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Pain and Stress in the Premature Infant

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Pain and Stress in the Premature Infant

by

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the requirements for the degree
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Each person whose signature appears below certifies that this dissertation in his/her opinion is adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.

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ABBREVIATIONS

ACTH	Adrenocorticotropic Hormone
ATP	Adenosine Triphosphate
CNS	Central Nervous System
CPAP	Continuous Positive Airway Pressure
CRH	Corticotropin-Releasing Hormone
dB	Decibel
FiO_2	Fraction of Inspired Oxygen
FTOE	Fractional Tissue Oxygen Extraction
GA	Gestational Age
GI	Gastrointestinal
HFNC	High Flow Nasal Cannula
HPA	Hypothalamic-Pituitary-Adrenal
HR	Heart Rate
Hz	Hertz
IFABP	Intestinal Fatty Acid Binding Protein
IRB	Institutional Review Board
MI	Mutual Information
mRNA	Messenger Ribonucleic Acid
NAVA	Neurally Adjusted Ventilatory Assist
NCPAP	Nasal Continuous Positive Airway Pressure
NICU	Neonatal Intensive Care Unit
NIVM	Nasal Intermittent Mandatory Ventilation

NIPPV	Noninvasive Positive Pressure Ventilation
NIRS	Near Infrared Spectroscopy
ROP	Retinopathy of Prematurity
SampEn	Sample Entropy
SD	Standard Deviation
ShanEn	Shannon Entropy
SNAPPE-II	Score for Neonatal Acute Physiology with Perinatal Extension-II
SpO_2	Peripheral Oxygen Saturation
StO_2	Mesenteric Oxygen Saturation
TDPs	Tissue Damaging Procedures

ABSTRACT OF THE DISSERTATION

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by

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Procedural pain and stress in premature neonates is currently assessed utilizing tools that lack objectivity and accuracy. This results in untreated or mismanaged pain. My dissertation utilized physiological methods and biochemical markers that identified pain in premature neonates and its effect on energy metabolism. The painful procedure that I examined is the retinopathy of premature (ROP) examination, a necessary and routine eye examination in the neonatal intensive care unit (NICU) that detects and identifies infants at risk for blindness due to retinal detachment. The effects of the ROP exam on peripheral and deep tissue oxyhemoglobin saturation and urinary purine degradation markers were examined. I conclude that these noninvasive metrics can be used to inform clinical decisions regarding the pain and stress status of the premature infant.

CHAPTER ONE

INTRODUCTION

Approximately 10% of infants born in the year 2016 in United States were born premature [1]. The mortality rate of premature infants has been steadily decreasing thanks to numerous technological advances and an increase in evidence-based clinical care surrounding this unique population [2,3]. Though the overall survival rate of premature infants has been increasing, numerous co-morbidities are associated with the premature state and persist until adulthood, including neurological, visual, auditory, metabolic, and behavioral disorders [4]. As premature infants fight to survive in the neonatal intensive care unit (NICU), they are exposed to many painful procedures and stressful events that are required for their care [5–8]. The culmination of the state of prematurity and the pain and stress contribute to increased adenosine triphosphate (ATP) catabolism and an energy deficient state in the premature infant [9–11]. The energy state of the premature infant is important because energy is required for stress adaptation, or allostasis [12]. Exposure to chronic stress and the inability to deal with the "wear and tear" of the body, or allostatic load [13], can induce long-term adaptive alterations of the physiological systems within the human body [14–16]. These alterations may be associated with disrupted purinergic signaling pathways caused by a substantial increase in ATP catabolism [17], and have widespread effects in the circulatory, digestive, endocrine, immune, nervous, renal, respiratory, and skeletal systems [18]. Despite the widespread effects of purinergic signaling pathways, a considerable amount of research may have a positive impact on the the premature infant population [19].

The Purpose of These Studies

The purpose of this dissertation is to explore the relationships between energy deficiency and physiological systems in the premature neonate. We begin our research by reviewing literature on the energy deficit state of the premature infant and the challenges they face within the NICU (**Chapter 2**). Next, we longitudinally quantified and correlated urinary ATP utilization markers and intestinal injury markers in premature infants with and without oxygen support in response to the retinopathy of prematurity (ROP) exam to better understand the connection between energy deficiency, respiratory insufficiency, and the gastrointestinal system (**Chapter 3**). The ROP exam is a necessary and routine eye exam that prevents blindness in the premature infant. However, administration of the exam is associated with increased pain and stress [20]. We concurrently evaluated physiological metrics such as heart rate, systemic peripheral oxygenation, and mesenteric tissue oxygenation with clinical events such as apnea, desaturation, bradycardia, and presence of gastric residuals in the same cohort of premature infants to further elucidate the effects of respiratory insufficiency on mesenteric oxygenation levels and clinical events in response to the ROP exam (**Chapter 4**). Lastly, a case study on two premature neonates demonstrated the use of nonlinear analysis techniques to quantify the regulation and crosstalk between physiological systems and discusses its potential impact on the future of premature infant clinical care (**Chapter 5**).

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CHAPTER TWO

THE ENERGY COSTS OF PREMATURITY AND THE NEONATAL INTENSIVE CARE UNIT (NICU) EXPERIENCE

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Abstract

Premature neonates are in an energy deficient state due to (1) oxygen desaturation and hypoxia events, (2) painful and stressful stimuli, (3) illness, and (4) neurodevelopmental energy requirements. Failure to correct energy deficiency in premature infants may lead to adverse effects such as neurodevelopmental delay and negative long-term metabolic and cardiovascular outcomes. The effects of energy dysregulation and the challenges that clinicians in the neonatal intensive care unit (NICU) face in meeting the premature infant's metabolic demands are discussed. Specifically, the focus is on the effects of pain and stress on energy homeostasis. Energy deficiency is a complex problem and requires a multi-faceted solution to promote optimum development of premature infants.

Background

Premature infants (less than 37 weeks gestational age) face numerous challenges during their stay in the neonatal intensive care unit (NICU). Not only do they face the challenge of being underdeveloped compared to term infants, they are also at risk for a variety of illnesses, as well as undernutrition and growth failure (also known as “failure to thrive”) [1,2], These factors lead to a state of energy deficiency and catabolism, with potential long-term effects such as impaired neuronal development [3,4] and metabolic diseases [5,6]. Critically ill neonates and premature infants in particular, also undergo multiple (from 4 to 16) tissue damaging procedures (TDPs) for clinical care or diagnostic purposes [7–9]. These procedures include tape removal, heel-stick, venipuncture, intravenous or central catheter placement, injections and tracheal suctioning and intubation. Analgesic treatments for TDPs usually come in the form of 0.5 mL to 2 mL/kg per dose of a 24% oral sucrose solution given with a pacifier, lingually or at the buccal mucosa [10–13]. However, sucrose may play an indirect role in the modulation of the premature infant’s stress physiology, metabolism and energy state [10,14–18]. With this in mind, the premature condition in the context of routine clinical procedures, energy metabolism and prospective long-term outcomes of energy deficit are of interest. The administration of oral sucrose and its potential effects on energy metabolism and stress physiology require particular attention. It is anticipated that, with improved understanding of the potential mechanisms of the development of energy deficit in premature neonates, rational preventive and treatment approaches will emerge. The objective of this narrative review is to summarize the complexity of the metabolic

demands of the premature neonate, as well as the potential consequences of not meeting these demands, leading to energy deficit.

Premature Infants Are in An Energy Deficient State

Preterm births steadily increased from 9.5% in 1981 to 12.7% in 2005 [19]. During the first few weeks of postnatal life, premature infants are most likely ill, physiologically unstable and lack adequate nutritional support [2]. Following birth, these infants simultaneously experience a loss of maternal nutritional support along with their own limited ability for energy storage [20,21]. Energy deficit occurs due to four possible reasons (see Figure 1 for a simplified diagram).

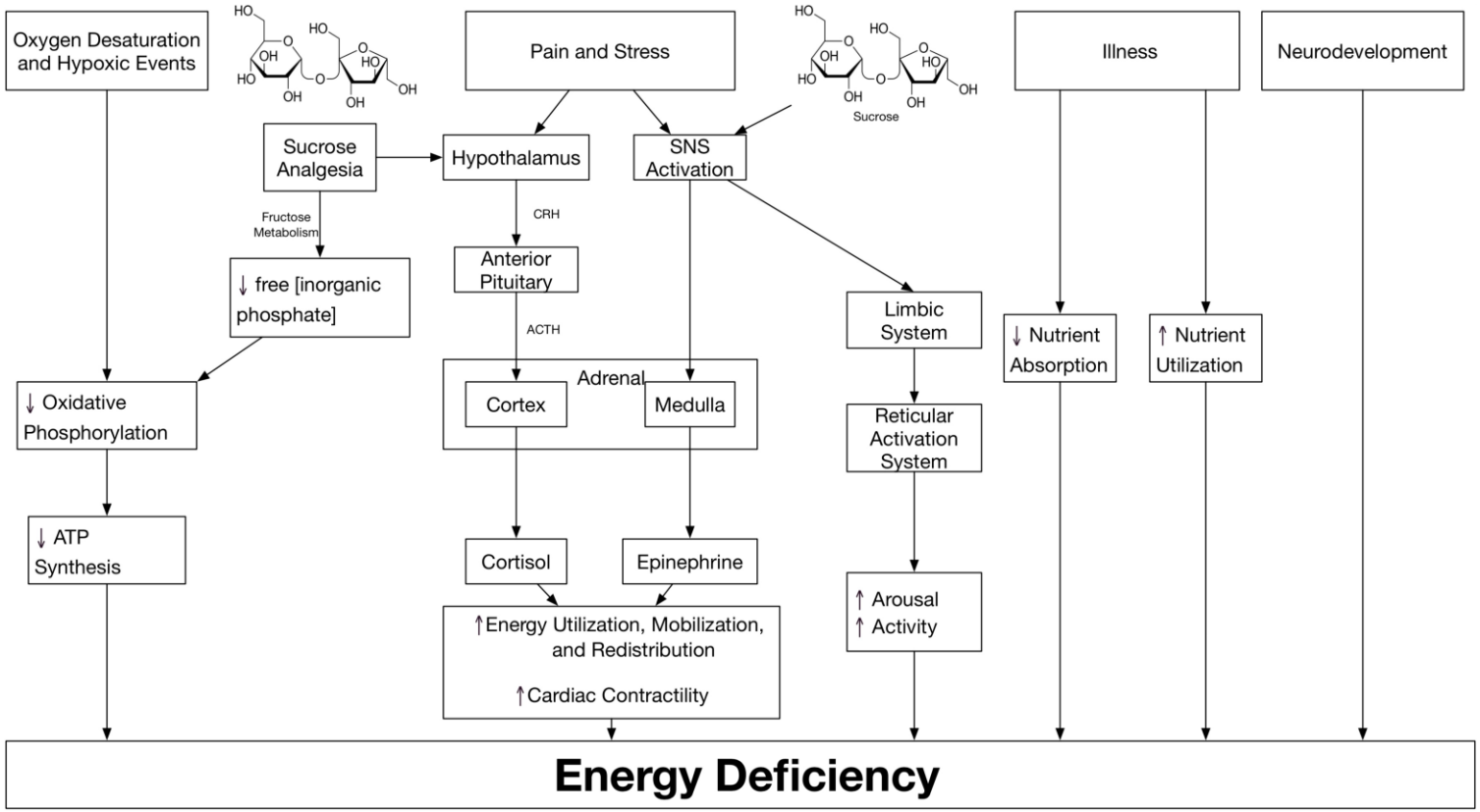

Figure 1. Causes of energy deficiency in the premature infant. (ATP, adenosine triphosphate; SNS, sympathetic nervous system)

First, there may be reduced energy stores due to oxygen desaturation events that occur frequently in the NICU setting, most often due to prematurity or respiratory disease [22]. Neonates experiencing multiple oxygen desaturation events are at risk for hypoxia, which refers to an inadequate oxygen supply to the tissues. This leads to a reduced rate of oxidative phosphorylation by aerobic respiration and to reduced adenosine triphosphate (ATP) synthesis [23]. Because of the lack of oxygen as a final electron acceptor, the mitochondria are unable to maintain the proton gradient necessary to form ATP from adenosine diphosphate (ADP) and inorganic phosphate [24]. Consequently, ATP production is reduced and possibly interrupted.

Second, energy stores may be reduced in response to stressful stimuli such as procedural pain. We explored the effects of tissue damaging procedures (TDPs) on ATP metabolism [25]. When the TDP was tape removal, performed along with the removal of a central or venous catheter, we found a significant increase in uric acid (UA) and malondialdehyde (MDA) thirty minutes after the painful stimulus. UA is a downstream product of ATP degradation. MDA is an oxidative stress marker formed by the oxidative degradation of polyunsaturated lipids by reactive oxygen species (ROS). The increase in ATP degradation in response to painful procedures may be due to energy expended through behavioral and physiological reactions to pain, such as crying, facial grimacing, flailing and tachycardia [26]. The increased oxidative stress could be due to increased purine degradation with concomitant production of ROS. Alternatively, oxidative stress could also be due to increased mitochondrial ATP synthesis activity, of which ROS is a byproduct, in order to meet the energy demands of increased ATP utilization [27]. Both the increased ATP utilization and oxidative stress can lead to energy deficit.

Third, energy stores can be reduced during illness [2]. Though current understanding for the neonatal population is modest, studies in adult and children show that critical illness changes metabolism significantly by decreasing the rate of absorption and utilization of nutrients [28–31]. Furthermore, elevated pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-10 play a role in increasing metabolic demand [29,32]. Such pro-inflammatory cytokines were found to be elevated in critically ill neonates, implying that increased metabolic demand can also occur in the premature neonate population [32,33]. An increase in metabolic demand will further decrease energy stores.
Finally, an increase in metabolic demand due to the high energy demands of the brain [34–36] can further reduce a premature neonate's energy stores. The neonatal brain accounts for 60% of total metabolism [37]. Most brain energy is used in the maintenance and manipulation of ion gradients for synaptic transmissions and cortical development [34]. As the brain develops during the early neonatal period, certain connections are pruned and ATP must be spent for the creation of cell components [38].

