept group BASEO2 / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.3957 DAYFDGORDER 0.5375 BASE02 0.0009	176.3 180.8
*Model D16; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder BASEO2 / solution; Random intercept studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP . DAYFDGORDER 0.2434 BASE02 .	175.9 179.7
*Model D17; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder BASEO2 / solution; Random intercept studyday BASEO2 / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP . DAYFDGORDER . BASE02 0.0021	175.9 179.7

*Model D18; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder BASEO2 / solution; Random intercept dayfdgorder BASEO2 / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP . DAYFDGORDER 0.2365 BASE02 0.0029	175.9 179.7
*Model D19; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder BASEO2 / solution; Random intercept group/ subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.3958 DAYFDGORDER 0.4002 BASE02 0.0006	176.3 180.8
*Model D20; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder BASEO2 / solution; Random intercept studyday/ subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.2781 DAYFDGORDER 0.2434 BASE02 0.0021	175.9 179.7
*Model D21; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder BASEO2 / solution; Random intercept dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.2826 DAYFDGORDER 0.2365 BASE02 0.0029	175.9 179.7
*Model D22; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder BASEO2 / solution; Random intercept BASEO2 / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.2970 DAYFDGORDER 0.2635 BASE02 0.0008	175.9 179.7
*Model D23; **Proc mixed** data=hrv covtest method=ML; Class id group; Model MEANFDGSaO2=group dayfdgorder BASEO2 /	None	GROUP 0.2606 DAYFDGORDER 0.2250	175.9 179.7

<table>
<tr><td>solution;
Random intercept / subject=id v=4 vcorr=4;
Run;</td><td></td><td>BASE02 0.0003</td><td></td></tr>
<tr><td colspan="4">Model D23 was the most parsimonious random effects model of the models that all had an AIC/BIC score of 175.9/179.9. Next, a residual analysis was run to evaluate the data for outliers.</td></tr>
<tr><td>*Model D23 with residual analysis;
Proc mixed data=hrv covtest method=ML;
Class id group;
Model MEANFDGSaO2=group dayfdgorder BASEO2 /
solution outpm=residls vciry;
Random intercept / subject=id v=4 vcorr=4;
Run;

data residls;
set residls;
label pred="Predicted Value"
scaledresid="Scaled Residual";
format pred scaledresid 7.2;
run;</td><td>None</td><td>No outliers outside of +/- 3.</td><td>175.9
179.7</td></tr>
<tr><td colspan="4"> 
</td></tr>
<tr><td colspan="4">To estimate the values for MeanFdgSa02 between groups, DAYFDGORDER and BASE02 were removed from the model</td></tr>
<tr><td>*Model D24 for estimating MeanFdgSa02 between groups;
Proc mixed data=hrv covtest method=ML;
Class id group;
Model MEANFDGSaO2=group / solution;</td><td>None</td><td></td><td></td></tr>
</table>

Random intercept / subject=id v=**4** vcorr=**4**; **Run**;			

Solution for Fixed Effects

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		92.4371	1.1026	13	83.84	<.0001
GROUP	0	-0.6393	1.0475	19	-0.61	0.5489
GROUP	1	0	.	.	.	.

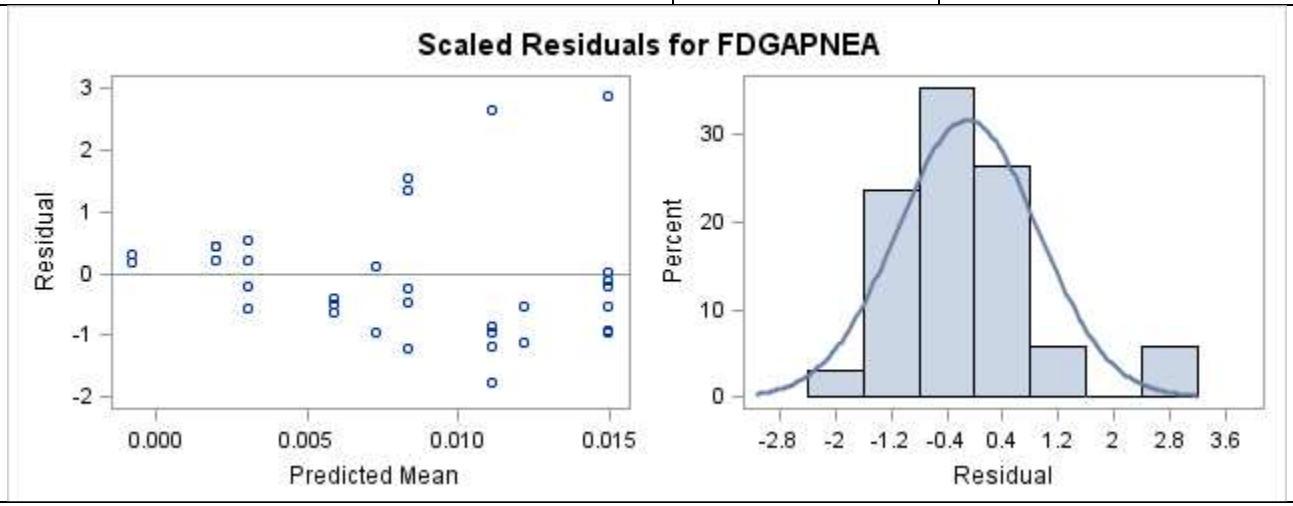
Interpretation: There was not a significant difference in mean Sa02 during feeding between the two feeding methods (p=0.26). The mean Sa02 during feeding of infants fed with the usual care method (group 0) was 92.4371 – 0.6393 = 91.8% and for the CoReg method was 92.4%.

E. Apneic Episodes: The number of feeding-related episodes of apnea (defined as absence of breath for more than 4 seconds (sec)) (Hanlon et al., 1997) [PAUSEMORE4]. A new variable was created called FDGAPNEA, which was calculated as PAUSEMORE4 divided by the total length of feeding (FDGTOT) in secs. The results for FDGAPNEA are in number of events per second.

SAS Code	Model Problems	Results	AIC BIC
The purpose of this analysis was to evaluate whether there was a difference between groups for the outcome variable FDGAPNEA, taking into account correlation within infant (ID was included in the class statement as well as group). Since there was only 1 measurement per feeding, there was no need to account for correlation within feeding. If the prior feeding was particularly stressful, there is the possibility for a carry-over effect on apnea, so order effect variables were included (studyday and dayfdgorder). First, the fixed effects model was determined, with all variables in the random effects model.			
*Model E1; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGAPNEA =group studyday dayfdgorder / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0464 STUDYDAY 0.1566 DAYFDGORDER 0.0473	-223.4 -219.6
*Model E2; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGAPNEA =group studyday / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0243 STUDYDAY 0.3339	-218.3 -214.4

SAS Code	Model Problems	Results	AIC BIC
*Model E3; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGAPNEA =group dayfdgorder / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0363 DAYFDGORDER 0.0684	-222.9 -219.8
*Model E4; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGAPNEA =group / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0184	-219.2 -216.0
Model E1 had the best AIC/BIC scores. Starting with model E1, add the interaction terms between group and the other variables, one at a time.			
*Model E5; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGAPNEA =group studyday dayfdgorder group*studyday / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.1683 STUDYDAY 0.2085 DAYFDGORDER 0.0570 STUDYDAY*GROUP 0.8845	-221.5 -217.0
*Model E6; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGAPNEA =group studyday dayfdgorder group*dayfdgorder / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0952 STUDYDAY 0.2699 DAYFDGORDER 0.1780 DAYFDGORDER*GROUP 0.6466	-221.8 -217.3
Adding the interaction terms did not improve the model. Using the fixed effects model in E1, now determine the random effects model.			
*Model E7; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGAPNEA =group studyday dayfdgorder / solution; Random intercept group studyday / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0430 STUDYDAY 0.1566 DAYFDGORDER 0.0473	-223.4 -219.6
*Model E8; **Proc mixed** data=hrv covtest method=ML; Class id group;	None	GROUP 0.0430	-223.4 -219.6

SAS Code	Model Problems	Results	AIC BIC
Model FDGAPNEA =group studyday dayfdgorder / solution; Random intercept group dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;		STUDYDAY 0.3442 DAYFDGORDER 0.0140	
*Model E9; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGAPNEA =group studyday dayfdgorder / solution; Random intercept studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.2676 STUDYDAY 0.4018 DAYFDGORDER 0.2861	-215.1 -211.2
*Model E10; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGAPNEA =group studyday dayfdgorder / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0430 STUDYDAY 0.1343 DAYFDGORDER 0.0116	-223.4 -219.6
*Model E11; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGAPNEA =group studyday dayfdgorder / solution; Random intercept studyday / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0521 STUDYDAY 0.4018 DAYFDGORDER 0.2826	-215.1 -211.1
*Model E12; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGAPNEA =group studyday dayfdgorder / solution; Random intercept dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0604 STUDYDAY 0.4054 DAYFDGORDER 0.2797	-215.1 -211.2
*Model E13; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGAPNEA =group studyday dayfdgorder / solution; Random intercept / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0390 STUDYDAY 0.3883 DAYFDGORDER 0.2689	-215.1 -211.2
Model E10 was the most parsimonious random effects model with the best AIC/BIC scores. This model was used to run a residual analysis to evaluate the data for outliers.			
*Model E10 with residual analysis; **Proc mixed** data=hrv covtest method=ML;	None	No outliers outside of +/- 3.	

SAS Code	Model Problems	Results	AIC BIC
Class id group; Model FDGAPNEA =group studyday dayfdgorder / solution outpm=residls vciry; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**; **data** residls; set residls; label pred="Predicted Value" scaledresid="Scaled Residual"; format pred scaledresid **7.2**; **run**;			
			
To estimate the values for FDGAPNEA, the variables other than group were removed from the fixed effects model.			
*Model E11; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGAPNEA =group / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0446	-217.5 -214.3
Solution for Fixed Effects			

SAS Code	Model Problems	Results	AIC BIC

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		0.002806	0.002925	9	0.96	0.3624
GROUP	0	0.008569	0.003674	9	2.33	0.0446
GROUP	1	0	.	.	.	.

Interpretation: Infants fed with the CoReg method had fewer feeding-related apneic events than infants fed with the usual care method (*p*=0.043). Infants fed with usual care had 0.002806+0.008569 = 0.011375 apneas/second and infants fed with CoReg had 0.002806 apneas/sec. Converted to minutes, infants fed with usual care had 0.68 apneas/minute and infants fed with CoReg had 0.17 apneas/minute.

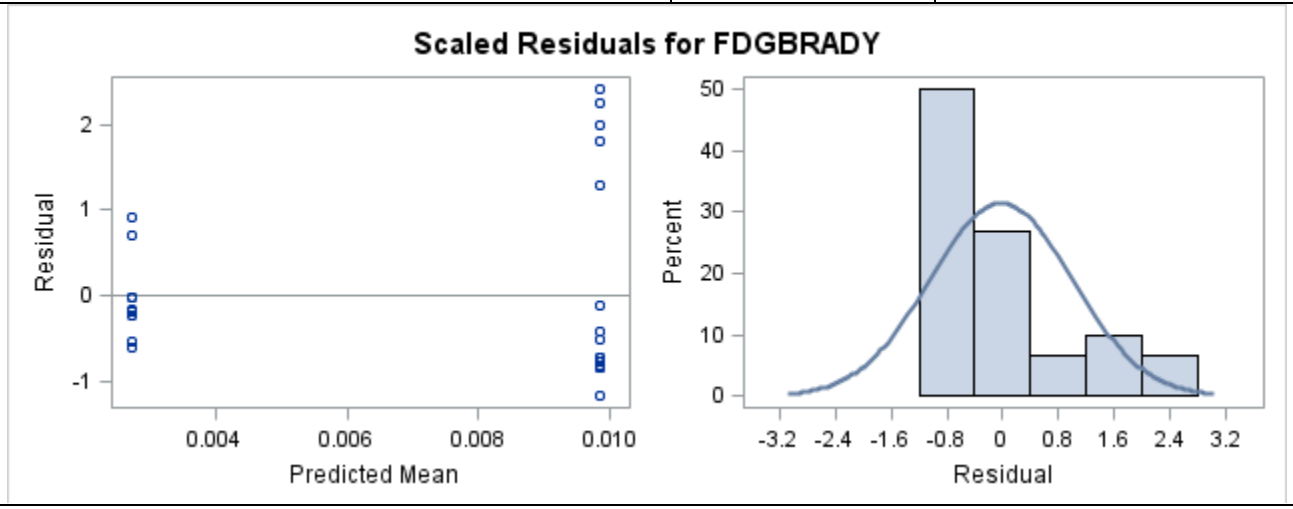
F. Bradycardia: Number of seconds of bradycardia (defined as heart rate less than 100 bpm) during feeding [SECBRADY]. A new variable was created called FDGBRADY, which was calculated as the SECBRADY divided by the total length of feeding (FDGTOT). The results of FDGBRADY are in number of seconds of bradycardia per second of feeding.

Model	Model Problems	Results	AIC BIC
The purpose of this analysis was to evaluate whether there was a difference between groups for the outcome variable FDGBRADY, taking into account correlation within infant (ID was included in the class statement as well as group). Since there was only 1 measurement per feeding, there was no need to account for correlation within feeding. If the prior feeding was particularly stressful, there is the possibility for a carry-over effect on bradycardia, so order effect variables were included (studyday and dayfdgorder). First, the fixed effects model was determined, with all variables in the random effects model.			
*Model F1; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGBRADY =group studyday dayfdgorder / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.1241 STUDYDAY 0.8800 DAYFDGORDER 0.6156	-168.9 -164.4
*Model F2; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGBRADY =group studyday / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.1248 STUDYDAY 0.9162	-170.6 -166.7

Model	Model Problems	Results	AIC BIC
*Model F3; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGBRADY =group dayfdgorder / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.1189 DAYFDGORDER 0.6238	-170.8 -167.0
*Model F4; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGBRADY =group / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.1238	-172.6 -169.4
Model F4 had the best AIC/BIC scores. Now add the interaction terms between group and the other variables.			
*Model F5; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGBRADY =group studyday dayfdgorder group*studyday / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	0.3322 STUDYDAY 0.8206 DAYFDGORDER 0.7640 STUDYDAY*GROUP .	-167.0 -161.9
*Model F6; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGBRADY =group studyday dayfdgorder group*dayfdgorder / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.2818 STUDYDAY 0.7497 DAYFDGORDER 0.7858 DAYFDGORDER*GROUP .	-165.1 -159.3
The interaction terms did not improve the model. Using the fixed effects model in F4, determine the random effects model.			
*Model F7; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGBRADY =group / solution; Random intercept group studyday / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.1282	-172.5 -169.3
*Model F8; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGBRADY =group / solution;	None	GROUP 0.1238	-172.6 -169.4

Model	Model Problems	Results	AIC BIC
Random intercept group dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;			
*Model F9; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGBRADY =group / solution; Random intercept studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP .	-174.4 -171.9
*Model F10; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGBRADY =group / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.1282	-172.5 -169.3
*Model F11; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGBRADY =group / solution; Random intercept studyday / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.1316	-174.4 -171.9
*Model F12; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGBRADY =group / solution; Random intercept dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.1316	-174.4 -171.9
*Model F13; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGBRADY =group / solution; Random intercept / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.1071	-174.4 -171.9
Model F13 was the random effects model that was the most parsimonious and had the lowest AIC/BIC scores. Next, a residual analysis was run to evaluate the data for outliers.			
*Model F13 with residual analysis; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGBRADY =group / solution outpm=residls vciry;		There were no outliers outside of +/- 3.	

Model	Model Problems	Results	AIC BIC
Random intercept / subject=id v=**4** vcorr=**4**; **Run**; **data** residls; set residls; label pred="Predicted Value" scaledresid="Scaled Residual"; format pred scaledresid **7.2**; **run**;			



Since no other variables were included in the fixed effects model, model F13 can be used for the estimates of the variable FDGBRADY.

Solution for Fixed Effects

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		0.002717	0.003582	12	0.76	0.4628
GROUP	0	0.007120	0.004171	16	1.71	0.1071
GROUP	1	0	.	.	.	.

Interpretation: There was not a significant difference in the number of seconds of bradycardia between the two feeding methods (p=0.11). Infants fed with usual care had 0.002717+0.007120 = 0.009837 seconds of bradycardia per second of feeding time. Infants fed with the CoReg method had 0.007120 seconds of bradycardia per second of feeding time. Converted to seconds of bradycardia per minute of feeding time, infants fed with usual care had 0.59 seconds of bradycardia per minute of feeding and infants fed with CoReg had 0.43 seconds of bradycardia per minute of feeding.

G. **Mean Heart Rate During Feeding (FMeanHR)**:

SAS Code	Model Problems	Results	AIC BIC
The purpose of this analysis was to evaluate whether there was a difference between groups for the outcome variable FMeanHR, taking into account correlation within infant (ID was included in the class statement as well as group). This measure was calculated for every 120-second bottle-in period that qualified for analysis of HRV. Some infants had multiple measurements within a feeding. To account for correlation within feeding, the variable FDGEVENT was added to the model. If the prior feeding was particularly stressful, there is the possibility for a carry-over effect on HR, so order effect variables were included (studyday and dayfdgorder). First, the fixed effects model was determined, with all variables in the random effects model.			
*Model G1; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution; Random intercept group studyday dayfdgorder FDGEVENT / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.3606 STUDYDAY . DAYFDGORDER . FDGEVENT .	455.2 459.7
*Model G2; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder / solution; Random intercept group studyday dayfdgorder FDGEVENT / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.3606 STUDYDAY 0.2752 DAYFDGORDER 0.2550	461.2 467.6
*Model G3; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday FDGEVENT / solution; Random intercept group studyday dayfdgorder FDGEVENT / subject=id v=**4**	None	GROUP 0.3606 STUDYDAY 0.5368 FDGEVENT .	461.2 467.6