To make up for increased metabolic requirements, clinicians need to respond appropriately by increasing nutritional availability to premature neonates. This can be a challenge because premature infants have immature digestive and absorptive capabilities [39]. Moreover, most immature infants must rely on total parenteral nutrition (TPN) for exogenous nutritional support [40]. TPN, however, can only safely provide a limited amount and concentration of nutrients. Furthermore, TPN use is associated with added potential complications which includes the following: (a) TPN may lead to a stunting of the neonatal intestinal development due to absence of trophic factors that are only released when nutrients are present in the intestinal lumen [41,42] and (b) Long-term use

of TPN is associated with an increased risk of metabolic liver dysfunction [43]. Because of such concerns, premature neonates are given early trophic feedings, weaned off TPN gradually and are cautiously introduced to enteral nutrition through a nasogastric or an orogastric tube [42,44].

Despite these attempts to provide adequate nutrition, preterm infants tend to experience growth failure. Reali et al. found that in their cohort of premature infants born with a weight appropriate for gestational age (AGA, 2.5–4.0 kg), as many as 71.4% weighed less than the 10th percentile at discharge from the hospital [45]. It is currently unclear whether the cause of growth failure is due to inadequate nutrient supply to the infant or due to non-nutritional mechanisms that can also restrict growth and energy stores, such as inflammation or illness [2,3]. Nonetheless, it is clear that premature infants are frequently in an energy-deprived state due to increased metabolic demands combined with inadequate nutrition.

Under circumstances of frequent energy deprivation, premature infants compensate through tissue breakdown and protein loss [46]. Tissue and energy stores are converted into readily available fuel. Amino acids are recycled through the liver as carbon sources for gluconeogenesis or degraded into ketones, serving as the brain's alternative fuel source [2]. As energy supply becomes increasingly scarce, tissue breakdown and energy storage utilization becomes necessary to provide fuel even for baseline cellular processes [47]. Under hypoxic conditions anaerobic metabolism is engaged, converting pyruvate to lactate and allowing the regeneration of nicotinamide-adenine dinucleotide (NAD^+), so glycolysis can continue to generate ATP. Nonetheless, the resulting ATP is expended quickly, resulting in a steady decrease of ATP stores [23].

Energy Deficiency Affects Long-Term Outcomes

Energy deficits were shown to be detrimental to neurodevelopment and cognitive functions, especially in neonates [48]. The brain is a rapidly developing organ, which is responsible for 60% of total body's energy requirements [37]. During development, a critical growth phase occurs, which refers to the period of time when the neonatal brain experiences a significant degree of neuroplasticity [49]. During this phase, the neonatal brain is highly influenced by nutritional availability [50]. Critical periods of growth are accompanied by an increase in metabolic demand, requiring adequate nutrition to support it. Stephens et. al identified the first week of life as a period of critical growth in extremely low birth weight infants (ELBW, infants who are born weighing less than 1000 g) and observed that increasing protein or energy intake during that time period correlated with improved neurodevelopmental outcomes at 18 months corrected age [51]. Despite the significant relationship between nutrition and growth and development, few tools exist that measure energy utilization and adequacy of nutrient intake in premature neonates. Current markers of nutritional status include growth velocity (15 g/k/day), weight gain (10–30 g/day) and head circumference (1 cm/week) [50,52]. However, these measures, are shown to only be applicable to a limited range of postnatal age [53]. For example, Fenton et al. [53] has shown that the commonly used weight growth velocity goal of 15 g/kg/d was only consistent with the preterm infant growth reference curves at about 34 weeks. Tools that measure the energy states of premature neonates throughout the wide range of gestational ages are needed. These tools need to be individualized to gestational age, birth weight, gender, and illness severity. For example, our laboratory examined urinary biochemical markers as a measure of ATP utilization in premature

neonates [10,54]. In the late pre-term neonates, we found urinary hypoxanthine to be highest in those with respiratory disease, showing the effect of illness on ATP degradation [54]. These data suggest that neonates with respiratory disease may require higher total energy intake [53]. In addition, we found an association between urinary allantoin levels and the incidence of severe intraventricular hemorrhage (IVH) [55]. Allantoin is an oxidation product of uric acid in the presence of reactive oxygen species, which may be produced during increased ATP utilization [10]. Few studies on IVH and nutrition exist but Sammallahti et al. [56] observed that those with IVH had lower total energy intake and lower energy intake from human milk. These data suggest the importance of careful monitoring and provision of adequate nutrition in this vulnerable population. An additional challenge for neonatal growth assessment is that optimal growth is currently not well defined. Additionally, the few reported studies in neonatal growth are impacted by confounding factors that reduce the value of their conclusions [57]. As previously described, current premature infant growth models only take into account simple measurements such as length, weight, head circumference and body mass index (BMI) [58]. Furthermore, it is unclear if these premature infant growth models are appropriate because they are based on a population of healthier term infants [2]. In view of the absence of correlation between rapid growth of premature neonates with long-term adult metabolic risks and clear association with a decreased infant morbidity and mortality, improved nutritional support may be warranted [59].

In contrast to the established long-term neurodevelopmental outcomes of energy deficit, evidence regarding long-term metabolic and cardiovascular outcomes is unclear. Once a premature infant is stabilized in the NICU, nutrition is given and accelerated

catch-up growth may occur within the first 24 months of postnatal life [60,61]. It is currently believed that catch-up growth is beneficial for the first 2 years of life [62], especially for neurodevelopment [3,50] but may have negative long-term consequences in adult metabolism [63]. Unfortunately, studies on the non-neurodevelopmental effects of catch-up growth are few and of poor quality [57]. Despite the benefits of catch-up growth, there are reports that preterm infants with accelerated weight gain after the first two years of life have higher relative body adiposity and cardiovascular complications compared to term infants [64–66]. In adults, such increased body adiposity is a risk factor for cardiovascular morbidity and metabolic syndrome [67]. Because of this, it was suggested that quality assessment of neonatal growth should include more comprehensive measures of body composition, such as fat mass (FM) vs. fat-free mass (FFM), instead of simple body length, weight, head circumference and BMI [58,68,69]. However, it remains unclear whether the increased FM in early postnatal life independently alters cardiovascular and metabolic health in adulthood.

Long-term effects of fetal growth, postnatal growth and early nutrition were studied with respect to cardiovascular and metabolic outcomes in preterm infants [70]. Adults, who were born prematurely, were found to have a significantly greater risk of developing hypertension and insulin resistance compared to those born at term. This difference, however, was not associated with body size, body composition, or FM distribution. Furthermore, growth between birth or expected term age and 12 to 18 months post-term, had no significant influence on blood pressure or metabolic syndrome in adulthood. Instead, it was suggested that growth during late infancy and childhood may have a more significant influence on later cardiovascular and metabolic health. This

is consistent with a recent longitudinal cohort study [71] of association between weight gain in infancy and childhood with biomarkers of metabolic syndrome in adolescents who were born preterm. No significant correlation between infant weight gain and long-term metabolic consequences was observed, regardless of catch-up growth rate. Instead, significant associations were reported between childhood weight gain (after 1 year of age) and later body composition changes ($p < 0.001$ for higher fat mass, high fat index, lean mass index and waist circumference), higher fasting insulin ($p = 0.002$), lower insulin sensitivity ($p < 0.001$), higher systolic and diastolic blood pressures ($p = 0.006$ and 0.005, respectively), lower high density lipoprotein (HDL) ($p = 0.001$) and a higher total cholesterol to HDL ratio ($p < 0.001$). These studies support the novel idea that the growth velocity after the first two years of life is a more accurate predictor of adult risk of metabolic disorders than the catch-up growth rate of premature infants during the first two years of life [3,4].

Prematurity and Chronic Stress, Energy Deficiency, and Neuroplasticity

Preterm infants are in a state of chronic physiological and biochemical stress due to prematurity, illness, medications and many unavoidable environmental stressors in the NICU. When the relationship between clinical handling procedures, stress, pain and energy expenditure was examined, it was observed that as the level of intervention increased, infant energy expenditure increased as well [72]. Additionally, a negative correlation was found between energy expenditure and oxygen saturation, supporting the hypothesis that oxygen desaturation events are likely to result in hypoxia, resulting in

decreased ATP synthesis coupled with an increased ATP utilization. Our lab found a similar association of increased ATP utilization in response to TDPs [25].

Because preterm infants lack the agency to limit external stressful stimuli, they must rely on their caregivers to limit their exposure to stressors. These environmental stressors include loud sounds and alarms from clinical equipment, noise from other infants, handling by the caregivers themselves and constant interruption of sleep for medical procedures. Furthermore, premature infants are also exposed to multiple tissue damaging procedures (TDPs) for clinical care or diagnostic purposes [8,73]. These can have long-term consequences, including neuroplastic modulation of the neonates' stress response. The stress response can be represented by two concepts: allostasis and allostatic load [74]. Allostasis refers to the active process of metabolic or physiological adaptations in response to stressful stimuli. Allostatic load refers to the "wear and tear" of the body that increases over time in response to chronic stress [75]. Allostatic load can manifest itself as a dysregulation of the stress response due to a lack of adaptation, prolonged response, or inadequate response [75].

A key regulator of allostasis is the hypothalamic-pituitary-adrenal (HPA) axis. While the development and function of this axis still remains to be fully characterized in the newborn, stress regulation involves three main steps. First, the paraventricular nucleus of the hypothalamus synthesizes and secretes corticotrophin-releasing hormone (CRH). Second, the anterior lobe of the pituitary gland releases adrenocorticotropic hormone (ACTH) in response to CRH. Finally, cortisol is released by the adrenal glands in response to ACTH. For a healthy allostatic response, baseline hormone levels are restored through cortisol's negative feedback loop to the hypothalamus and the pituitary

gland. In the premature infant, the allostatic response via the HPA axis may be irreversibly altered due to chronic stress exposure. The allostatic load may be elevated due to insufficient cortisol production caused by illness or an underdeveloped HPA axis [76]. Heckmann et al. found that a mature adrenal response, defined in clinically stable premature infants as a tripled level of cortisol in response to stress, was present only in 27% (12 out of 44) of ill preterm infants [77]. However, these high responders were more prone to central nervous system (CNS) bleeds. Additionally, it was demonstrated that during the first 7 days of life, the pituitary gland is responsive to human CRH but cortisol production was suboptimal [78]. This may be due to an underdeveloped adrenal cortex or cortisol synthesis. This cortisol deficiency disappeared by day of life 14 [78]. Thus, allostasis may be inadequate in early life.

The HPA axis also plays a role in energy homeostasis. As part of the allostatic response, the metabolic demand for energy is augmented to increase chances of survival. One group [72] showed a significant correlation between stress and energy expenditure in premature neonates. Stress was defined as (1) a heart rate of less than 100 bpm or more than 160 bpm or an increased baseline of 5 bpm or more, (2) irregular respiratory rate of less than 40 or more than 60 breaths/min, or a baseline increase of 7 breaths/min or more and (3) oxygen saturation of less than 90% or a decrease of 2.5% or more. Energy expenditure was measured as follows:

$$E = \frac{M \times t}{H_T}$$

where E = energy expenditure per heartbeat $\left(\frac{calories}{kg}\right)$, M = mean metabolic rate $\left(\frac{calories}{kg \cdot min}\right)$, t = duration of study (min) and H_T = total accumulated heartbeats.

Stressful experiences may also have lasting developmental impact. Allostatic load can manifest itself through the dysregulation and neuroplastic modification of the HPA axis. Recently, an "ACTH-cortisol" dissociation was reported in critically ill adults, referring to low circulating ACTH coupled with elevated plasma cortisol [79,80]. Furthermore, high levels of chronic stress can alter HPA axis reactivity and shift the baseline set point of cortisol, blunting the allostatic response to acute stress [81]. A prolonged period is required to return to pre-stress hormone levels and higher concentrations of cortisol is required to respond to subsequent stressors [82]. Prolonged exposure to elevated cortisol levels may lead to increased proteolysis, which can negatively impact the overall growth of the neonate [83]. Chronic stress may also be associated with increased cognitive and behavioral problems and metabolic risks [84–87]. This can modify the structure and synaptic connections of the prefrontal cortex, the area of the brain associated with personality expression, decision-making and social behavior [88]. In rats, chronic stress was shown to increase synaptic inhibition of prefrontal glutamatergic output neurons, resulting in decreased control of stress reactivity and behavior [89]. Furthermore, chronic stress decreases synaptic density in the prefrontal cortex as well as in the hippocampus [90]. Thus, there are good reasons to expect, even in the absence of newborn studies, that allostatic modifications in response to chronic stress may influence the neuronal development of the premature infant's brain.

Sucrose and Stress Relief

Oral sucrose with non-nutritive sucking was shown in many studies to reduce procedural pain scores [11–13]. The evidence for the analgesic effects of sucrose is strongest for

single event painful procedures such as heel lance, venipuncture, or intramuscular injection [7]. Analgesic benefits of sucrose for other painful procedures such as arterial puncture, subcutaneous injection, insertion of nasogastric or orogastric tubes, bladder catheterization, eye examinations or echocardiography examinations are less certain [7]. Most studies on sucrose examine pain behavior as the study variable. There is a limited amount of research on other outcome variables, such as cortisol. Some of these studies are outlined in Table 1.

Table 1. Summary table of studies that examined other variables besides pain response after sucrose administration. (CNS, central nervous system; ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; mRNA, messenger ribonucleic acid).