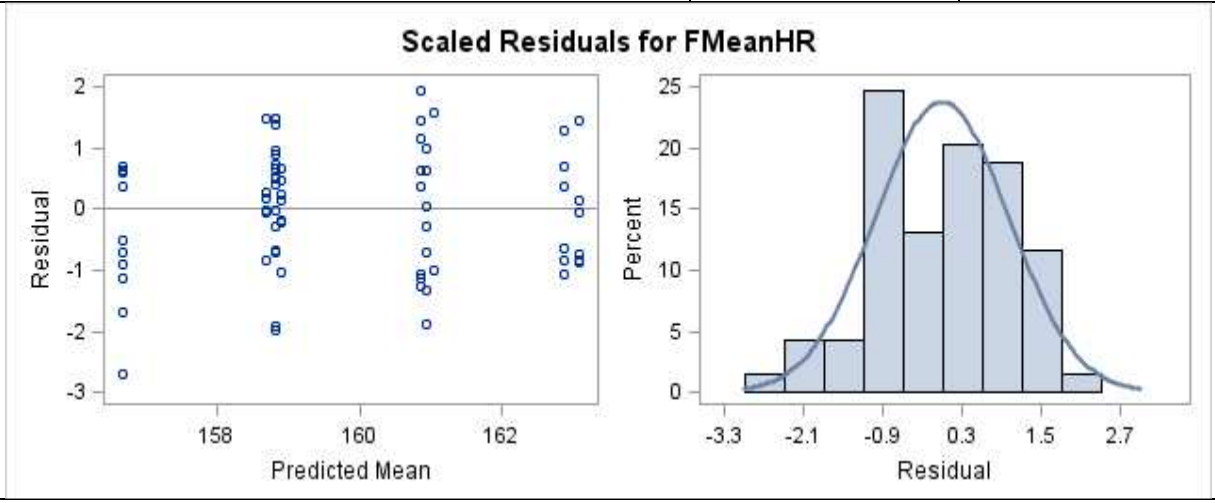
SAS Code	Model Problems	Results	AIC BIC
vcorr=**4**; **Run**;			
*Model G4; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group dayfdgorder FDGEVENT / solution; Random intercept group studyday dayfdgorder FDGEVENT / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.3606 DAYFDGORDER 0.5549 FDGEVENT .	461.2 467.6
*Model G5; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group FDGEVENT / solution; Random intercept group studyday dayfdgorder FDGEVENT / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.3147 FDGEVENT .	459.5 465.3
*Model G6; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday / solution; Random intercept group studyday dayfdgorder FDGEVENT / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.2842 STUDYDAY 0.2023	460.4 466.2
*Model G7; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group dayfdgorder / solution; Random intercept group studyday dayfdgorder FDGEVENT / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.3949 DAYFDGORDER 0.2218	458.3 463.4
*Model G8; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group / solution; Random intercept group studyday dayfdgorder FDGEVENT / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.3531	459.7 464.8
Model G1 had the lowest AIC/BIC scores. Using this fixed effects model, the random effects model was then determined.			

SAS Code	Model Problems	Results	AIC BIC
*Model G9; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.3783 STUDYDAY . DAYFDGORDER . FDGEVENT .	453.5 457.3
*Model G10; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution; Random intercept group studyday FDGEVENT / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.3861 STUDYDAY . DAYFDGORDER . FDGEVENT .	451.5 454.7
*Model G11; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution; Random intercept group dayfdgorder FDGEVENT / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.3510 STUDYDAY . DAYFDGORDER . FDGEVENT .	453.3 457.1
*Model G12; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution; Random intercept studyday dayfdgorder FDGEVENT / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0760 STUDYDAY . DAYFDGORDER . FDGEVENT .	454.5 457.7
*Model G13; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution; Random intercept group studyday / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.4778 STUDYDAY . DAYFDGORDER . FDGEVENT .	454.3 457.5
*Model G14; **Proc mixed** data=hrv covtest method=ML; Class id group;	None	GROUP 0.1884 STUDYDAY .	456.0 459.2

SAS Code	Model Problems	Results	AIC BIC
Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution; Random intercept group dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;		DAYFDGORDER . FDGEVENT .	
*Model G15; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution; Random intercept group FDGEVENT / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.3833 STUDYDAY . DAYFDGORDER . FDGEVENT .	451.5 454.7
*Model G16; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution; Random intercept studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.1200 STUDYDAY . DAYFDGORDER . FDGEVENT .	455.9 459.1
*Model G17; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution; Random intercept studyday FDGEVENT / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0819 STUDYDAY . DAYFDGORDER . FDGEVENT .	455.8 458.3
*Model G18; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution; Random intercept dayfdgorder FDGEVENT / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0759 STUDYDAY . DAYFDGORDER . FDGEVENT .	454.5 457.7
*Model G19; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.3693 STUDYDAY . DAYFDGORDER . FDGEVENT .	457.3 459.9

SAS Code	Model Problems	Results	AIC BIC
*Model G20; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution; Random intercept studyday / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.1727 STUDYDAY . DAYFDGORDER . FDGEVENT .	460.0 462.5
*Model G21; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution; Random intercept dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0469 STUDYDAY . DAYFDGORDER . FDGEVENT .	457.8 460.3
*Model G22; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution; Random intercept FDGEVENT / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0811 STUDYDAY . DAYFDGORDER . FDGEVENT .	455.8 458.3
*Model G23; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution; Random intercept / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.1524 STUDYDAY . DAYFDGORDER . FDGEVENT .	461.5 463.4
Model G15 was the most parsimonious model with the best AIC/BIC scores. This model was used to run a residual analysis to evaluate the data for outliers.			
*Model G15 with Residual analysis; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group studyday dayfdgorder FDGEVENT / solution outpm=residls vciry; Random intercept group FDGEVENT / subject=id v=**4** vcorr=**4**; **Run**; **data** residls;		No outliers outside of +/- 3.	

SAS Code	Model Problems	Results	AIC BIC
set residls; label pred="Predicted Value" scaledresid="Scaled Residual"; format pred scaledresid **7.2**; **run**;			



To estimate the values for Mean HR during feeding for the two groups, the model was run without the other variables in the fixed effects model.

*Model G16; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FMeanHR=group / solution; Random intercept group FDGEVENT / subject=id v=**4** vcorr=**4**; **Run**;			

Solution for Fixed Effects

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		157.12	3.0890	9	50.86	<.0001
GROUP	0	2.2790	2.5863	9	0.88	0.4011
GROUP	1	0	.	.	.	.

Interpretation: There was no significant difference in HR during feeding between the two groups (p=0.38). Heart rate during feeding was for infants fed with usual care = 2.279 + 157.12 = 159.4 beats per minute and for infants fed with CoReg 157.12 beats per minute.

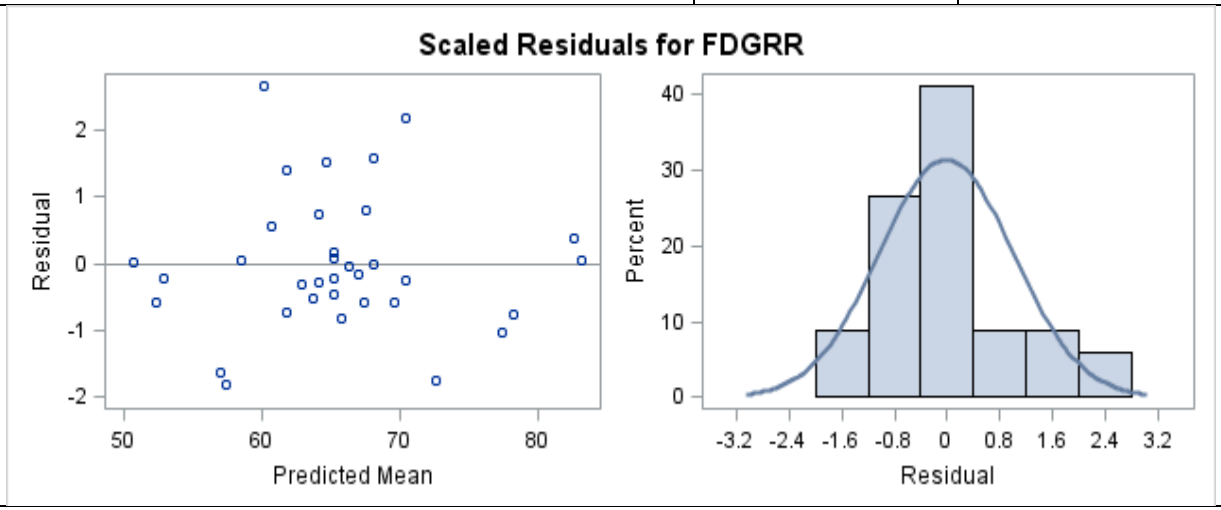
H. Respiratory Rate During Feeding (FDGRR): This variable measured the mean respiratory rate during feeding in breaths per minute.

SAS Code	Model Problems	Results	AIC BIC
The purpose of this analysis was to evaluate whether there was a difference between groups for the outcome variable FDGRR, taking into account correlation within infant (ID was included in the class statement as well as group). There was only one measurement per feeding, so there was no need to account for correlation within feeding. If the prior feeding was particularly stressful, there is the possibility for a carry-over effect on RR, so order effect variables were included (studyday and dayfdgorder). Baseline RR was used as a covariate in the analysis. First, the fixed effects model was determined, with all variables in the random effects model.			
*Model H1; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group studyday dayfdgorder BASERR / solution; Random intercept group studyday dayfdgorder BASERR / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0656 **STUDYDAY** 0.8565 **DAYFDGORDER** 0.1990 **BASERR** 0.1536	261.0 266.1
*Model H2; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group studyday dayfdgorder / solution; Random intercept group studyday dayfdgorder BASERR / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0860 **STUDYDAY** 0.8609 **DAYFDGORDER** 0.7872	271.0 275.5
*Model H3; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group studyday BASERR / solution; Random intercept group studyday dayfdgorder BASERR / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0571 **STUDYDAY** 0.9180 **BASERR** 0.1702	261.3 265.8
*Model H4; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group dayfdgorder BASERR / solution; Random intercept group studyday dayfdgorder BASERR / subject=id v=**4** vcorr=**4**;	None	**GROUP** 0.0631 **DAYFDGORDER** 0.2005 **BASERR** 0.1529	259.0 263.5

SAS Code	Model Problems	Results	AIC BIC
Run;			
*Model H5; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group studyday / solution; Random intercept group studyday dayfdgorder BASERR / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0792 **STUDYDAY** 0.8727	269.1 273
*Model H6; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group dayfdgorder / solution; Random intercept group studyday dayfdgorder BASERR / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0841 **DAYFDGORDER** 0.7943	269.1 272.9
*Model H7; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group BASERR / solution; Random intercept group studyday dayfdgorder BASERR / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0569 **BASERR** 0.1698	259.3 263.1
*Model H8; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group / solution; Random intercept group studyday dayfdgorder BASERR / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0809	267.1 270.3
Model H7 was the most parsimonious model with the best AIC/BIC scores. Use this model and determine the random effects model.			
*Model H9; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group BASERR / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0569 **BASERR** .	259.3 263.1
*Model H10; **Proc mixed** data=hrv covtest method=ML;	None	**GROUP** 0.0569	259.3 263.1

SAS Code	Model Problems	Results	AIC BIC
Class id group; Model FDGRR = group BASERR / solution; Random intercept group studyday BASERR / subject=id v=**4** vcorr=**4**; **Run**;		**BASERR** 0.0353	
*Model H11; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group BASERR / solution; Random intercept studyday dayfdgorder BASERR / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** . **BASERR** 0.0644	260.4 264.2
*Model H12; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group BASERR / solution; Random intercept group studyday / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0569 **BASERR** 0.0353	259.3 263.1
*Model H13; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group BASERR / solution; Random intercept group dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0595 **BASERR** 0.1712	259.4 263.2
*Model H14; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group BASERR / solution; Random intercept group BASERR / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0595 **BASERR** 0.0084	259.4 263.2
*Model H15; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group BASERR / solution; Random intercept studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** . **BASERR** .	260.4 264.2
*Model H16; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group BASERR / solution;	None	**GROUP** 0.2596 **BASERR** 0.0056	260.4 264.2

SAS Code	Model Problems	Results	AIC BIC
Random intercept studyday BASERR / subject=id v=**4** vcorr=**4**; **Run**;			
*Model H17; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group BASERR / solution; Random intercept dayfdgorder BASERR / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.2603 **BASERR** 0.0099	258.4 261.6
*Model H18; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group BASERR / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0594 **BASERR** 0.0055	259.4 263.2
*Model H19; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group BASERR / solution; Random intercept studyday / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0459 **BASERR** 0.0046	260.4 264.2
*Model H20; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group BASERR / solution; Random intercept dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0543 **BASERR** 0.0075	258.4 261.6
*Model H21; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group BASERR / solution; Random intercept BASERR / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0543 **BASERR** 0.0034	258.4 261.6
*Model H22; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group BASERR / solution; Random intercept / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0331 **BASERR** 0.0016	258.4 261.6

SAS Code	Model Problems	Results	AIC BIC
Model H22 is the most parsimonious model with the best AIC/BIC scores. Next, a residual analysis was run to evaluate the data for outliers.			
*Model H22 with residual analysis; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group BASERR / solution outpm=residls vciry; Random intercept / subject=id v=**4** vcorr=**4**; **Run**; **data** residls; set residls; label pred="Predicted Value" scaledresid="Scaled Residual"; format pred scaledresid **7.2**; **run**;		No outliers outside of +/- 3.	
			
To estimate the values for RR during feeding for each group, the model was run without the other variables in the fixed effects model.			
*Model H23; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FDGRR = group / solution; Random intercept / subject=id v=**4** vcorr=**4**; **Run**;			

SAS Code	Model Problems	Results	AIC BIC

Solution for Fixed Effects

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		70.0025	3.3462	13	20.92	<.0001
GROUP	0	-7.9722	3.5400	19	-2.25	0.0363
GROUP	1	0	.	.	.	.

Interpretation: Infants fed with the CoReg method had a significantly higher mean RR than infants fed with the usual care method (*p*=0.03). Mean RR for infants fed with the usual care method was 70.0025 – 7.9722 = 62 breaths per minute. Infants fed with the CoReg method had a mean RR of 70 breaths per minute.

Question 3: Is there a difference between the feeding methods for heart rate variability outcome measures?

An analysis was run to determine if there was a difference in heart rate variability outcomes between the intervention and usual-care feeding methods [GROUP]. The specific measures evaluated included: HF (high frequency) Power, LF (low frequency) Power, LF/HF Ratio, and SD12. These variables had only one measurement per feeding, so there was no need to account for correlation within feedings, but correlation between feedings of the same infant was evaluated. Order of study feedings was also considered. When baseline values for a variable were available and appropriate, these were considered in the covariance structure.

I. High Frequency Power in milliseconds squared (FHFms):

Model	Model Problems	Results	AIC BIC
The purpose of this analysis was to evaluate whether there was a difference between groups for the outcome variable FHFms, taking into account correlation within infant (ID was included in the class statement as well as group). This outcome variable had multiple measurements per feeding. Correlation between measures within the same feeding were accounted for using the term FDGEVENT. If the prior feeding was particularly stressful, there is the possibility for a carry-over effect on HF, so order effect variables were included (studyday and dayfdgorder). First, the fixed effects model was determined, with all variables in the random effects model.			

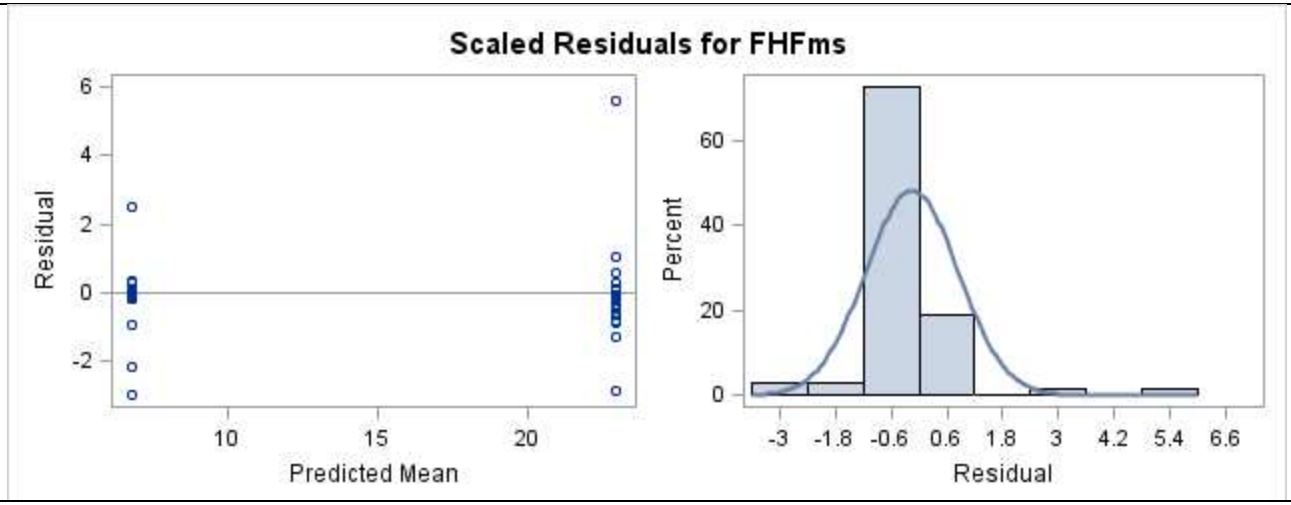
*Model I1; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group studyday dayfdgorder fdgevent / solution; Random intercept group studyday dayfdgorder fdgevent / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite. Model overparametized; not able to estimate.	GROUP 0.2368 STUDYDAY . DAYFDGORDER . FDGEVENT .	641.2 643.7
*Model I2; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group studyday dayfdgorder / solution; Random intercept group studyday dayfdgorder fdgevent / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.2368 STUDYDAY 0.8380 DAYFDGORDER 0.8393	647.2 651.7
*Model I3; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group studyday fdgevent / solution; Random intercept group studyday dayfdgorder fdgevent / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.2368 STUDYDAY 0.7739 FDGEVENT .	647.2 651.7
*Model I4; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group dayfdgorder fdgevent / solution; Random intercept group studyday dayfdgorder fdgevent / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.2368 DAYFDGORDER 0.7815 FDGEVENT .	647.2 651.7
*Model I5; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group studyday / solution; Random intercept group studyday dayfdgorder fdgevent / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.2384 STUDYDAY 0.8266	645.2 649.1