Population	Parameter Measured	Effect	Sucrose Dose	Control	Ref
Premature Infant	ATP Utilization, Oxidative Stress	Increased, Increased	0.2 mL	Placebo (Sterile Water)	[10]
Premature Infant	Salivary Cortisol	No Significant Difference	0.1 – 0.3 mL per Painful Procedure	Placebo (Sterile Water)	[91]
Premature Infant	Plasma Cortisol	No Significant Difference	Pacifier Dipped in Sucrose	Water	[92]
Adult Human Females	Salivary Cortisol, Regional Brain Responses	Decreased, Increased in Left Hippocampus	3 Servings of Study Beverage per Day	Aspartame	[93]
Premature Infant	Neurodevelopmental Assessment	No Significant Difference	0.5 mL per Painful Procedure	Placebo (Sterile Water)	[94]
Mouse Pups	CNS White Matter Regions CNS Gray Matter Regions	Decreased, Decreased	0.1 – 0.2 g Sucrose per kg Body Weight	Vehicle (Sterile Water)	[95]
Adult Rat	Plasma ACTH, Corticosterone, CRH mRNA	Decreased, Decreased, Decreased	Up to 4 mL Twice a Day	Saccharin and/or Water	[96, 97]

A few studies in premature neonates measured the effects of sucrose administration on cortisol. Boyer et al. [91] found no significant difference in salivary cortisol concentration 30 minutes after a painful procedure in premature infants receiving either 24% sucrose or a placebo of sterile water. Stang et al. [92] found no significant difference in plasma cortisol 30 minutes after circumcision in groups receiving a dorsal penile nerve block agent and sucrose or water. Although sucrose had no significant effect on plasma cortisol levels, the pain scores decreased in both studies, which suggest that

sucrose may only mask pain behavior with little effect on the glucocorticoid response [98]. A different response has been observed in animals. Ulrich-Lai and her colleagues showed that in rats exposed to chronic stress, sucrose consumption decreased corticotropin-releasing hormone messenger ribonucleic acid (CRH mRNA) in the paraventricular nucleus of the hypothalamus [96]. They also showed that the palatable and rewarding properties of sucrose are responsible for a decrease in adrenocorticotropic hormone (ACTH) and corticosterone [97]. The basolateral amygdala is altered in response to sucrose consumption and this alteration is long-lasting due to neuroplasticity [97]. Furthermore, increased duration and/or frequency of sucrose administration played a larger role in the dampening of the HPA axis than the volume of sucrose given, suggesting that sucrose may alter neuroplasticity and stress relief [99].

It was calculated that a 1000-gram infant receiving an average of 10 doses of 24% oral sucrose per day, at 0.5–1 mL per dose, is equal to a one year old infant receiving ½ can of regular Coke Classic per day [11]. The effect of this much sucrose on human premature infants is unknown. In adults, a 19-day study compared salivary cortisol levels between two groups that consumed either sucrose or aspartame along with a standardized, low-sugar baseline diet [93]. It was found that sucrose but not aspartame, reduced salivary cortisol levels after a comprehensive imaging stress test. In the same study, sucrose consumption correlated with a significant increase in activity levels in the left hippocampus, implying that sucrose inhibits stress induced deactivation of the hippocampus, perhaps through HPA axis suppression. These data suggest that oral sucrose consumption may modify the brain's response to stress, specifically in the paraventricular nucleus of the hypothalamus and the basolateral amygdala. Interestingly,

Stevens et al. showed that preterm infants <31 weeks' gestational age who received >10 doses of sucrose per 24 h in the first week of life had poorer neurologic development compared with infants who received fewer sucrose doses [12]. However, in older premature neonates that are over 32 weeks gestational age, Banga et al. showed that repeated dosages of sucrose administration for procedural pain in premature infants for the first seven days after enrollment had no significant impact on neurobehavioral outcomes at 40 weeks post conception [94]. Additional studies are required to clarify the effect of sucrose analgesia on the newborn's brain.

The mechanism for sucrose's analgesic effect is unknown but is thought to be due to (a) the release of endogenous opioids two minutes after sucrose administration [100], although evidence to substantiate this hypothesis in humans is lacking, or (b) the occurrence of ingestion analgesia [101]. Ingestion analgesia occurs when hedonic foods are eaten and functions to defend eating from ending and stops when eating is over. Hedonic food is specific to the animal's homeostatic state. For example, sodium becomes hedonic when effective circulating volume is low [102]. In a complementary manner, the sensation of thirst increases when plasma osmolality rises above normal levels [103]. Similarly, sucrose, an inherently hedonic food due to its sweet taste, has been shown to reduce stress via brain reward pathways [97]. Chronic stress modifies the brain's reward pathway to increase the hedonic value of palatable high-calorie foods through the actions of glucocorticoids [16]. Incidentally, there is strong evidence that an animal's energy stores may play a role in the regulation of the HPA axis [16]. The hedonic value of oral sucrose may be elevated in chronically stressed premature infants that are energy

deficient, which may contribute to its effectiveness in decreasing the behavioral signs of pain.

Though sucrose may have a role in pain and stress relief, it may come at the cost of long-term neurologic and metabolic consequences and altered brain stress and reward pathways. Acutely, a single dose of oral sucrose administration for heel lance has been associated with increased ATP utilization and oxidative stress [10], perhaps due to the high metabolic cost of the fructose moiety of sucrose [104]. In a mouse pup model, the effects of early repeated sucrose treatment before an intervention on long-term brain structure was examined [95]. These mice pups received an oral dose of vehicle (sterile water) or 24% sucrose via a micropipette, two minutes before an intervention. The mice pups were separated into three different intervention groups: a needle-prick on the paw, light tactile paw pressure with a cotton swab, or only handling in a similar manner as the other groups. The mice pups received 10 interventions daily from post-natal day 1 (P1) to P6 to model the NICU experience. Adult brains were collected between P85 and P95 and were scanned using magnetic resonance imaging (MRI). Early repetitive sucrose exposure in mice resulted in smaller white matter volumes in the corpus callosum, stria terminalis and fimbria ($p < 0.0001$) and smaller cortical and subcortical gray matter in the hippocampus and cerebellum ($p < 0.0001$), regardless of intervention. This suggests that sucrose may affect brain development independent of procedural pain. The modulation of the HPA axis and the increased hedonic values placed on sweet solutions may also play a role in the increased risk of long-term negative metabolic outcomes. In the interim, however, there is not enough evidence to recommend the cessation of oral sucrose administration for procedural pain in the NICU. More studies are required to examine the

analgesic effectiveness of metabolically “cheaper” sweet solutions, such as glucose, as well as other pharmacologic and non-pharmacologic methods to reduce pain.

Conclusions

In conclusion, premature infants are in a state of energy deficiency due to hypoxia, pain and stress, illness and neurodevelopment. Each of these factors increases ATP utilization, reducing energy stores. In addition, oral sucrose, a commonly used intervention for pain was recently shown to acutely increase ATP utilization as evidenced by increased biochemical markers of hypoxia and oxidative stress over time [10]. Nutritional support specific to a neonate’s age, weight, gender and illness severity needs to be provided to prevent energy deficit and tools that monitor energy states and efficacy of nutritional intake need to be developed and tested. Management of a neonate’s nutritional status is complex and requires prospective studies that will yield evidence-based methods and techniques.

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CHAPTER THREE

THE EFFECTS OF THE ROP EXAM ON PREMATURE INFANT URINARY ATP UTILIZATION MARKERS AND INTESTINAL INJURY

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Abstract

Objective: To compare urinary markers of ATP utilization and intestinal injury between premature infants receiving oxygen support, and those breathing room air unassisted, for 24 hours following the retinopathy of prematurity (ROP) exam.

Study Design: We collected urine from 48 premature infants (room air = 20, oxygen support = 28) and compared urinary markers of ATP utilization (hypoxanthine, xanthine, and uric acid) and a marker for intestinal injury known as intestinal fatty acid binding protein (IFABP). All markers were normalized by creatinine measurements.

Results: We found significant differences between groups in urinary ATP utilization markers in the period of 18-24 hours, but not from 0-18 hours. We also found significant differences between groups in IFABP/creatinine ratios for the time periods of 6-12 hours and 12-18 hours. There was a significant positive correlation between hypoxanthine/creatine at 6-12 hours and IFABP/creatinine at 12-18 hours, and between uric acid/creatinine at 12-18 hours and IFABP/creatinine at 12-18 hours.

Conclusion: Premature infants on oxygen support may have a higher risk of intestinal injury as well as increased ATP catabolism. There may be some crosstalk between premature infants' energy deficient state and the pathology of intestinal injury.

Background

Premature infants undergo an eye examination called the retinopathy of prematurity (ROP) exam during their care at the NICU (neonatal intensive care unit). The ROP exam is a necessary and routine eye examination in the NICU that detects and identifies infants at risk for blindness due to retinal detachment [1]. However, a number of adverse physiological side effects may occur with this procedure. These side effects include an increase in both systolic and diastolic blood pressure, increased heart rate during the eye exam, and a drop in oxygen saturation [2]. An increase in the number of apnea events was consistently observed in response to the eye exam [2–8]. This may be a result of respiratory disorders or due to illness severity, although the mechanism has not yet been clearly established [3].

The impact of the ROP exam on the energy expenditures of premature infants is similarly not well understood. Premature infants are in an energy-deficient state due to respiratory challenges, procedural pain and stress, illness, and neurodevelopmental energy requirements [9]. When left in a continued state of energy deprivation, premature infants undergo tissue breakdown and protein loss, which can decrease the quality of life and may be associated with higher morbidity and mortality [10]. Peng et al. established a negative correlation between oxygen saturation and energy expenditure in premature infants [11]. We reported a link between respiratory disorders and urine hypoxanthine, a marker of hypoxia in late pre-term infants (infants born between 34 weeks and 36 6/7 weeks gestational age) [12]. Similarly, increased urinary uric acid was reported in asphyxiated premature neonates [13]. Generally, prolonged hypoxia inhibits oxidative phosphorylation and reduces ATP synthesis, leading to accumulation of ATP catabolic

markers [14]. The final product of ATP catabolism is uric acid, which may be increased after hypoxia and reperfusion injury. Then uric acid enters circulation from stressed or damaged tissues and is eventually excreted in urine.

Another important aspect of the ROP exam is its effect on the digestive system. Increased gastric residuals were reported in association with the ROP exam, possibly due to the systemic effects of mydriatic eye drops given before the eye exam [4,8,15]. Other reported side effects include vomiting, increased incidence of necrotizing enterocolitis (NEC), and upper digestive tract hemorrhage, especially in premature infants less than 31 weeks post-conceptional age [2]. Urinary intestinal fatty acid binding protein (IFABP) is an early marker of intestinal injury and may predict or detect the occurrence of NEC [16–18]. IFABP is a cytosolic protein that is primarily located within the small intestine and is released into circulation in response to enterocyte injury or cell death [19–21]. It is readily excreted in urine because of its relatively small size. Because IFABP is not expressed within the urinary tract, the concentration in the urine directly reflects intestinal injury.

Purine signaling plays a major role in the digestive tract and the pathophysiology of a number of disorders such as inflammatory bowel disease, ischemia, diabetes, and cancer [22]. In rats subjected to ischemia followed by reperfusion, decreased ATP levels in the intestine correlated with an increase in intestinal permeability, suggesting a loss in epithelial cell lining integrity [23]. Furthermore, the purinergic P2 receptor, which is sensitive to ATP, was found to play a role in a mouse model in the modulation of gastrointestinal motility [24]. To our knowledge, the relationship between energy levels and intestinal health has never been explored in premature neonates. The use of urinary

markers for both ATP utilization and intestinal injury in the context of the ROP exam may lead to a more thorough understanding of the crosstalk between pain and stress, energy expenditure, and the intestinal tract function.

The aim of this study is to noninvasively evaluate the effects of the ROP exam on urinary markers of ATP utilization and intestinal injury (IFABP) in two groups of neonates. One group includes premature infants that are receiving extraneous oxygen support, and the other group includes premature infants who are stable in room air. Uric acid, hypoxanthine, xanthine, and IFABP were quantified in a ratio with creatinine in a 12-hour period before the ROP exam and during 24-hours after the ROP exam. The exam was chosen as a painful procedure because of its documented effects on cardiovascular, respiratory and gastrointestinal systems.

Methods

We conducted a prospective study at Loma Linda University Children's Hospital to measure the physiological effects of the ROP exam in premature neonates. The Loma Linda University Children's Hospital Institutional Review Board approved the study protocol and informed consent documents. Subjects included in the study were hospitalized premature infants who: (1) required the retinopathy of prematurity eye examination, (2) met the screening recommendations of the American Academy of Pediatrics and Ophthalmology [25] and (3) were included in the study by parental consent. Exclusion criteria covered infants who were: (1) scheduled for laser eye surgery on the day of the examination, (2) with intraventricular hemorrhage ≥ grade 3 (Papile classification) [26] diagnosed by head ultrasound, (3) receiving the following

medications: morphine, fentanyl, methadone, midazolam, lorazepam, muscle relaxants, phenobarbital, phenytoin, and levetiracetam, (4) with renal injury defined by plasma creatinine > 1.5 mg/dL, or (5) with severe cyanotic congenital heart disease, severe respiratory distress or GI dysfunction. After consent was obtained, investigators collaborated with the clinical staff to obtain urine samples. These were collected by placing cotton balls over the urethral meatus. Urine-soaked cotton balls were removed from the diaper with every diaper change and stored at 4°C. Urine collection was separated into two time-points: 12 hours before the eye exam and 24 hours after the eye exam collected in 6-hour increments. Urine was extracted from the cotton using pressure, centrifuged for 10 minutes at 20 000 × g and 4°C, and stored at −80°C until further processing, as previously described [12].

Urinary uric acid, hypoxanthine, xanthine, and creatinine concentrations were measured as previously published by our group [12]. Specifically, urine samples were thawed and sonicated before 200 μL was transferred to an Eppendorf tube containing 1×10^{-7} mol of 2-aminopurine (internal standard). The samples were then analyzed on an HPLC (Waters 996 PDA, Waters 600 controller, and 717plus autosampler; Millipore Corp) by injecting 35 μL onto a Supelcosil LC-18-S 15 cm × 4.6 mm, 5 μm column (SGE; Austin, TX), with the following isocratic conditions: 10 mM potassium dihydrogen phosphate buffer, pH 4.7, flow rate 1.0 mL/min. Creatinine, hypoxanthine, and 2-aminopurine were quantitated by obtaining peak areas at the appropriate retention times (~3.5, 8, and 13.5 minutes, respectively) and wavelengths (230, 248, and 305 nm, respectively). The area ratios of each compound to 2-aminopurine were determined and converted into concentration using standard curves. Samples were analyzed in triplicate

and values with a coefficient of variation less than 10% were included in the final analysis. The limits of detection were 3.2 μM for creatinine, 1.58 μM hypoxanthine, 1.32 μM xanthine, and 5.0 μM uric acid.