*Model I6; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group dayfdgorder / solution; Random intercept group studyday dayfdgorder fdgevent / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.2385 DAYFDGORDER 0.8286	645.2 649.1
*Model I7; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group fdgevent / solution; Random intercept group studyday dayfdgorder fdgevent / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.2438 FDGEVENT .	645.3 649.1
*Model I8; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random intercept group studyday dayfdgorder fdgevent / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.2450	643.3 646.5
Model I1 had the best AIC/BIC scores, but was overparametized. All of the models had an "estimated G matrix not positive definite." Of the models that were not overparametized, model I8 had the best AIC/BIC scores. Use this fixed effects model and determine the random effects model.			
*Model I9; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random intercept group studyday dayfdgorder / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.2450	643.3 646.5
*Model I10; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random intercept group studyday fdgevent / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.2448	643.3 646.5

*Model I11; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random intercept group dayfdgorder fdgevent / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.2450	643.3 646.5
*Model I12; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random intercept studyday dayfdgorder fdgevent / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.0397	666.0 668.6
*Model I13; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random intercept group studyday / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.2450	643.3 646.5
*Model I14; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random intercept group dayfdgorder / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.2450	643.3 646.5
*Model I15; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random intercept group fdgevent / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.2450	643.3 646.5
*Model I16; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random intercept studyday dayfdgorder / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.0397	666.0 668.6

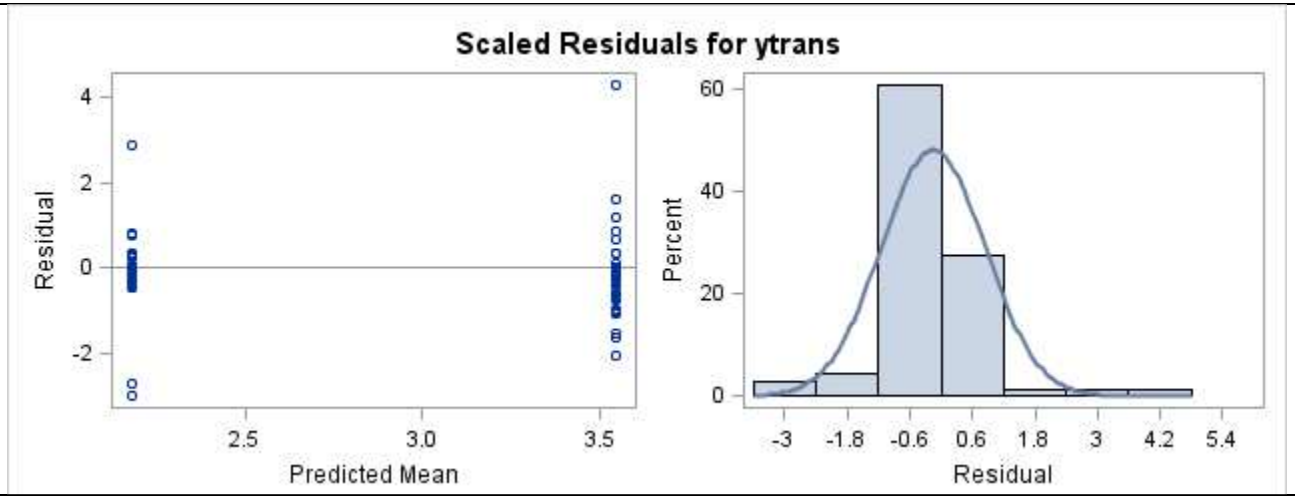
*Model I17; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random intercept studyday fdgevent / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.0397	666.0 668.6
*Model I18; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random intercept dayfdgorder fdgevent / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.0397	666.0 668.6
*Model I19; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random intercept group / subject=id v=8 vcorr=8; **Run**;	None	GROUP 0.2449	643.3 646.5
*Model I20; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random intercept studyday / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.0383	666.0 668.6
*Model I21; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random intercept dayfdgorder / subject=id v=8 vcorr=8; Run;	Estimated G matrix is not positive definite.	GROUP 0.0386	666.0 668.6
*Model I22; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random intercept fdgevent / subject=id v=8 vcorr=8; **Run**;	Estimated G matrix is not positive definite.	GROUP 0.0388	666.0 668.6

*Model I23; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random intercept / subject=id v=8 vcorr=8; **Run**;	None.	GROUP 0.0374	666.0 668.6
*Model I24; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution; Random group / subject=id v=**8** vcorr=**8**; **Run**;	None.	GROUP 0.2337	641.3 643.9
Model I24 had the best AIC/BIC scores. This model was used to run a residual analysis to evaluate the data for outliers.			
*Model I24 with residual analysis; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FHFms=group / solution outpm=residls vciry; Random group / subject=id v=**8** vcorr=**8**; **Run**; **data** residls; set residls; label pred="Predicted Value" scaledresid="Scaled Residual"; format pred scaledresid **7.2**; **run**;		Outliers present outside of +/- 3.	



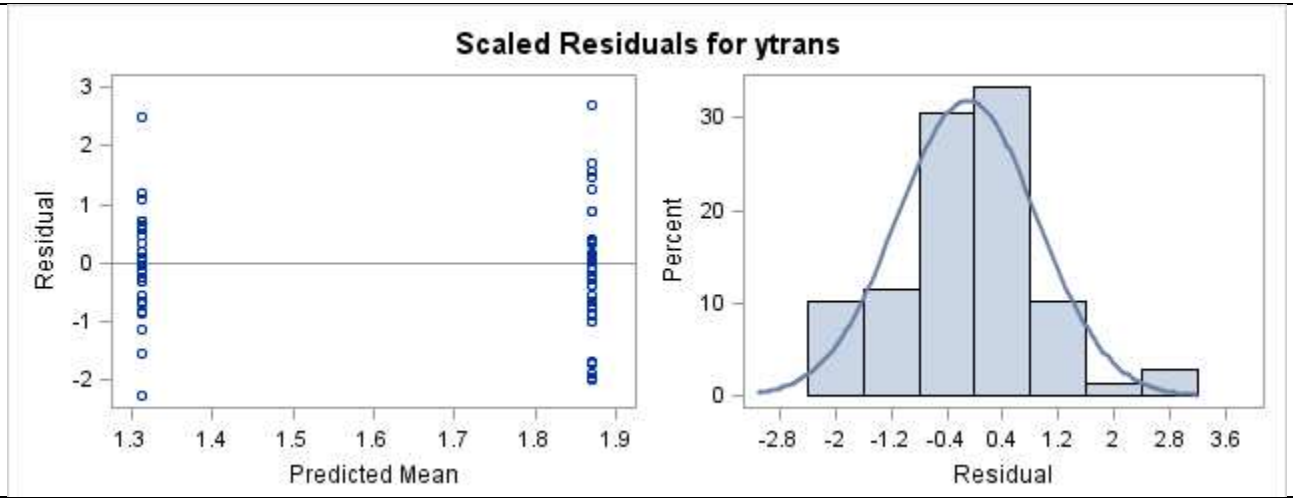
Since there were outliers outside of +/- 3, the data was transformed, first using the square root.

*Square Root Transformation; %let ypower=0.5; **data** adjusted; set hrv; ytrans=fhfms**&ypower; label ytrans="FHFms to Power &ypower"; **run**; *Model I24 Square Root Transformed; **Proc mixed** data=adjusted covtest method=ML; Class id group; Model ytrans=group / solution outpm=residls vciry; Random group / subject=id v=**8** vcorr=**8**; **Run**;	None	GROUP 0.1864 There were still outliers outside of +/- 3.	



Since there were still outliers, a log transformation was used.

*Model I24 with Log transformation; **data** adjusted; set hrv; ytrans=log(fhfms); label ytrans="FHFms to Log"; **run**; **Proc mixed** data=adjusted covtest method=ML; Class id group; Model ytrans=group / solution outpm=residls vciry; Random group / subject=id v=**8** vcorr=**8**; **Run**;	None	GROUP 0.2662 No outliers outside of +/- 3.	



Model I24 was used to estimate group values for HF Power.

Solution for Fixed Effects

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		6.8023	10.0136	22	0.68	0.5040
GROUP	0	16.1180	13.1613	22	1.22	0.2337
GROUP	1	0	.	.	.	.

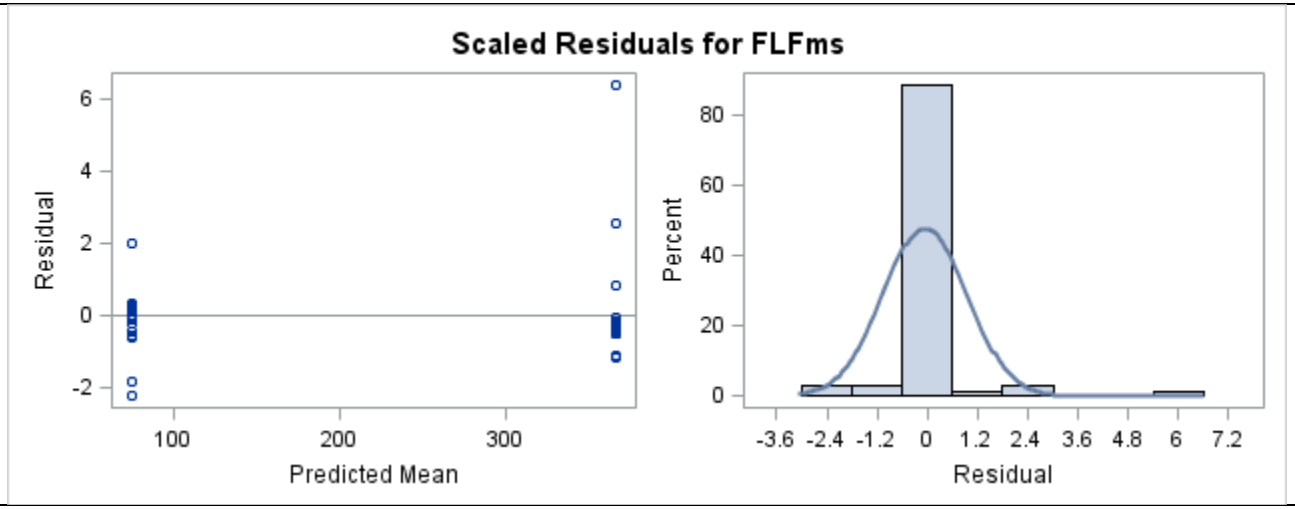
Interpretation: There was not a significant difference between the two feeding methods for HF Power (p=0.26). The estimates for HF Power (ms^2) were for the usual care group 16.1180+6.8023=22.92 and for infants fed with the CoReg method 6.80.

J. Low Frequency Power in milliseconds squared [FLFms]:

Model	Model Problems	Results	AIC BIC
The purpose of this analysis was to evaluate whether there was a difference between groups for the outcome variable FLFms, taking into account correlation within infant (ID was included in the class statement as well as group). This outcome variable had multiple measurements per feeding. Correlation between measures within the same feeding were accounted for using the term FDGEVENT. If the prior feeding was particularly stressful, there is the possibility for a carry-over effect on HF, so order effect variables were included (studyday and dayfdgorder). The random effects model identified from the analysis of FHFms was used. The fixed effects model was determined.			

*Model J1; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFms=group studyday dayfdgorder fdgevent / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	Estimated G matrix not positive definite. Model overparameterized.	**GROUP** 0.3192 **STUDYDAY** . **DAYFDGORDER** . **FDGEVENT** .	1079.8 1081.7
*Model J2; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFms=group studyday dayfdgorder / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	Estimated G matrix not positive definite.	**GROUP** 0.3192 **STUDYDAY** 0.3606 **DAYFDGORDER** 0.7012	1085.8 1089.6
*Model J3; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFms=group studyday fdgevent / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	Estimated G matrix not positive definite.	**GROUP** 0.3192 **STUDYDAY** 0.9303 **FDGEVENT** 0.7012	1085.8 1089.6
*Model J4; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFms=group dayfdgorder fdgevent / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	Estimated G matrix not positive definite.	**GROUP** 0.3192 **DAYFDGORDER** 0.9303 **FDGEVENT** 0.3606	1085.8 1089.6
*Model J5; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFms=group studyday / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	Estimated G matrix not positive definite.	**GROUP** 0.3045 **STUDYDAY** 0.3797	1083.9 1087.1
*Model J6; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFms=group dayfdgorder / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	Estimated G matrix not positive definite.	**GROUP** 0.3510 **DAYFDGORDER** 0.7673	1084.6 1087.8
*Model J7; **Proc mixed** data=hrv covtest method=ML; Class id group;	Estimated G matrix not positive definite.	**GROUP** 0.3211 **FDGEVENT** 0.3308	1083.8 1087

Model FLFms=group fdgevent / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;			
*Model J8; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFms=group / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	Estimated G matrix not positive definite.	**GROUP** 0.3373	1082.6 1085.2
Model J8 had the best AIC/BIC scores of the models above, with the except of model J1, which was overparameterized. Since model J8 still got an error message stating the estimated G matrix was not positive definite, the random effects model was simplified.			
*Model J9; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFms=group / solution; Random intercept / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0792	1099.8 1102.3
Next, the data were evaluated for outliers using a residual analysis.			
*Model J9 with residual analysis; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFms=group / solution outpm=residls vciry; Random intercept / subject=id v=**4** vcorr=**4**; **Run**; **data** residls; set residls; label pred="Predicted Value" scaledresid="Scaled Residual"; format pred scaledresid **7.2**; **run**;		There are outliers outside of +/- 3.	



Since there were outliers outside of +/- 3, the data were transformed. A log transformation was used since this was what worked best for FHFms.

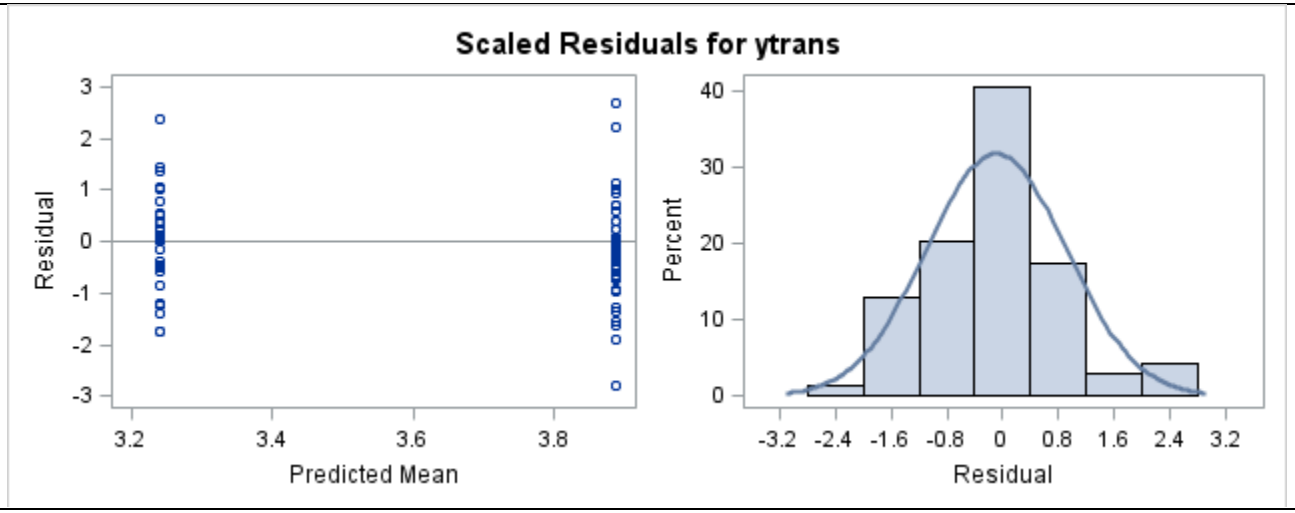
```
*Log transformation;
data adjusted;
set hrv;
ytrans=log(flfms);
label ytrans="FLFms to Log";
run;

*Once log transformed, run procmixed modeling ytrans;

Proc mixed data=adjusted covtest method=ML;
Class id group;
Model ytrans=group / solution outpm=residls vciry;
Random intercept / subject=id v=4 vcorr=4;
Run;
```

No outliers outside of +/- 3.

GROUP	0.0608



Model J9 was used to estimate values for FLFms for the two groups.

Solution for Fixed Effects

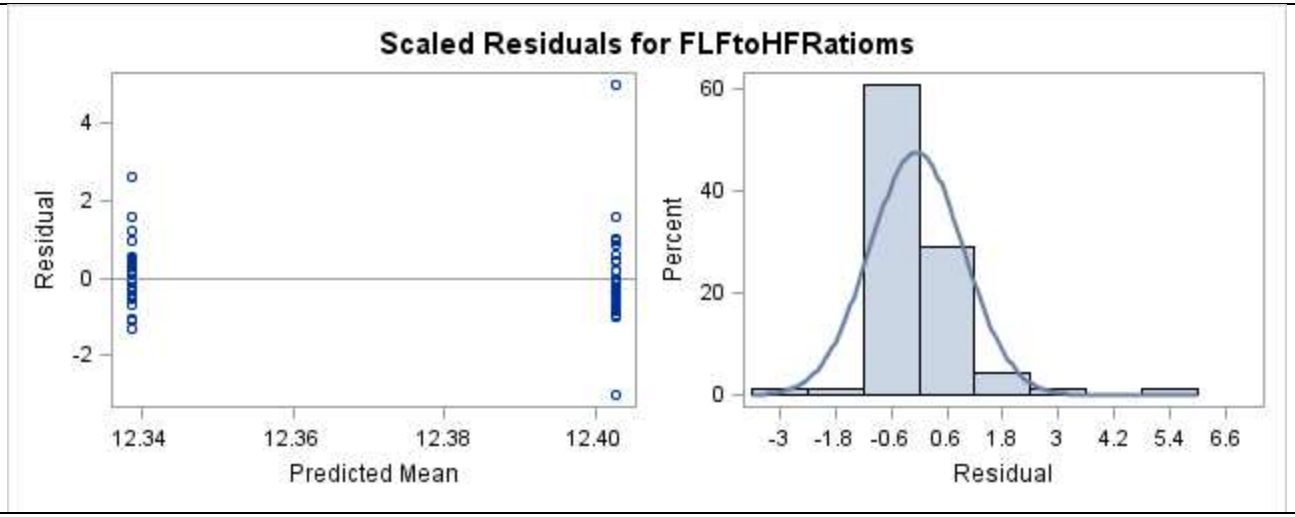
Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		75.1561	157.14	13	0.48	0.6404
GROUP	0	290.90	162.60	54	1.79	0.0792
GROUP	1	0	.	.	.	.