Intestinal fatty acid binding protein (IFABP) was measured in urine using the ELISA kit from Hycult Biotech (Hycult Biotech, PA). Standards and samples were added to the 96-well plate in duplicates, with appropriate washing, incubation, and reaction times as indicated by the manufacturer. The plate absorbance was read at 450 nm within 30 min after the addition of the stop solution. The urine concentrations were standardized by the creatinine levels measured in the HPLC assay.

Statistics

To analyze the data, assumptions of normality and equal variance were assessed. Demographic data for categorical variables were analyzed using Chi-square test. The Mann-Whitney U test was performed at each time epoch to assess the difference in urinary purines and IFABP between groups. Pearson correlation, Chi-Square and Mann-Whitney U were performed using SPSS statistics for Windows Version 25.

Results

Subject Demographics

We enrolled a total of 48 premature subjects; 28 subjects were receiving oxygen support at the time of the ROP exam while 20 subjects were stable in room air.

Table 1. Subject Demographics and Clinical Data. Mean ± SD. A zone of 2.5 indicates that one eye had a zone of 2 and the other eye had a zone of 3. SNAPPE-II: score for neonatal acute physiology with perinatal extension-II [#]Independent samples t-test [+]Chi-square

	On Room Air (n=20)	**Receiving Oxygen (n=28)**	**P value[#]**
Birthweight (g)	12101 ± 418	885 ± 257	0.002
Weight @ Exam time	2314 ± 725	1792 ± 568	0.011
Gestational Age (wks)	29.5 ± 2.1	27.0 ± 1.9	< 0.001
Gestational Age @ Exam time	36.4 ± 2.6	34.5 ± 2.7	0.016
Apgar-1 min	4.9 ± 1.9	3.6 ± 2.4	0.048
Apgar-5 min	7.1 ± 1.3	6.5 ± 2.1	0.162
Gender	Female: 10 Male: 10	Female: 11 Male: 17	0.305[+]
SNAPPE-II	14.35 ± 11.16	24.21 ± 14.15	0.010
Ethnicity	African-American: 6 Asian: 2 Hispanic/Latino: 1 Caucasian: 9 Other/More than one race: 0 Unknown: 2	African-American: 4 Asian: 2 Hispanic/Latino: 2 Caucasian: 17 Other/More than one race: 2 Unknown: 1	0.342[+]
ROP Exam Number	2.3 ± 1.7	2.1 ± 1.5	0.692
ROP Stage	0 : 13 1 : 2 2 : 2 Mature : 1	0 : 19 1: 0 2: 8 Mature: 1	0.065
ROP Zone	0 : 0 2: 9 3 : 4 Mature : 1	0 : 4 2 : 10 2.5 : 1 3 : 2 Mature : 1	0.258

Subjects who were receiving oxygen support had a significantly lower birth weight ($P < 0.005$), weight at time of exam ($P < 0.05$), gestational age ($P < 0.001$), gestational age at the time of exam ($P < 0.05$), one minute Apgar score ($P < 0.05$), and SNAPPE-II (Score for Neonatal Acute Physiology with Perinatal Extension-II) [27] ($P < 0.05$) compared to

subjects who were on room air (Table 1). There were no significant differences between groups in gender, ethnicity, ROP exam number, ROP stage and zone. As expected, the group had significantly different FiO_2 before, during and after the ROP exam (Table 2).

Table 2. Oxygen support and FiO_2 information. CPAP: continuous positive airway pressure, HFNC: high flow nasal cannula, NAVA: neurally adjusted ventilatory assist, NCPAP: nasal continuous positive airway pressure, NIMV: nasal intermittent mandatory ventilation, NIPPV: noninvasive positive pressure ventilation. $^{+}$Chi-square

	On Room Air (n=20)	**Receiving Oxygen (n=28)**	**P value**
Mode of oxygen support	Spontaneous room air = 20	CPAP: 5 HFNC: 13 Nasal cannula: 4 NAVA: 2 NCPAP: 2 NIMV: 1 NIPPV: 1	$< 0.001^{+}$
FiO_2 Min 24 hours before exam (%)	21 ± 0	23.7 ± 3.6	0.002
FiO_2 Max 24 hours before exam (%)	21 ± 0	29.5 ± 8.6	< 0.001
FiO_2 During Exam	21 ± 0	26.2 ± 6.4	< 0.001
FiO_2 Min 24 hours after exam (%)	21 ± 0	23.4 ± 4.3	0.017
FiO_2 Max 24 hours after exam (%)	21 ± 0	33.6 ± 11.7	< 0.001

Effect of ROP Exam on Urinary IFABP

We found no significant differences in urinary IFABP/creatinine ratio at baseline and up to 6 hours after the ROP exam. However, urinary IFABP/creatinine ratio was significantly higher in the oxygen support group compared to the room air group at 6-12 hours and 12-18 hours after the exam ($P < .05$ for both, Figure 1). Urinary

IFABP/creatinine ratio remained elevated until 18-24 hours, but the data for that time interval was no longer significant.

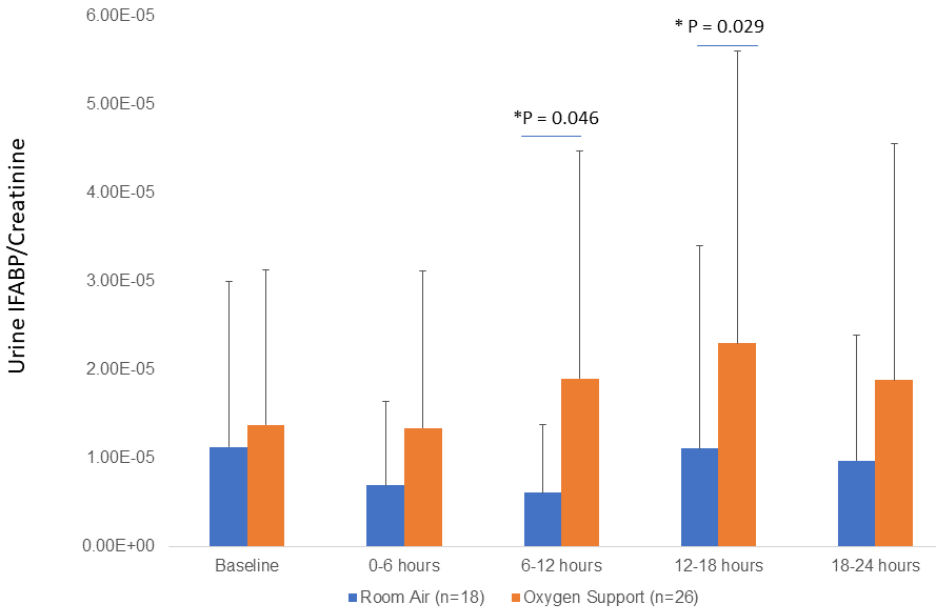

Figure 1. Longitudinal bar plots of the IFABP/Creatine ratio of both groups. Mean ± Standard Deviation. *Mann-Whitney U Test

Effect of ROP Exam on Urinary ATP Utilization Markers

We found no significant differences in hypoxanthine/creatinine between the room air and the oxygen support groups at baseline and up to first 18 hours after the exam. However, the ratio of hypoxanthine to creatinine became significantly elevated in the oxygen support group, compared to the room air group, at 18-24 hours after the ROP exam (Figure 2). We observed similar elevations in xanthine/creatinine (Figure 3) and uric acid/creatinine (Figure 4).

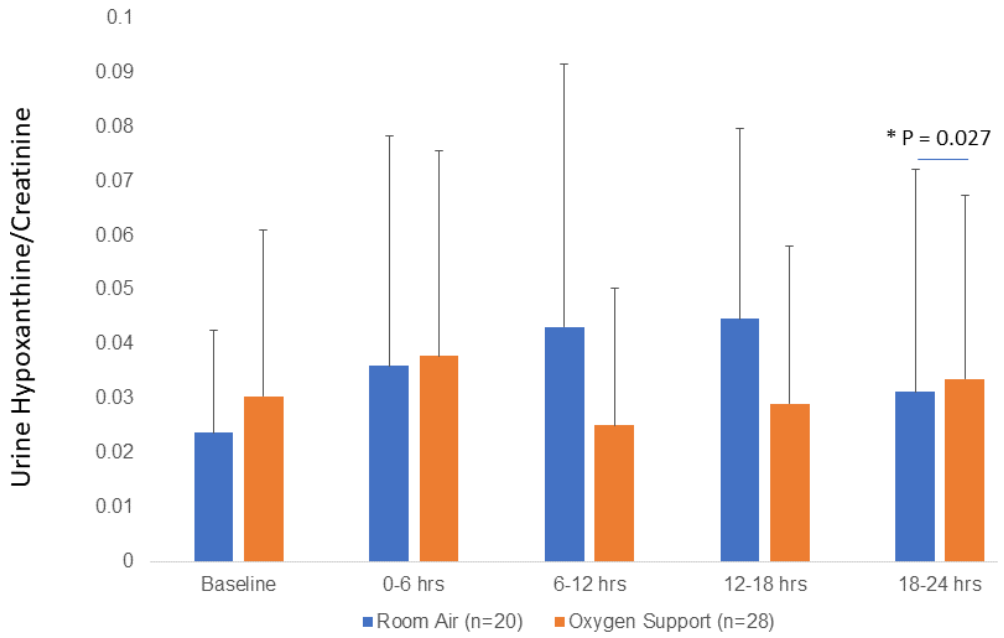

Figure 2. Longitudinal bar plots of the Hypoxanthine/Creatine ratio of both groups. Mean ± Standard Deviation. *Mann-Whitney U Test

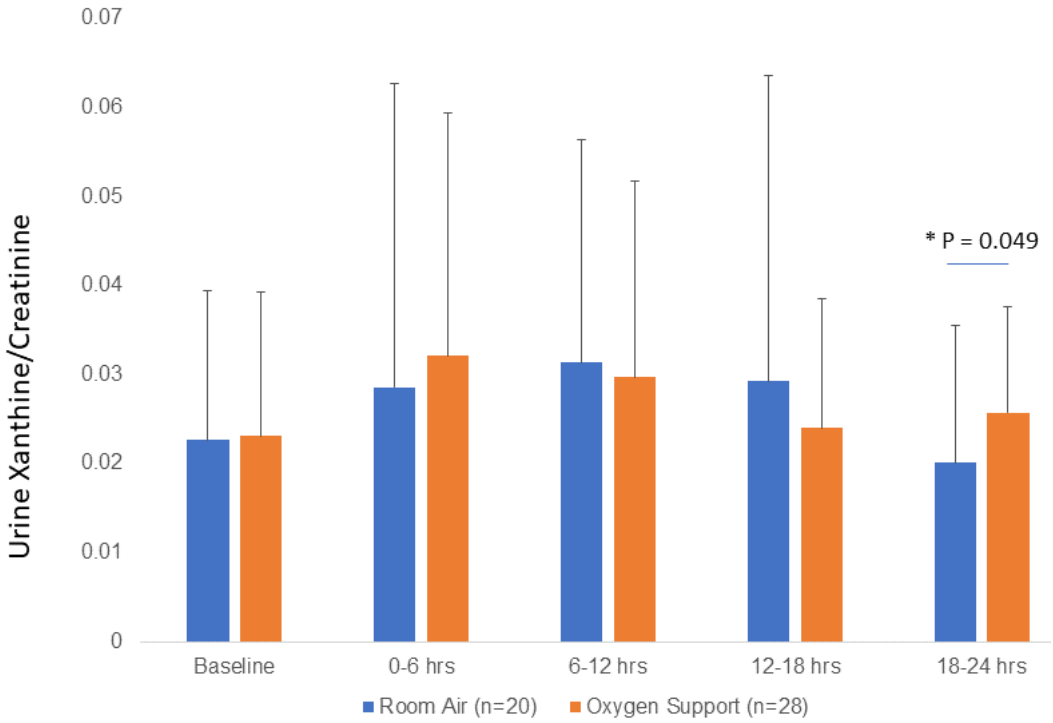

Figure 3. Longitudinal bar plots of the Xanthine/Creatine ratio of both groups. Mean ± Standard Deviation. *Mann-Whitney U Test

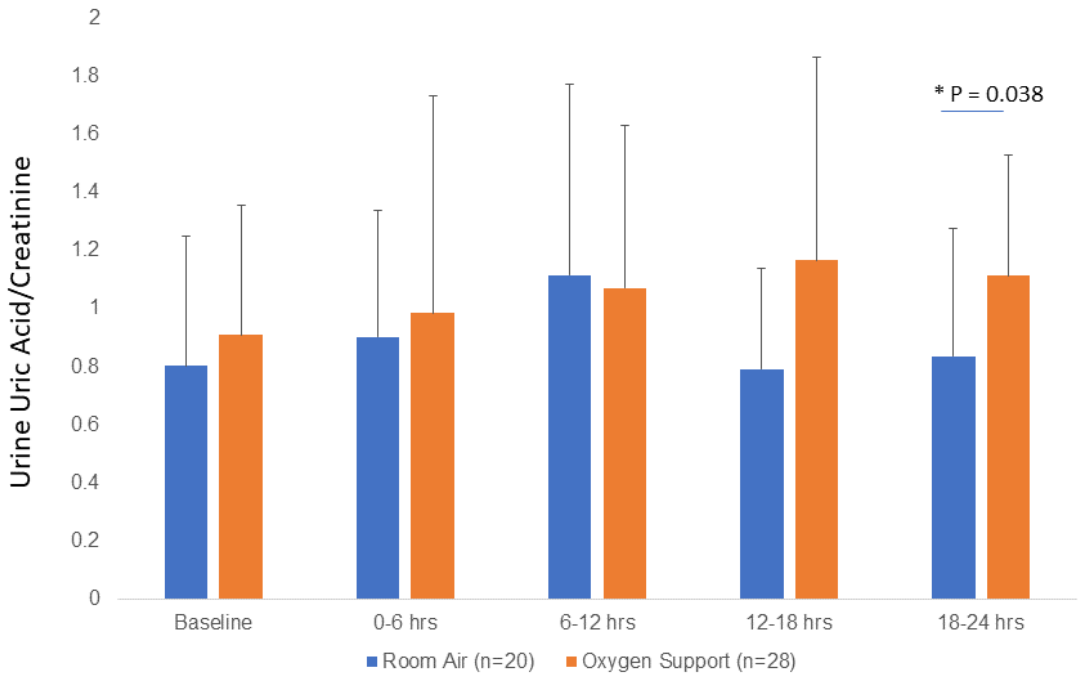

Figure 4. Longitudinal bar plots of the Uric Acid/Creatine ratio of both groups. Mean ± Standard Deviation. *Mann-Whitney U Test

Correlation between Intestinal Injury and ATP Utilization Markers

We examined the relationship between IFABP/creatinine ratio and the ATP utilization marker ratios of hypoxanthine/creatinine, xanthine/creatinine and uric acid/creatinine. Significant positive correlations were found between 6-12 hour hypoxanthine/creatinine and 12-18 hour IFABP/creatinine (Table 3). Similarly, significant positive correlation was found between uric acid/creatinine and IFABP/creatinine during 12-18 hour time interval (Table 3).

Table 3. Pearson correlation table between purines and IFABP measurements. IFABP: Intestinal fatty acid binding protein. CR: Creatinine.

Purine/Creatinine	Time Group	IFABP/CR 12-18 Hours Correlation	Significance
Hypoxanthine/CR	6 – 12 Hours	R = 0.444	P = 0.001
Uric Acid/CR	12 – 18 Hours	R = 0.426	P = 0.008

Discussion

Although the ROP exam is a necessary part of the clinical care of the premature infant, it was linked to adverse cardiorespiratory [5,6] and intestinal [15,28,29] events. Premature infants receiving oxygen or ventilatory support were shown to be at a higher risk for such adverse effects compared to neonates on room air [3,4]. We examined this relationship by measuring markers of ATP utilization and intestinal injury following a required ROP exam. ATP utilization markers were previously linked to cardiorespiratory events [12], and it is well known that ATP is used in signaling pathways in the intestinal tract [22]. However, the relationship between ATP utilization, intestinal injury, and cardiorespiratory characteristics of the premature infant have not been examined.

Effect of the ROP Exam on Intestinal Injury

We found a significant increase in the intestinal injury marker IFABP after the ROP exam in premature infants receiving oxygen support compared to those on room air. IFABP remained elevated as early as 6 hours and up until 18 hours after the ROP exam. This finding supports other studies that premature infants receiving oxygen support have an increased risk of intestinal injury compared to premature infants that are stable in room air [30]. This intestinal injury may stem from pain, since it is well-documented that pain medications given before the ROP exam were ineffective in reducing pain scores [31]. Pain activates the sympathetic nervous system which leads to vasoconstriction of intestinal arterioles shunting blood away from the GI tract. This decreases gastrointestinal perfusion, increasing the risk for ischemia-related cell injury. Significant alterations in IFABP and ATP degradation markers in the oxygen support group may reflect greater ischemic insult due to (i) an increased allostatic load [9], (ii) pain-induced sympathetic nervous system activation and (iii) enhanced response to mydriatic eye drops, cyclopentolate and phenylephrine. Plasma levels of cyclopentolate were found to be significantly higher in neonates receiving oxygen compared to those in room air [4], which suggests a significant systemic distribution of this drug. Cyclopentolate inhibits the parasympathetic nervous system, leaving an unopposed sympathetic response to pain.

Effect of the ROP Exam on ATP Utilization Markers

We found significantly elevated hypoxanthine, xanthine, and uric acid levels in the oxygen support group compared to the room air group during the 18-24 hours after the ROP exam. This documents the time course for purine metabolism and urinary

excretion after a painful event. The reduction in hypoxanthine/creatinine 6-12 hours after the ROP exam in the oxygen support group may reflect the activation of the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) pathway, to compensate for this group's increased need for ATP. In the HGPRT pathway, hypoxanthine is converted into inosine monophosphate (IMP) by transferring the 5-phosphoribosyl group from 5-phosphoribosyl 1-pyrophosphate (PRPP) to hypoxanthine. The activation of the HGPRT pathway and the catabolism of hypoxanthine to xanthine and uric acid may be the reason for the reduction in hypoxanthine/creatinine at 6-12 hours. Elevated levels of these purines at 18-24 hours after the ROP exam suggest a sustained requirement for ATP in the oxygen group due to the pain and stress of the exam as well as due to the respiratory disease [12].

Correlation between Intestinal Injury Markers and ATP Utilization Markers

We found significant positive correlations between hypoxanthine/creatinine at 6-12 hour and IFABP/creatinine at 12-18-hour (Table 3). A significant positive correlation was also found between uric acid/creatinine and IFABP/creatinine during the 12-18 hour time interval (Table 3). Increased hypoxanthine/creatinine at 6-12 hours correlated with increased urinary IFABP at 12-18 hours, after the ROP exam, respectively. This suggests that an early increase in ATP utilization, as evidenced by an increase in urinary hypoxanthine/creatinine at 0-6 and 6-12 hours after the exam may be used to predict intestinal injury. Significant positive correlation between the 18-24 hour uric acid/creatinine and IFABP levels supports the suggested relationship between ATP utilization, intestinal permeability and epithelial cell lining degradation [23].

Conclusion

Our results suggest that premature infants receiving oxygen support may exhibit increased ATP breakdown and an increased risk for intestinal injury. ATP breakdown products play multiple roles in signaling pathways, especially in the intestine. Because of the versatility of ATP and its breakdown products, elucidating the specific pathways that ATP catabolism takes may provide insight on how energy deficiency is affecting the premature infant at the cellular level. The correlation between urinary ATP breakdown markers and IFABP provides a glimpse at the crosstalk between energy breakdown, pain and stress, intestinal injury and respiratory morbidities. An understanding of this relationship may be necessary to help optimize nutritional support for premature infants and prevent intestinal injury. Limitations of this study include a low sample size and a potential bias due to lack of randomization of study subjects. Further research is necessary to determine the effect of gestational age and respiratory distress on ATP metabolism in the context of the ROP exam.

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CHAPTER FOUR

DIFFERENTIAL EFECTS OF THE RETINOPATHY OF PREMATURITY EXAM ON THE PHYSIOLOGY OF PREMATURE INFANTS

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Abstract

Objective: To compare the differential effects of the retinopathy of prematurity (ROP) examination on the physiology of premature infants with and without oxygen support.

Study Design: We collected data from 42 premature infants (room air = 19, oxygen support = 23) and compared physiological metrics such as heart rate (HR), systemic peripheral oxygenation (SpO_2), mesenteric tissue oxygenation (StO_2), and fractional tissue oxygen extraction (FTOE) and clinical events (apnea, desaturation, bradycardia, and gastric residuals).

Results: We found significant differences between groups in HR during and briefly after the exam, and in StO_2, during eye drops administration, eye exam, and up to 8 minutes after the exam. SpO_2 was significantly different between the group in all time points, while FTOE was not. Gastric residuals were higher after the exam in oxygen support infants, compared to baseline.

Conclusion: Premature infants on oxygen support may be at a higher risk of adverse effects in response to the ROP exam.

Introduction

The retinopathy of prematurity (ROP) eye exam is a necessary and routine examination in the NICU that detects and identifies infants at risk for blindness due to retinal detachment [1]. Before the ROP exam can be performed, the pupils must be dilated with mydriatic eye drops (Cyclomydril: cyclopentolate 0.2% and phenylephrine 1%), administered in three total doses: two drops in each eye every five minutes, one hour before the examination. To reduce pain, a 0.2 mL dose of Sweet-Ease (24% sucrose) is orally administered to the neonate's buccal mucosa via syringe. One minute before the eye exam, topical anesthetic eye drops (proparacaine HCl 0.5%) are administered into each eye. The eyes are examined one at a time using a binocular indirect ophthalmoscope. A speculum is used to keep the eyelids open while a depressor is used to manipulate the globe into various positions as required by the ophthalmologist. Although these eye examinations are the standard of care for the detection of ROP, there are aspects of the ROP exam that could result in adverse effects in neonates.

Despite the administration of oral sucrose and topical anesthetic, studies show that the ROP exam resulted in signs of pain and stress [2–4], as well as increased apnea events [5,6], and increased incidence of delayed gastric emptying [7]. The characteristics of these negative after-effects are currently unknown and only an undetermined subset of the premature infant population present negative complications. The onset, extent, and duration of complications are unclear.

The mydriatic eye drop used at the study site before the ROP exam is composed of cyclopentolate and phenylephrine, both of which dilate the pupils to allow the ophthalmologist to clearly assess ROP progression. Adverse effects, including pain, have

been linked to the use of mydriatic eye drops [8]. Transient ileus [9], or a temporary arrest of intestinal peristalsis, and necrotizing enterocolitis [10] have also been observed in neonates that have received mydriatic eye drops. Furthermore, mydriatic eye drops, specifically, cyclopentolate, have varying clearance rates in premature infants [5]. An hour after mydriatic eye drop administration, plasma cyclopentolate concentration was shown to be significantly higher in premature infants receiving oxygen therapy compared to premature infants without exogenous oxygen support [5], which may explain some of the side effects of the ROP exam.

The primary purpose of this prospective pilot study was to compare the physiological effects of the ROP exam on two groups of subjects: premature infants receiving oxygen support at least 24 hours before and at the time of their exam, and premature infants that are on spontaneous room air. While heart rate and systemic peripheral oxygenation (SpO_2) is a variable of interest in many studies, local mesenteric tissue oxygenation (StO_2) and the calculated metric fractional tissue oxygen extraction (FTOE) [11] have not yet been examined in premature infants undergoing the ROP exam. The physiological effects that we examined included longitudinal changes over time in heart rate, SpO_2, StO_2, and FTOE (i) before the exam, (ii) during mydriatic eye drop administration, (iii) during the ROP exam, (iv) eight minutes after the ROP exam, and (v) 12 hours after the ROP exam. We also documented the number of apnea and bradycardia events 12 hours before and 12 hours after the ROP exam and gastric residuals 24 hours before and 24 hours after the exam.

Subjects and Methods

We conducted a prospective study at Loma Linda University Children's Hospital to measure the physiological effects of the ROP exam in premature neonates. The Loma Linda University Children's Hospital Institutional Review Board (IRB) approved the study protocol and informed consent documents. Subjects were premature infants who (i) required the retinopathy of prematurity eye examination, (ii) met the screening recommendations of the American Academy of Pediatrics and Ophthalmology [12] and (iii) parental consent. Exclusion criteria were infants (i) scheduled for laser eye surgery on the day of the examination, (ii) with intraventricular hemorrhage ≥ grade 3 (Papile classification) [13] diagnosed by head ultrasound, (ii) receiving the following medications: morphine, fentanyl, methadone, midazolam, lorazepam, muscle relaxants, phenobarbital, phenytoin, and levetiracetam, (iv) with renal injury defined as plasma creatinine > 1.5 mg/dL and (v) with severe cyanotic congenital heart disease, severe respiratory distress and gastrointestinal dysfunction.

After consent was obtained, we collaborated with the ophthalmology team to schedule the ROP exam. The experimental procedure is described in Figure 1. Twelve hours before the ROP exam, we obtained baseline heart rate and SpO_2 using the Masimo Radical 7 Pulse Oximeter (Masimo, Irvine CA) and the CASMED FORE-SIGHT (Branfort CT) to collect StO_2. FTOE [14] was calculated using the equation below:

$$FTOE = \frac{SpO_2 - StO_2}{SpO2} * 100\%$$

where SpO_2 is the peripheral oxygen saturation obtained from the Masimo Radical 7 and StO_2 is the mesenteric oxygen saturation obtained from the CASMED FORE-SIGHT.

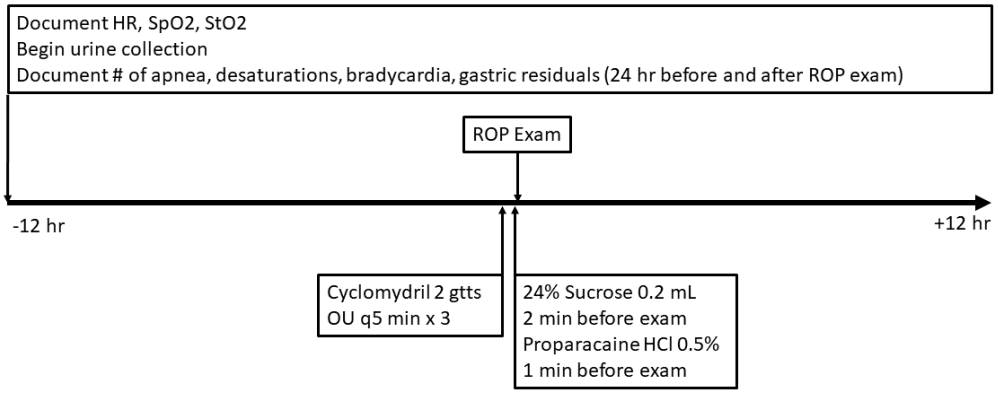

Figure 1. Flowchart of experimental procedure.

The physiological variables were grouped in different time periods defined as follows: the baseline value is the mean value of every recorded measurement 12 hours before the ROP exam. All measurements beside the baseline were averaged into one-minute intervals. The "Eyedrop" epoch values represent the three doses of mydriatic eye drops given every five minutes, totaling a 15-minute period. The "Procedure" epoch values reflect the measurements during the ROP exam. We worked with the ophthalmology team to standardize the ROP exam to a duration of four minutes, and the values were grouped in one-minute averages. The "Post" epoch values represent the eight-minute study period after the ROP exam. See statistical analysis below for further information.