Interpretation: There was no significant difference between the two feeding methods for LF Power, although there was a trend toward significance (p=0.06). Infants fed with the usual care group had LF Power 75.1561 + 290.90 = 366.06 ms^2 and infants fed with the CoReg method had LF Power of 75.16 ms^2. If LF Power is an indicator of increased sympathetic input (i.e., more stress), then these results suggest that infants fed with the CoReg method had less sympathetic input (i.e., less stress).

K. Low Frequency to High Frequency Ratio [FLFtoHFRatioms]:

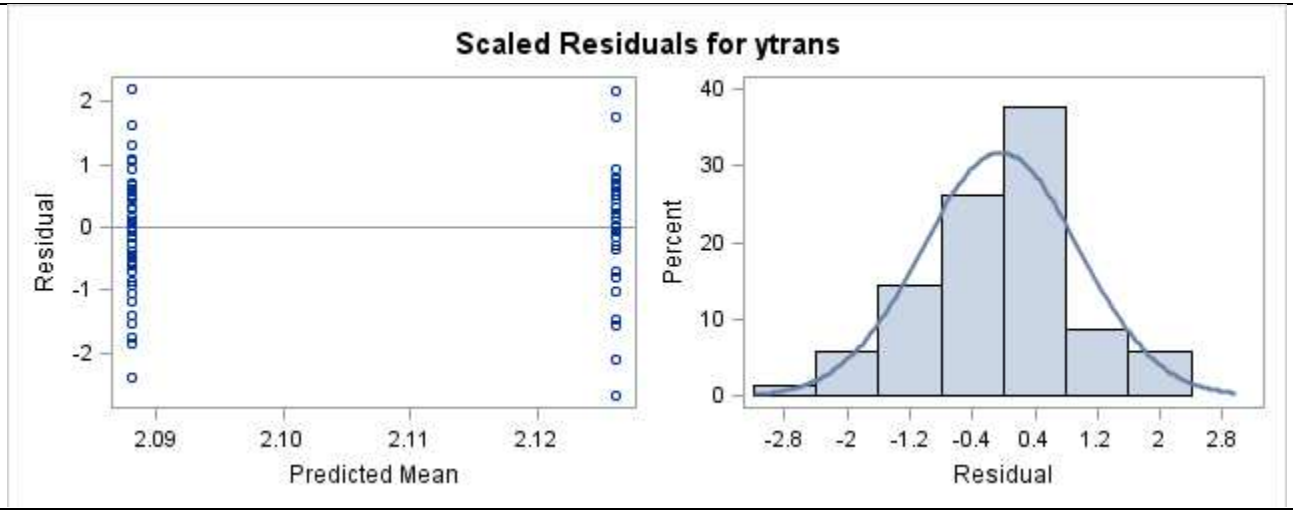
Model	Model Problems	Results	AIC BIC
The purpose of this analysis was to evaluate whether there was a difference between groups for the outcome variable FLFtoHFRatioms, taking into account correlation within infant (ID was included in the class statement as well as group). This outcome variable had multiple measurements per feeding. Correlation between measures within the same feeding were accounted for using the term FDGEVENT. If the prior feeding was particularly stressful, there is the possibility for a carry-over effect on LF/HF ratio, so order effect variables were included (studyday and dayfdgorder). The random effects model identified from the analysis of FHFms was used. The fixed effects model was determined.			
*Model K1; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFtoHFRatioms=group studyday dayfdgorder fdgevent / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	Estimated G matrix not positive definite. Model overparameterized.	**GROUP** 0.9425 **STUDYDAY** . **DAYFDGORDER** . **FDGEVENT** .	515.7 517.6
*Model K2; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFtoHFRatioms=group studyday dayfdgorder / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	Estimated G matrix not positive definite.	**GROUP** 0.9425 **STUDYDAY** 0.8683 **DAYFDGORDER** 0.3873	521.7 525.5
*Model K3; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFtoHFRatioms=group studyday fdgevent / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	Estimated G matrix not positive definite.	**GROUP** 0.9425 **STUDYDAY** 0.4089 **FDGEVENT** 0.3873	521.7 525.5
*Model K4; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFtoHFRatioms=group dayfdgorder fdgevent / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	Estimated G matrix not positive definite.	**GROUP** 0.9425 **DAYFDGORDER** 0.4089 **FDGEVENT** 0.8683	521.7 525.5
*Model K5; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFtoHFRatioms=group studyday / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	Estimated G matrix not positive definite.	**GROUP** 0.9949 **STUDYDAY** 0.9267	520.4 523.6

*Model K6; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFtoHFRatioms=group dayfdgorder / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	Estimated G matrix not positive definite.	**GROUP** 0.9695 **DAYFDGORDER** 0.3940	519.7 522.9
*Model K7; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFtoHFRatioms=group fdgevent / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	Estimated G matrix not positive definite.	**GROUP** 0.9483 **FDGEVENT** 0.7938	520.3 523.5
*Model K8; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFtoHFRatioms=group / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	Estimated G matrix not positive definite.	**GROUP** 0.9793	518.4 520.9
Model K8 had the best AIC/BIC scores of the above models that were not overparameterized. Since the G matrix was not positive definite, the random effects model was simplified.			
*Model K9; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFtoHFRatioms=group / solution; Random intercept / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.9788	518.4 520.9
Model K9 did not have any model problems and had the same AIC/BIC as model K8. This model was used to run a residual analysis.			
*Model K9 with residual analysis; **Proc mixed** data=hrv covtest method=ML; Class id group; Model FLFtoHFRatioms=group / solution outpm=residls vciry; Random intercept / subject=id v=**4** vcorr=**4**; **Run**; **data** residls; set residls; label pred="Predicted Value" scaledresid="Scaled Residual"; format pred scaledresid **7.2**; **run**;	None	**There are outliers outside of +/- 3.**	



Since there were outliers, the data were log transformed, as was done with LF and HF Power.

*Log transformation; **data** adjusted; set hrv; ytrans=log(FLFtoHFRatioms); label ytrans="FLFtoHFRatioms to Log"; **run**; **Proc mixed** data=adjusted covtest method=ML; Class id group; Model ytrans=group / solution outpm=residls vciry; Random intercept / subject=id v=**4** vcorr=**4**; **Run**;	None	**No outliers outside of +/- 3.** **GROUP** 0.8620	



Model K9 was used to estimate values for LF/HF Ratio for the groups.

Solution for Fixed Effects

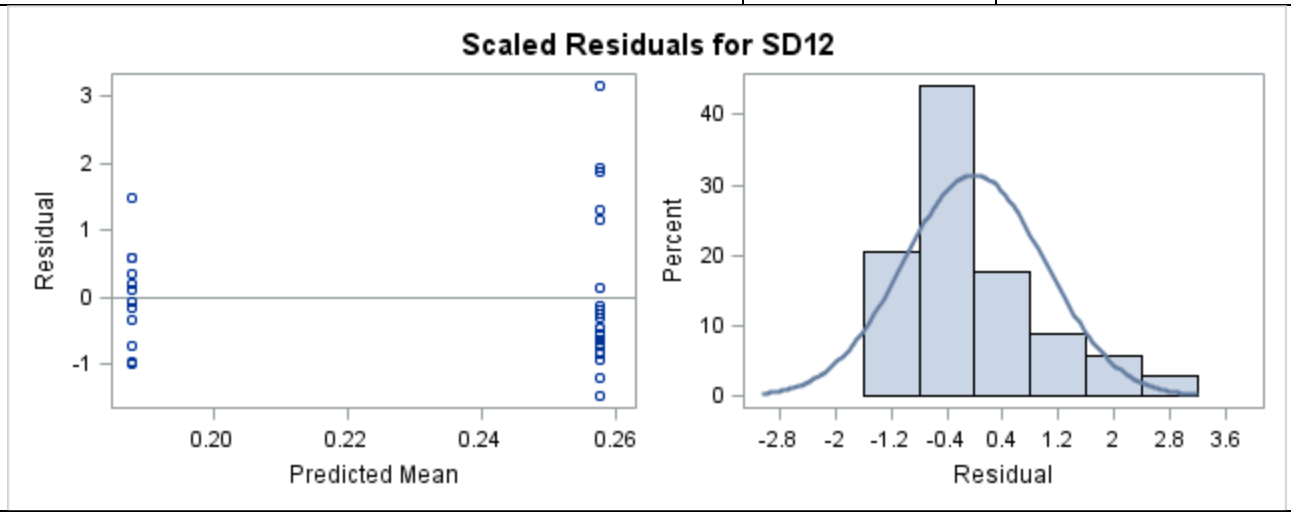
Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		12.3387	2.4233	13	5.09	0.0002
GROUP	0	0.06399	2.3936	54	0.03	0.9788
GROUP	1	0	.	.	.	.

Interpretation: There was no significant difference in LF to HF Ratio between the two feeding methods (p=0.86). The LF to HF Ratio for infants fed with the usual care method was 12.3387 + 0.06399 = 12.4 and for the CoReg method was 12.34.