We prospectively reviewed the medical record 12 hours before and after the ROP exam for apnea, desaturation, and bradycardia events. Apnea was defined as a cessation of respiration for 20 seconds. Desaturations were defined as followed: mild (85–89% SpO_2), moderate (81–84% SpO_2), and severe (≤80% SpO_2) [15]. Bradycardia was defined as a heart rate less than 80 beats per minute

Statistical Analysis

RStudio version 1.1.383 (https://www.rstudio.com/), a user-friendly interface to the *R statistical language* (https://www.r-project.org/) was used for all imputations and analyses. For the short-term statistical analysis (eye drops and up to eight minutes post-exam), the method is as follows. Missing values for this time series data were imputed using the *Amelia* package (https://cran.r-project.org/web/packages/Amelia/Amelia.pdf) to account for the correlation across time points with appropriate boundary restrictions for each outcome, ensuring percentages stay within the range of 0 – 100 and pulse rate values remain above 0. Some subjects had no data available for certain outcomes—these were not used in the imputation step and subsequent analyses.

Data was averaged into one-minute intervals. Each eye drop period consisted of 5 minutes, the eye exam procedure period consisted of 4 minutes, and up to 8 minutes of post-procedure time was used. A repeated measures analysis of variance (RMANOVA) approach with random intercepts was used to detect the timing of changes in SpO_2, StO_2, HR, and FTOE values relative to baseline and between the two room air groups. Time was modelled as a categorical variable to allow for post-hoc tests of individual time points with baseline time. A threshold alpha level of 0.05 was used throughout the analysis.

Results

Subject Demographics and Baseline SpO_2, StO_2, HR, and FTOE

Subjects were grouped based on their oxygen requirement (FiO_2). Subjects breathing spontaneously on room air had significantly higher weight and greater gestational age at the time of birth and at the time of the exam (Table 1).

Table 1. Subject Demographics. Mean ± SD. A zone of 2.5 indicates that one eye had a zone of 2 and the other eye had a zone of 3. [#]Independent samples t-test [+]Chi-square

	On Room Air (n=19)	**Receiving Oxygen (n=23)**	**P value[#]**
Birthweight (g)	1220 ± 426	886 ± 279	0.004
Weight @ Exam time	2352 ± 724	1804 ± 575	0.009
Gestational Age (wks)	29.5 ± 2.2	27.1 ± 2.1	0.001
Gestational Age @ Exam time	36.5 ± 2.6	34.6 ± 2.8	0.030
Apgar-1 min	4.8 ± 1.9	3.9 ± 2.5	0.157
Apgar-5 min	7.1 ± 1.2	6.5 ± 2.1	0.300
Gender	Female: 9 Male: 10	Female: 8 Male: 15	0.408[+]
SNAPPE-II	15.1 ± 10.9	22.9 ± 15.2	0.06
Ethnicity	African-American: 6 Asian: 2 Hispanic/Latino: 1 Caucasian: 6 Other/More than one race: 0 Unknown: 2	African-American: 1 Asian: 1 Hispanic/Latino: 2 Caucasian: 14 Other/More than one race: 4 Unknown: 1	0.29[+]
ROP Exam	2.2 ± 1.7	2.3 ± 1.6	0.909
ROP Stage	0 : 12 1 : 0 2 : 1 Mature : 1	0 : 9 1: 0 2: 8 Mature: 1	.065
ROP Zone	0 : 0 2: 9 2.5 : 0 3 : 4 Mature : 1	0 : 4 2 : 10 2.5 : 1 3 : 2 Mature : 1	.258

As expected, FiO_2 requirement was significantly different 12 hours before, during and 12 hours after the examination (Table 2). There were no significant differences between the groups with regards to Apgar scores, number of ROP exams, ROP stage and zone, gender and ethnicity**.** Mean Score for Neonatal Acute Physiology with Perinatal Extension-II (SNAPPE-II) [16] scores of the oxygen support group was higher than the room air group

(22.9 ± 15.2 vs. 15.1 ± 10.9, respectively), but the difference was not statistically significant (P = 0.06). We found no statistical or clinically significant difference in baseline heart rate, SpO_2, StO_2 and FTOE between the two groups.

Table 2. Oxygen support and FiO_2 information. CPAP: continuous positive airway pressure, HFNC: high flow nasal cannula, NAVA: neurally adjusted ventilatory assist, NCPAP: nasal continuous positive airway pressure, NIMV: nasal intermittent mandatory ventilation, NIPPV: noninvasive positive pressure ventilation. $^{+}$Chi-square

	On Room Air (n=19)	**Receiving Oxygen (n=23)**	**P value**
Mode of oxygen support	Spontaneous room air = 19	CPAP: 4 HFNC:11 Nasal cannula: 3 NAVA: 2 NCPAP: 1 NIMV: 1 NIPPV: 1	$< 0.001^{+}$
Time between the third eye drop and ROP exam (minutes)	67 ± 48	70 ± 27	0.775
FiO_2 Min 24 hours before exam (%)	21 ± 0	24.0 ± 3.7	0.001
FiO_2 Max 24 hours before exam (%)	21 ± 0	30.4 ± 9.6	$< 0.001^{+}$
FiO_2 During Exam	21 ± 0	26.3 ± 6.0	< 0.001
FiO_2 Min 24 hours after exam (%)	21 ± 0	23.3. ± 3.9	0.017
FiO_2 Max 24 hours after exam (%)	21 ± 0	33.6 ± 11.7	$< 0.001^{+}$

Effect of Eye Drops and ROP Exam on Heart Rate

When comparing groups, we found no statistically significant difference in heart rate in response to the first two eyedrops (Figure 2A). However, we found that in neonates receiving oxygen, heart rate significantly increased above baseline one minute after the third eye drop administration, from baseline values of 155.3 ± 16 to 163.1 ± 17,

a 5% increase ($P < 0.01$). This increase in heart rate was sustained throughout the last 4 minutes after the third eye drop. We did not find any sustained change in heart rate compared to baseline in neonates breathing room air.

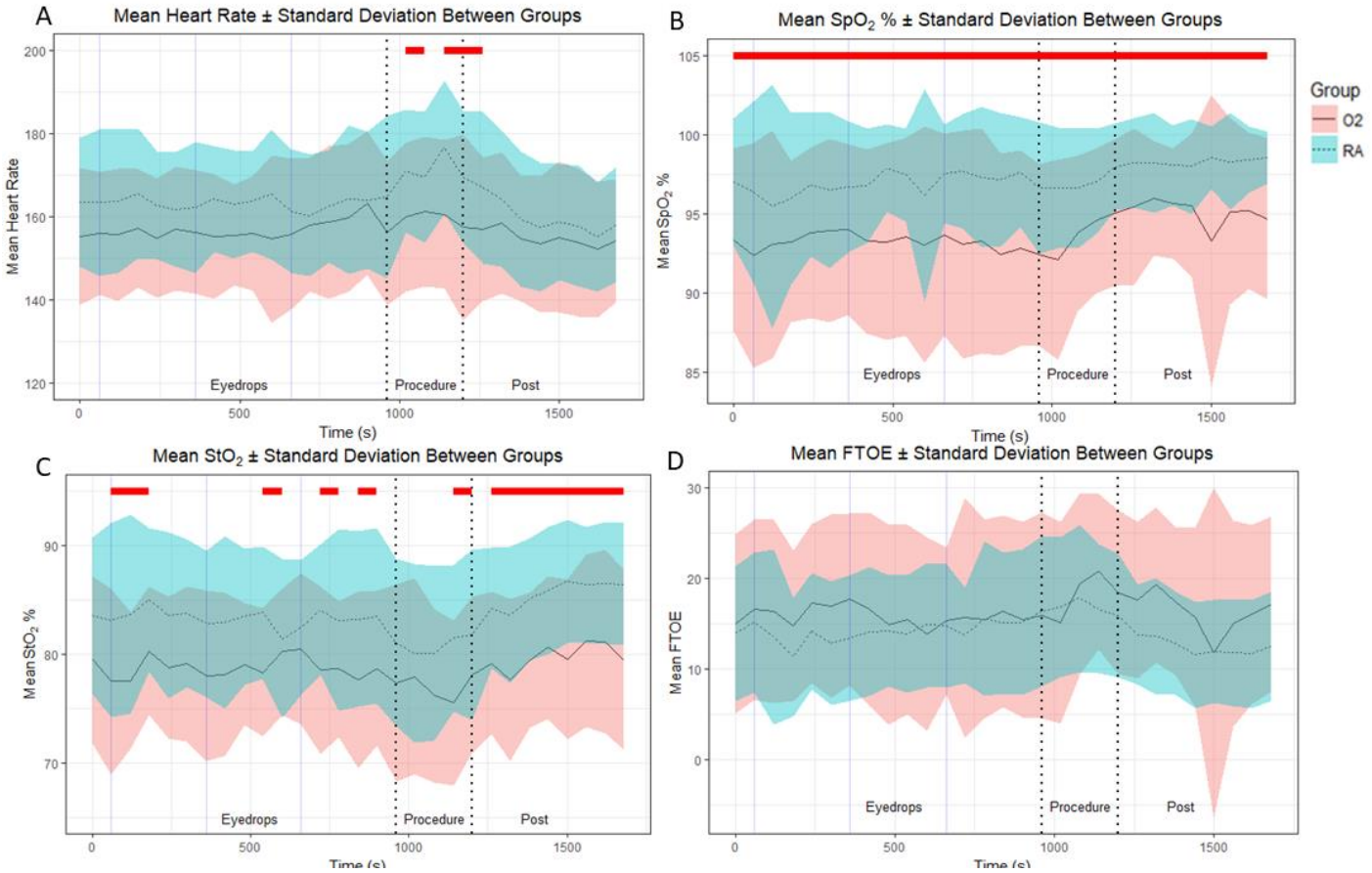

Figure 2: Mean and Standard Deviations for A) Heart Rate B) SpO_2% C) mesenteric StO_2% and D) FTOE. Red lines indicate time points with significant differences between groups. Blue lines indicate start of eye drop administration.

During the ROP exam, we found that heart rate significantly increased in neonates on room air, from baseline values of 163 ± 15 to 177 ± 16 bpm, an 8.5% increase ($P < 0.01$). In neonates requiring oxygen support, heart rate increased from 155 ± 16 to 161 ± 18 bpm, a 3.2% increase compared to baseline. When comparing groups, we observed significant differences in heart rate during the 2nd and 4th minute of the eye exam, with the difference persisting up to the first minute post eye exam. During the second minute of the exam, subjects on room air had a significantly higher mean heart rate of 171 ± 15 per minute compared to the group receiving oxygen support, whose mean heart rate was 160 ± 18. As the eye exam procedure continued to its fourth and final minute, mean heart rate continued to increase in neonates on room air, while heart rate plateaued in neonates receiving oxygen support. Heart rate returned to baseline within two minutes of completion of eye exam. There was no significant difference in heart rate between groups during the 12 hours post ROP exam.

Effect of Eye Drops and ROP Exam on SpO_2

As shown in Figure 2B, we found a statistical but not a clinically significant difference in baseline SpO_2 values between the two groups. For neonates receiving oxygen, baseline SpO_2 was 93% ± 6; for the room air group, baseline oxygen saturation was 97% ± 4.0. There were also significant differences in SpO_2 between the groups during all three eye drop administrations, with the oxygen group having an average range of SpO_2 of 92% ± 7 to 94% ± 5, and the room air group having an average range of SpO_2 of 95% ± 8 to 98% ± 3. There was also a significant difference in SpO_2 between groups during the eye exam. The mean SpO_2 of neonates in the oxygen group ranged from 92%

± 6 to 95% ± 5 while the room air group stayed at an average SpO_2 of 97% ± 4. The difference between groups persisted until the end of our analysis 12 hours after the exam ($P < 0.005$).

Effect of Eye Drops and ROP Exam on Mesenteric StO_2

As shown on Figure 2C, we observed no statistically significant group differences in baseline StO_2. However, we noted that within two minutes after the third eye drop, StO_2 began to decrease significantly in neonates in the oxygen support group. This reduction in StO_2 was sustained throughout the four minutes after the eye drop and throughout the entire eye exam procedure, with StO_2 decreasing to 75% ± 8 in neonates in the oxygen group compared to 81% ± 7 in the room air group ($P < 0.01$). Group differences remained statistically different during the eight minutes after the eye exam, with mean values ranging from 78% ± 7 to 81% ± 8 in the oxygen group and 82% ± 8 to 86 ± 5 in the room air group. No significant differences in StO_2 were observed during the 12-hour period post-exam.

Effect of Eye Drops and ROP Exam on FTOE

As shown in Figure 2D, we found no significant group differences in FTOE at baseline. There were also no significant differences in FTOE during all eye drop administrations. FTOE values ranged from 14 ± 11 to 18 ± 10 in the oxygen support group and 11 ± 7 to 16 ± 9 in the room air group. There were also no significant differences between groups during the eye exam. The values ranged from 15 ± 11 to 21 ± 9 in the oxygen group, and the room air group had values ranging from 16 ± 8 to 18 ± 8.

There were no significant differences between groups after the eye exam, both in the short-term and the long-term analyses.

Effect of Eye Drops and ROP Exam on Apnea, Bradycardia, Oxygen Desaturation, and Gastric Residuals

Prospective medical record review twelve hours before and after the ROP exam showed significant differences between groups in the number of events of apnea ($p < 0.05$) and bradycardia ($p < 0.005$). Histograms of the increased apnea and bradycardia events in neonates in the oxygen group compared to neonates on spontaneous room air are on Figure 3A and 3B, respectively.