L. SD12:

SAS Code	Model Problems	Results	AIC BIC
The purpose of this analysis was to evaluate whether there was a difference between groups for the outcome variable SD12, taking into account correlation within infant (ID was included in the class statement as well as group). This outcome variable had only one measurement per feeding. If the prior feeding was particularly stressful, there is the possibility for a carry-over effect on SD12, so order effect variables were included (studyday and dayfdgorder). First, the fixed effects model was determined, with all variables in the random effects model.			
*Model L1; **Proc mixed** data=hrv covtest method=ML; Class id group; Model SD12=group studyday dayfdgorder / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0417 STUDYDAY 0.9026 DAYFDGORDER 0.4528	-48.7 -43.6
*Model L2; **Proc mixed** data=hrv covtest method=ML; Class id group; Model SD12=group studyday / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0496 STUDYDAY 0.9079	-50.2 -45.7
*Model L3; **Proc mixed** data=hrv covtest method=ML; Class id group; Model SD12=group dayfdgorder / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0384 DAYFDGORDER 0.4536	-50.7 -46.2
*Model L4; **Proc mixed** data=hrv covtest method=ML; Class id group; Model SD12=group / solution; Random intercept group studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0495	-52.2 -48.3
Model L4 had the best AIC/BIC scores. Use this fixed effects model and now determine the random effects model.			
*Model L5; **Proc mixed** data=hrv covtest method=ML; Class id group; Model SD12=group / solution; Random intercept group studyday / subject=id v=**4** vcorr=**4**;	None	GROUP 0.0510	-54 -50.8

SAS Code	Model Problems	Results	AIC BIC
Run;			
*Model L6; **Proc mixed** data=hrv covtest method=ML; Class id group; Model SD12=group / solution; Random intercept group dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0495	-52.2 -48.3
*Model L7; **Proc mixed** data=hrv covtest method=ML; Class id group; Model SD12=group / solution; Random intercept studyday dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.2418	-53.8 -50.6
*Model L8; **Proc mixed** data=hrv covtest method=ML; Class id group; Model SD12=group / solution; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0510	-54 -50.8
*Model L9; **Proc mixed** data=hrv covtest method=ML; Class id group; Model SD12=group / solution; Random intercept studyday / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0332	-55.8 -53.2
*Model L10; **Proc mixed** data=hrv covtest method=ML; Class id group; Model SD12=group / solution; Random intercept dayfdgorder / subject=id v=**4** vcorr=**4**; **Run**;	None	GROUP 0.0367	-53.8 -50.6
*Model L11; **Proc mixed** data=hrv covtest method=ML; Class id group; Model SD12=group / solution; Random intercept / subject=id v=**4** vcorr=**4**; **Run**;	None	**GROUP** 0.0233	-55.8 -53.2
Model L11 is the most parsimonious model with the best AIC/BIC scores. A residual analysis was run to evaluate the data for outliers.			

SAS Code	Model Problems	Results	AIC BIC
*Model L11 with residual analysis; **Proc mixed** data=hrv covtest method=ML; Class id group; Model SD12=group / solution outpm=residls vciry; Random intercept / subject=id v=**4** vcorr=**4**; **data** residls; set residls; label pred="Predicted Value" scaledresid="Scaled Residual"; format pred scaledresid **7.2**; **run**;	None	There were outliers outside of +/- 3.	
			
The data were log transformed.			
*Log transformation; **data** adjusted; set hrv; ytrans=log(sd12); label ytrans="SD12 to Log"; **run**; **Proc mixed** data=adjusted covtest method=ML;	None	No outliers outside of +/- 3. GROUP 0.0407	

SAS Code	Model Problems	Results	AIC BIC
Class id group; Model ytrans=group / solution outpm=residls vciry; Random intercept group / subject=id v=**4** vcorr=**4**; **Run**;			

The results of model L11 were used to estimate group values.

Solution for Fixed Effects

Effect	GROUP	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		0.1878	0.03053	13	6.15	<.0001
GROUP	0	0.06988	0.02832	19	2.47	0.0233
GROUP	1	0	.	.	.	.

Interpretation: Infants fed with the usual care method had a significantly higher SD12 than infants fed with the CoReg method (p=0.04). Infants fed with the usual care method had an SD12 of 0.06988 + 0.1878 = 0.25768 and infants fed with CoReg had an SD12 of 0.1878.

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CHAPTER 5: DISCUSSION

The research presented in the three studies that compose this dissertation add significantly to the literature on feeding medically fragile infants. In chapter two, the milk flow rates and variability in milk flow from 29 types of bottle nipples used in hospitals for feeding medically fragile infants was presented. This data is highly relevant to clinical practice as it may help guide clinicians in choosing appropriate nipples for supporting safe oral feeding in medically fragile infants. The information gained in this study also guided the selection of the bottle nipples for the study of feeding an infant with hypoplastic left heart syndrome (HLHS), which was presented in chapter three.

Chapter three presented the first of its kind study of the physiologic and behavioral responses of an infant with HLHS to the challenge of oral feeding with either a standard-flow or slow-flow nipple. The results of this study suggest that despite a slow-flow nipple, this infant experienced significant distress during oral feeding. This data confirms anecdotal evidence from clinicians that infants with HLHS experience significant distress during feeding. This study supports the need for further investigation into the responses of infants with HLHS to oral feeding and the identification of oral feeding interventions for this extremely fragile population.

Finally, chapter four presented a study evaluating heart rate variability (HRV) as a new outcome measure for assessing feeding-related stress. The findings of this study were not conclusive regarding the usefulness of HRV for this purpose, but support further evaluation of this measure, particularly non-linear methods of HRV analysis. The findings were also supportive of a gentle, co-regulated approach to feeding, which involved a side-lying position, minimal stimulation, and enhanced auditory assessment by the feeder of the infant's swallowing and breathing. This feeding technique may prove useful for other populations of medically fragile infants, like infants with HLHS.

Future Directions For Research

Mastering oral feeding is usually a criteria for discharge from the hospital for medically fragile infants. The study presented in chapter two will help to guide clinicians in choosing bottle nipples that will

be supportive of these fragile infants while in the hospital, but the transition to home is often a difficult one. The nipples that are available in the hospital are typically single-use nipples that are supplied to the hospital by formula companies (e.g., Enfamil and Similac). These nipples are not readily available for purchase outside of the hospital, so parents must make decisions about which nipples to purchase for use at home. While there are abundant options, there is currently no data available on the milk flow rates of bottle nipples used for feeding infants after discharge. To build upon the data presented in chapter two, a study will be conducted to test milk flow rates of bottle nipples available in the community setting. This data will help clinicians to guide parents in selecting a bottle nipple that will be of similar flow to that with which the infant has been successful in the hospital.

Further study of the responses of infants with HLHS to oral feeding is critically needed. The results of the study presented in chapter three suggest that an even slower-flow nipple than was used in the study may be more appropriate for these fragile infants. Given their fragile physiologic state and multiple risk factors for feeding difficulty, it is likely that a combination of interventions will be most successful in supporting oral feeding. A study of a larger sample of infants with HLHS using a combination of a slower-flow nipple (e.g., Dr. Brown's Ultra-Preemie) and a gentle, co-regulated approach to feeding, such as that used in the study presented in chapter four, would be a valuable future study in this population. Additionally, a study evaluating the physiologic and behavioral responses to breast-feeding in infants with HLHS is needed. Using HRV as an outcome measure in these studies may provide additional information about the stress responses of these infants to different feeding methods.

Further evaluation of the use of HRV as an outcome measure is also needed. An analysis of the HRV outcomes of the data presented in chapter three will be conducted to contribute to the development of this potential outcome measure and to assess the nervous system response to the different flow conditions. Secondary analyses of HRV outcomes are also planned for data from a study that evaluated the effect of side-lying positioning in preterm infants (Park, Thoyre, Knafl, Hodges, & Nix, 2014) and a larger study of the co-regulated approach to feeding preterm infants (Thoyre, NIH, Grant Number R21 NR012507).

Both populations of medically fragile infants that were included in this dissertation are at risk for long-term feeding difficulty. The Feeding Flock Feeding Interest Group at the University of North Carolina

at Chapel Hill, including Suzanne Thoyre, Jinhee Park, Hayley Estrem, Eric Hodges, Cara McComish, and myself are in the process of developing a set of parent-report instruments that may aid in the early identification of children with feeding problems and the assessment of the efficacy of treatment interventions. The Pediatric Eating Assessment Tool (Pedi-EAT) is a measure of problematic feeding behaviors for children ages 6 months to 7 years (Thoyre, et al., 2014). The Pedi-EAT has been validated and the Feeding Flock has plans to test this with a large sample of children with feeding problems as well as those who are typically developing. The Pedi-EAT will be a valuable tool for the long-term follow-up of children with HLHS and those born preterm as they transition from a liquid diet to solid foods.

Additional measures that are in development include the Child Oral and Motor Proficiency Scale (ChOMPS), which is a parent-report measure of a child's skills related to eating and the Family Management Measure: Feeding (FaMM Feed), which is an adaptation of Knafl and colleagues (2011) Family Management Measure. The purpose of the FaMM Feed is to assess the family's approach to managing their child's eating problem. The ChOMPS and FaMM Feed have undergone content validity testing by clinical and research experts in the field of pediatric feeding and plans are underway to further evaluate the content validity of these measures through cognitive interviewing of experiential experts (i.e., parents). These instruments will be useful for the long-term evaluation of the skill acquisition of preterm infants and those with HLHS as they enter childhood and the ways in which families manage the challenges of feeding difficulty.

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