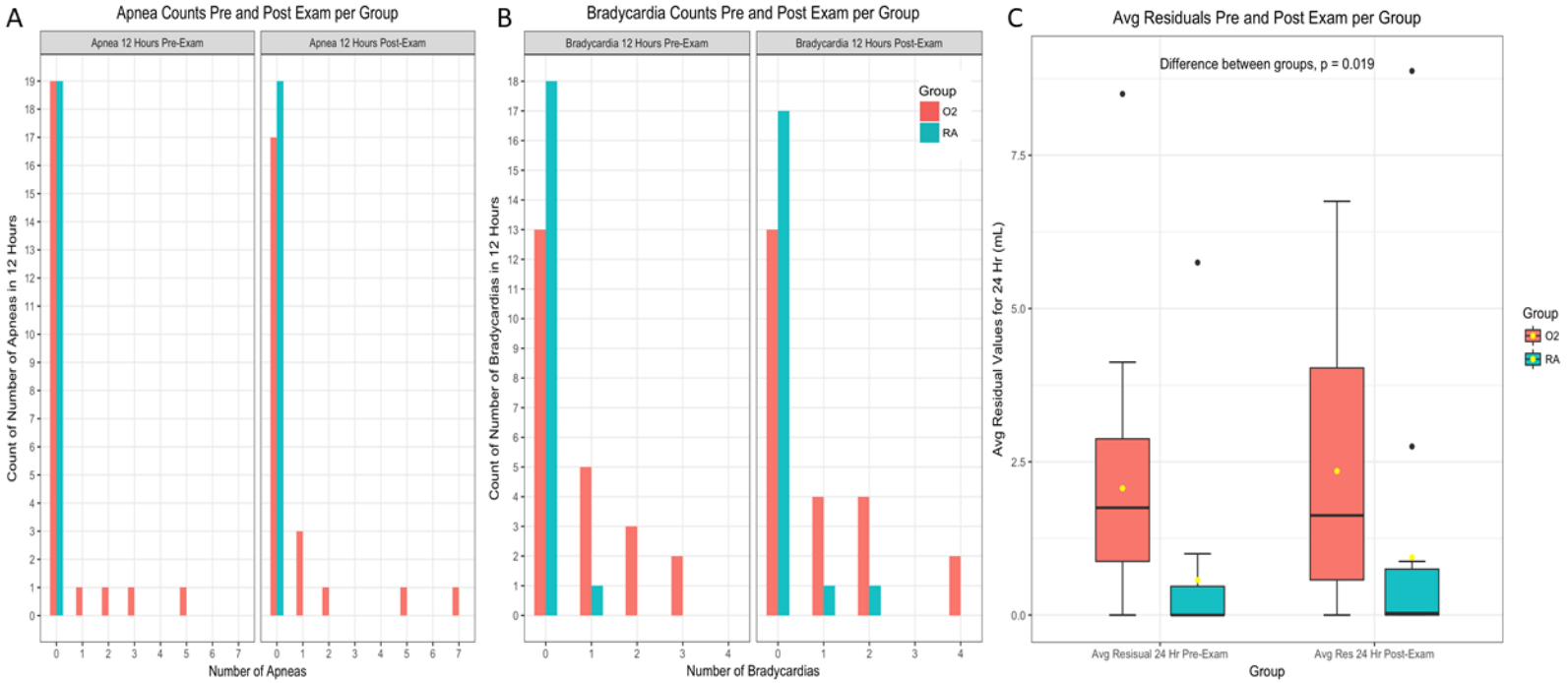

Figure 3: Histogram of A) Apnea and B) Bradycardia Events 12 Hours Before and After the ROP Exam. C) Bar plots of average gastric residuals of both groups 24 Hours Before and After the ROP Exam.

We also reviewed the medical record in the same time span for clinically relevant oxygen desaturations stratified into three different categories: mild (85–89% SpO_2), moderate (81–84% SpO_2), and severe (≤80% SpO_2) [15]. The differences between groups were statistically significant for all categories (mild: $P < 0.005$, moderate: $P < 0.05$, severe: $P < 0.005$). The ranges of clinically recorded desaturations in premature infants receiving oxygen support were as follows: before the ROP exam, mild (0 to 6), moderate (0 to 5), severe (0 to 6) and after the ROP exam, mild (0 to 9), moderate (0 to 3), and severe (0 to 10). For premature infants in the room air group: before the ROP exam, mild (0 to 1), moderate (0 to 0), severe (0 to 0) and after the ROP exam, mild (0 to 1), moderate (0 to 1), and severe (0 to 1). Histograms of desaturations are included in Figure 4A (mild), Figure 4B (moderate), and Figure 4C (severe)

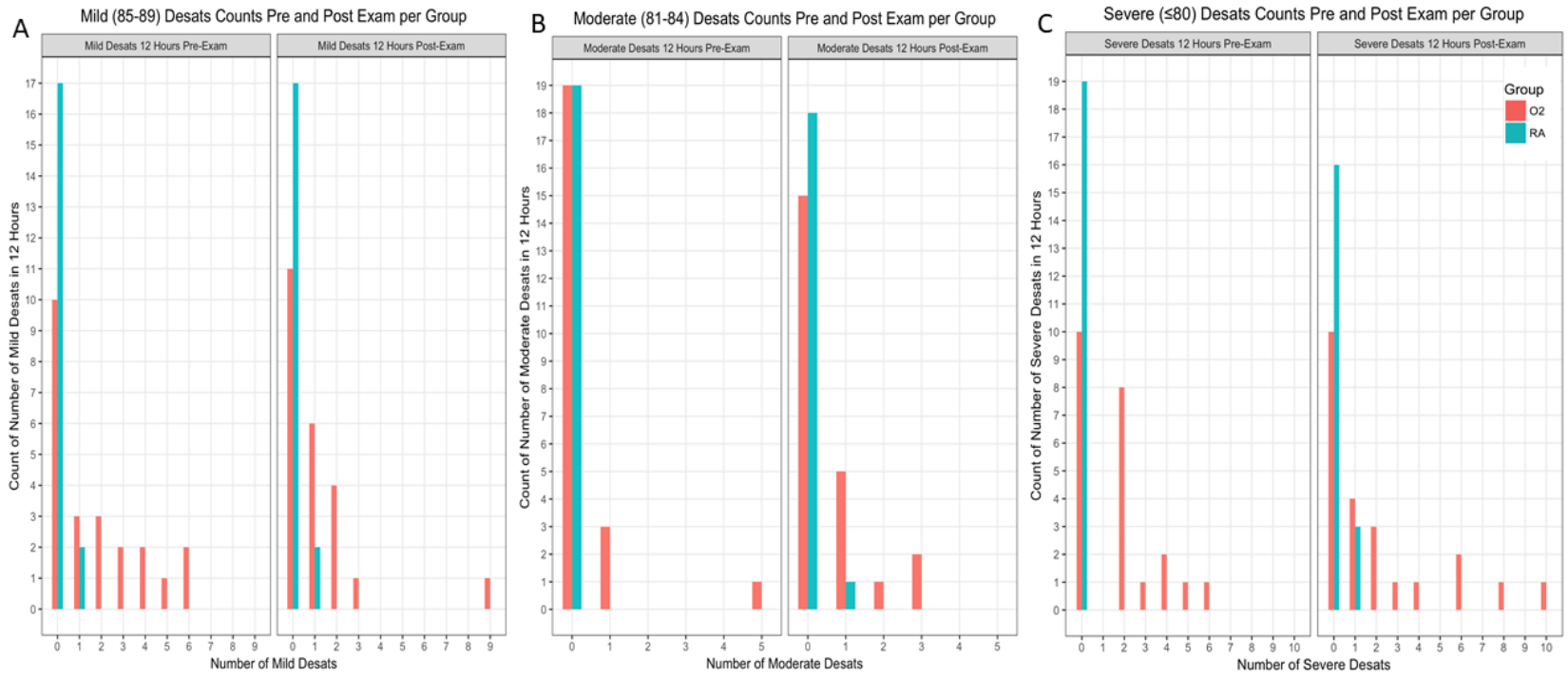

Figure 4: Histogram of A) Mild Desaturation Events (85-89%), B) Moderate Desaturation Events (81-84%), and C) Severe Desaturation Events (≤80%) 12 Hours Before and After the ROP Exam

The volume of gastric residuals was also prospectively reviewed for up to 24 hours before and after the ROP exam. The average residual for subjects before the ROP exam was 0.57 mL for room air subjects and 2.07 mL for oxygen support subjects. After the ROP exam, the average residual for room air subjects increased to 0.94 mL and oxygen support subjects increased to 2.35 mL. The difference between groups was statistically significant ($P < 0.05$); however, the clinical significance is unclear. See Figure 3C for a box plot of the gastric residual data.

Discussion

It is well documented that the ROP eye examination procedure, which includes administration of topical mydriatics, can result in signs of pain and stress [2–4] increased apnea events [5,6] and increased incidence of delayed gastric emptying [7]. However, it is unclear which groups of infants will present with these adverse effects and which component of the eye exam alters normal physiology. To begin to clarify the characteristics of patients who may respond poorly to this exam and to understand the effects of each component of the eye exam, we examined physiological (heart rate, SpO_2, StO_2) and clinical (apnea, desaturation, bradycardia, gastric residuals) variables before, during and after a single ROP exam procedure in 42 premature neonates. Nineteen of the neonates were on spontaneous room air before, during and after the exam, while 23 neonates received exogenous oxygen support during the same study period.

Effect of Eye Drops on Heart Rate

We found the heart rate response to be significantly different between neonates on oxygen compared to neonates on room air. Heart rate increased significantly after the third eye drop in neonates on oxygen while heart rate remained stable during that same time period in neonates on room air, compared to baseline. This increase in heart rate in neonates on oxygen may be due to higher plasma levels of the muscarinic antagonist, cyclopentolate, shown by Mitchell et al. [5] to be higher in neonates on oxygen, suggesting increased systemic absorption of the drug in this population. Predictably, higher circulating levels of a drug that inhibits the vagus, administered with an adrenergic agonist, will cause an increase in heart rate. The relationship between oxygen support and higher plasma cyclopentolate requires further investigation, which may include a prolonged half-life and differences in drug metabolism and clearance. The data also suggest the need to re-evaluate the dose of cyclopentolate given to neonates receiving oxygen support.

Effect of ROP Exam on Heart Rate

In response to the ROP eye exam, we found that neonates receiving oxygen support had a minimal increase in heart rate (3.2% increase) compared to neonates on room air, whose heart rate increased by 8.5% compared to baseline. The increase in heart rate in the room air group may be a delayed response to cyclopentolate, although Mitchell et al, found that plasma cyclopentolate levels were significantly lower in neonates on room air [5]. Alternatively, the larger increase in heart rate in neonates on room air may be in response to scleral depression, especially if the ROP was in a zone that was difficult

to visualize. However, we did not find any significant difference in ROP stage or zone between the two groups. The larger increase in heart rate in neonates on room air may be a nociceptive response to the exam, since the efficacy of proparacaine is not well-established in premature infants. Marsh et al. noted that although pain scores were lower in neonates who received proparacaine, 68% of these neonates still had a pain score of more than 10, which suggest moderate pain [17]. Although the eye examination involves manipulation of the conjunctival fornix and touching the cornea is avoided, it is well-documented that the cornea is 300–600 times more sensitive to pain than the skin [18]. It is unclear why the heart rate response was muted in the oxygen group, especially because of the well-documented correlation between chemoreceptors and autonomic activity [19]. This diminished response may be indicative of this group's limited ability to respond to the added allostatic load [20,21], We did not observe a bradycardic response during speculum application, which precluded activation of the oculocardiac reflex. Heart rate returned to baseline soon after the eye exam in the oxygen group but dipped below baseline in the room air group.

Effect of Eye Drops on StO_2

We found significant reductions in mesenteric StO_2 compared to baseline in response to the third eye drop administration. The reduction in StO_2 is greater in the oxygen group. As described earlier, elevated levels of cyclopentolate have been documented in neonates receiving oxygen support. Administering the mydriatic drug Cyclomydril, which inhibits the parasympathetic nervous system (cyclopentolate) and enhances the sympathetic nervous system (phenylephrine) will reduce gastric and

duodenal motility [19] and delay gastric emptying [7]. Mesenteric StO_2 was shown to directly correlate with peristaltic motility as measured by transabdominal ultrasonography [22]. Specifically, high StO_2 values were associated with normal or hyperactive intestinal motility, and lower StO_2 values were associated with slower intestinal motility.

Effect of ROP Exam on Mesenteric StO_2

StO_2 continued to decrease during the exam in both groups, with the reduction greatest in neonates in the oxygen group. In both groups, StO_2 decreased below baseline during the exam. This finding may reflect increased sympathetic nervous system activation, due to the pain and stress related to the exam, shown to still be present despite proparacaine administration [17]. Systematic reviews examining the analgesic effect of proparacaine report inconsistent results [23–25]. This suggests that proparacaine only blocks mechano-nociception within the eye and not other types of nociceptors such as polymodal nociceptors (which respond to heat, irritation, and inflammation) or cold thermo-nociceptors (which respond to moderate temperature changes) [26]. Furthermore, older neonates of the room air group that have a larger and more rigid sclera may require a greater magnitude of scleral depression, causing additional discomfort due to increased intraocular pressure [27].

Though the sources of nociception related to the eye exam are currently unknown, the presence of pain is reflected in the continuous stepwise increase in heart rate (sinoatrial α-adrenergic activation) and a significant reduction in StO_2 (α-adrenergic activation) during the exam. The reduction in StO_2, which may reflect reduced gastric and

intestinal motility during the exam [22], was significantly greater in the oxygen support group. This finding further highlights the physiologic vulnerability of this group of neonates.

Effect of Eye Drops and Eye Exam on SpO_2

Despite the significant effects of eye drops and ROP exam on heart rate and StO_2, we found no statistical or clinically significant difference in SpO_2 between the oxygen group and the room air group. We also did not observe any statistical or clinically significant change in SpO_2 during and after eye drop administration and ROP eye exam compared to baseline. SpO_2 remained above 90% in both groups before, during and after the procedure. This finding highlights the reduced utility of peripheral oxygen saturation monitoring in determining the regional effects of the ROP eye exam.

Effect of Eye Drops and ROP Exam on Apnea, Bradycardia and Gastric Residuals

We found that neonates on oxygen had significantly higher episodes of apnea and bradycardia, and significantly higher volume of gastric residuals, compared to neonates on spontaneous room air. It is unclear which specific component of the procedure resulted in these findings. The change in gastric residuals may be due to the mydriatic drugs and the ROP exam, which jointly enhanced sympathetic nervous system activity to the intestines. Increased episodes of bradycardia during the 12 hours after the exam may be due alterations in neurotransmitter release, since neonates are documented to have lower baseline concentration of epinephrine in the adrenal gland [28]. Neonates on oxygen are specifically at higher risk for bradycardia, since regulation of neurotransmitter

exocytosis involves adenosine triphosphate (ATP) [29], an energy source that may be limited in neonates with underlying respiratory pathology that just experienced a painful event [20]. Energy deficit may also explain the increase in apnea episodes in neonates on oxygen, since ATP is required for respiratory muscle contraction and relaxation as well as purinergic activity in the respiratory centers of the brain [19,30]

Limitations

The major limitation of this study is the small sample size. Future studies are needed, incorporating larger sample sizes that follow patients through multiple eye examinations. Another limitation is the intermittent loss of signal in near infrared spectroscopy (NIRS) as well as some sporadic movement artifacts appearing during the ROP exam itself. This limitation was addressed through data imputation, as described under "Statistical Analysis." Lastly, this study relied on the medical record for documentation of apnea, bradycardia, and oxygen desaturations events, as well as gastric residuals volume. Although nurses are trained on how to clearly identify and document these variables, the number of nurses involved in the study may increase the variability of the data.

Conclusion

Our findings suggest that neonates on oxygen undergoing the ROP exam may be at higher risk for adverse outcomes compared to neonates on room air. This may be due to the systemic effects of Cyclomydril as well as scleral manipulation. Future studies are needed to determine the appropriate plan of care for this group of infants, with a specific

focus on the dose of mydriatics and the need for removal of residual eye drops to prevent systemic absorption. The timing and reinstitution of oral feeding must be evaluated on a case-by-case basis and must be balanced with the neonate's nutritional needs. Additionally, risk factors that can increase cardiorespiratory events need to be further qualified in a study with a larger sample size. These factors include the effect of serial exams and the degree of difficulty of the ROP exam. In addition, interventions that may limit the occurrence of adverse effects, such as prevention of pain, positioning, sensory stimulation [31], and kangaroo mother care [32–34] require further investigation.

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CHAPTER FIVE

CASE-STUDY OF NONLINEAR SYSTEMS ANALYSIS IN PREMATURE INFANTS AFTER THE ROP EXAM

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Abstract

Nonlinear systems analysis has only recently begun to be applied in premature infant clinical research. Most studies have been done on the brain and the heart using electroencephalography (EEG) and electrocardiography (ECG), respectively. This longitudinal case study applies entropy analysis methods, power spectral density analysis, and Poincaré plot analysis to physiological biomedical signals obtained via pulse oximetry and near infrared spectroscopy at the bedside of two premature neonates. Our findings showcase the potential of these nonlinear metrics to further understand the crosstalk between organ systems and the autonomic nervous system, consequently improving the ability of the clinical staff to make informed decisions.

Background

Traditional linear systems analysis is the basis of many discoveries in clinical research. Standard metrics such as mean, median, and range all play a role in the understanding of data [1]. These metrics are part of the reductionist idea of simplifying systems into their major components to understand them individually, and, eventually, as an interrelated whole. However, the reductionist application of linear systems analysis to the human body leaves much to be desired. By looking only at the linear components of signals and features of the human body, we are missing much of the larger picture since the human body largely behaves as a nonlinear system [2]. Examples of these nonlinear systems include the cardiovascular, respiratory, and gastrointestinal systems. These systems all have complex mechanisms that are involved in constant feedback, autoregulation, and crosstalk between systems. These complex mechanisms are in constant communication with each other to reach a common goal: survival via adaptation to changes in environment or state.

Nonlinear systems analysis has only recently picked up traction and begun to be applied in premature infant clinical research. Nonlinear systems analysis provides an important suite of tools to study in premature infants because they exhibit immature and underdeveloped regulatory systems that increases the morbidity and mortality risks. Premature infants exhibit altered cardiovascular regulation, which increases hypoxia and hemorrhage risk [3]. Compared to term infants, premature infants have impaired autonomic cardiovascular control as shown by less parasympathetic control of the heart along with greater respiratory-mediated changes and lower sympathetic control of blood pressure [4]. Premature infants also have immature respiratory control that is exacerbated

by co-morbidities and presents itself most commonly as apnea of prematurity [5]. Furthermore, gastrointestinal disorders common in the premature infant may be due to a combination of immature development of swallowing and feeding mechanisms and altered autonomic control of the gastrointestinal tract [6–8]. Thus, applying nonlinear systems analyses to the premature infant is highly clinically relevant and may provide predictive value for assessing infant outcome.

The main nonlinear analysis techniques used in this case study is the use of entropy quantifications, power spectral density analysis, and Poincaré plots. Each of these techniques have been previously applied in clinical research in various fields. The most prominent datasets that have been analyzed with these nonlinear methods are electrocardiography (ECG) and electroencephalography (EEG). Though these nonlinear methods have been applied to cerebral tissue oxygenation in the premature infant [9], they have not been applied to mesenteric tissue oxygenation.

Entropy Quantifications

The three nonlinear entropy metrics used are sample entropy (SampEn), Shannon entropy (ShanEn), and mutual information (MI). Sample entropy (SampEn) is a modification of approximate entropy that was originally developed and promoted by Steve Pincus as a method of assessing cardiac health [10]. It is used for the assessment of the regularity and complexity of physiological time-series signals. Suppose two time series exists:

> Series 1: (0, 5, 10, 15, 20, 0, 5, 10, 15, 20,…) which has a pattern of multiples of 5 and repeats at 20.

Series 2: (0, 5, 20, 15, 5, 0, 10, 10, 10, 20,…) for which each point has an equal chance of selecting either 0, 5, 10, 15, or 20.

Linear analysis metrics will not be able to distinguish between these two series. However, because Series 1 is more regular and less complex compared to Series 2, it will have lower SampEn than Series 2.

Shannon entropy was developed by Claude Shannon in 1948 [11]. Shannon entropy is defined by the equation

$$H(X) = -\sum_{i=0}^{N-1} p_i log_b p_i$$

here p_i is the probability of the number i appearing in the stream of the time series and b is the log base used. In other words, it is a way to quantify the amount of information stored within a variable by giving it a "measure of uncertainty." A basic example would be to examine a coin toss and a die throw. A coin toss has two outcomes and would have lower Shannon entropy than a die that has six possible outcomes. Shannon entropy can be considered as an analog to variance.

Mutual information is a quantification of the mutual dependence between two datasets. High mutual information indicates a large reduction in uncertainty, low mutual information indicates a low reduction in uncertainty, and zero mutual information between two variables means that they are independent. Normalization of the mutual information is calculated and returns a value between 0 to 1. The normalized mutual information becomes analogous to the Pearson correlation coefficient [12].

Power Spectral Density

Conversion of time series data into the frequency domain allows for the visualization and quantification of which specific frequencies are the most prominent within a time epoch [13]. We encounter many of these types of signals in everyday life, such as the pitches within a music, the wavelength of your favorite radio station, and our ability to only see specific wavelengths of visible light. Conversion of these signals into the frequency domain can tell us which pitch was the most prominent within a song, which wavelength can we tune into to have the clearest signal of the radio station, or which color of light is the most abundant within the daytime. The application of power spectrum anaylsis is widespread in clinical research, especially in cardiology. Power spectral density analysis of heart rate variability is capable of the early detection of dysregulation of cardiac control between the sympathetic nervous system and the vagus [14]. This method and subsequent findings further clarify the understanding of morbidities that are correlated with the dysregulation of the autonomic nervous system, such as the association between heart rate variability, insulin resistance, and the metabolic syndrome [15].

Power spectral density analysis is performed by the Fast Fourier Transform [16]. This method allows us to evaluate the relative power (or amplitude) of all the frequencies within a time series. The result is a transformed data-set with frequency as the new independent variable and power as the new dependent variable. In this case, power is described in decibels for ease of visualization.

Poincaré Plots

Poincaré plot analysis is a method rooted in chaos theory and assumes that the human body is a chaotic system. Chaos behavior is defined by several properties. Chaos is deterministic, sensitive to initial conditions, a sense of organization and order, exhibits self-similarity and recurrence properties, strange attractors, and constrained values [2,17]. Determinism within a chaotic system is the idea that chaotic behavior can be described by mathematical rules and equations. The sensitivity to initial conditions is more commonly known as the "butterfly effect" and described the idea that a small change in the initial conditions of a chaotic system may have a large effect on the system, given enough time. Chaotic systems tend to be organized and contain an emerging pattern, which is contrasted by true randomness. Self-similarity and recurrence is the concept that a part of an object is similar to its whole. Biological examples include similarities between bronchiole tubes and alveoli, and villi and microvilli within the intestines. Strange attractors are stable states that chaotic systems tend to gravitate towards, especially in response to a disturbance to the system. It is said that R-R interval oscillations exhibit a strange attractor pattern due to their lack of periodicity and nonlinear temporal relationship with cardiac autonomic control [18]. Constrained values within a chaotic system describes the limits within a relatively narrow range of values over an infinite period of time, restricting the values from becoming infinitely large or small.

The Poincaré plot is a type of recurrence plot that quantifies self-similarity in a signal [19]. These plots describe how a system changes over time and can be used to visualize the variability. It is used to distinguish between systems that exhibit randomness, chaos, and periodicity. The plot is a simple scatter plot, but the x and y axis

points are shifted with a time lag. That is, a series of data y_{n+1} is plotted with points (y_1, y_2), (y_2, y_3), (y_3, y_4) and so on.

Associated with Poincaré plots are quantification metrics SD1 and SD2. A 45-degree diagonal line from the origin describes the semi-axis for long-term variability (SD2), and a line perpendicular to that line describes the semi-axis for short-term variability (SD1). SD1 and SD2 is calculated from the equation

$$SD = \sqrt{\frac{1}{n}\sum_{i=1`}^{n}(d_i)^2}$$

where d_i is the distance between Poincaré plot points along either semi-axis [20]. An ellipse is fitted and superimposed onto the plot for better visualization of SD1 and SD2. The ratio of SD1/SD2 is also calculated as a measure of randomness [19].

The aim of this study is to analyze long-term measurements of heart rate (HR), mesenteric tissue oxygenation saturation (StO_2), peripheral capillary oxygen saturation (SpO_2), and fractional tissue oxygenation extraction (FTOE) in preterm infants using data analysis techniques for nonlinear dynamics systems to investigate the characteristics and interdependence between systemic and local hemodynamic fluctuations and crosstalk. A secondary aim of this study is to longitudinally extract nonlinear quantifiable variables from power spectral densities and Poincaré plots to visualize gut blood flow dynamics in StO_2.

Methods

Data collection was described in **Chapter 4**. Description of the retinopathy of prematurity (ROP) exam and corresponding mydriatic eye drops were also described.

Twelve hours before the ROP exam, we obtained baseline heart rate and SpO_2 using the Masimo Radical 7 Pulse Oximeter (Masimo, Irvine CA) and the CASMED FORE-SIGHT (Branfort CT) to collect StO_2. FTOE was calculated using the formula:

$$FTOE = \frac{SpO_2 - StO_2}{SpO2} * 100\%$$

where SpO_2 is the peripheral oxygen saturation obtained from the Masimo Radical 7 and StO_2 is the mesenteric oxygen saturation obtained from the CASMED FORE-SIGHT. The NIRS probe was placed on the left lower abdominal quadrant as per manufacturer recommendation. The sample rate of data acquisition was 0.5 Hz.

A total of 48 subjects were enrolled. Two neonates with at least six hours of sufficient data after the eye exam were selected for the present analysis.

Table 1. Description of the case study sample. GA = gestational age.

Characteristics	**Neonate 1**	**Neonate 2**
GA at birth (weeks)	24.43	30.86
GA at exam time (weeks)	36.86	34.57
Birth weight (kg)	0.59	1.41
Weight at Exam Time	2.32	1.87
Apgar (1, 5 ,10)	1, 5, 7	4, 7, N/A
Respiration	High flow nasal cannula	Spontaneous

All code was written in Python 2.7 using the Jupyter IDE. All code used in this project is available at https://github.com/jctan05/dissertation. Excerpts of specific functions are included. Savitzky-Golay filter function was imported from SciPy (https://www.scipy.org) Entropy code was imported from pyEntropy

(https://github.com/nikdon/pyEntropy) and mutual information code was imported from scikit-learn (http://scikit-learn.org/stable/).

Linear Analysis Visualization

Data was sliced into two main time points: one hour before ROP exam eye drop administration, and one hour after the ROP exam ended. The exam itself was excluded from the visualizations, because they are included in another chapter (**Chapter 4**). SpO_2, StO_2, and FTOE were plotted together to increase the visibility of similar systems dynamics. HR and FTOE were plotted together after z-score normalization and Savitzky–Golay filter [21] smoothing (window size = 31, polynomial order = 2) to increase the visibility of the similar long-term variability of both signals while retaining information about peaks in events.

Entropy Analysis

Data was sliced into 11 discrete time epochs:

1. "PRE" : a time period of baseline (1 hour before eye drop administration)
2. "ED1" : 5 mins worth of data during 1st set of eyedrop administration
3. "ED2" : 5 mins worth of data during 2nd set of eyedrop administration
4. "ED3" : Variable amount of data during 3rd set of eyedrop administration until the eye exam started.
5. "DUR" : 4 mins worth of data during the eye exam
6. "1 H" : 1 hour worth of data up to 1 hour after the eye exam
7. "2 H" : 1 hour worth of data from hour 1 to hour 2 after the eye exam

8. “3 H” : 1 hour worth of data from hour 2 to hour 3 after the eye exam
9. “4 H” : 1 hour worth of data from hour 3 to hour 4 after eye exam
10. “5 H” : 1 hour worth of data from hour 4 to hour 5 after eye exam
11. “6 H” : 1 hour worth of data from hour 5 to hour 6 after eye exam

Sample entropy (m = 2, r = 0.2 * standard deviation of dataset), Shannon entropy, and mutual information of HR:FTOE and SpO_2:StO_2 were calculated for each time epoch.

Power Spectral Density and Poincaré Plot Analysis

Data slices were increased to 17 discrete time epochs but with the addition of 6 more hours after the eye exam for a total of 12 hours. Missing data was dropped from the data set to ensure proper function execution. Data was smoothed using the same Savitzky-Golay filter as described above. Power spectral density and Poincaré plots were created for each time epoch. Maximum decibels, frequency at maximum decibels, SD1, SD2, and SD1/SD2 ratio were calculated for each time epoch.

Results and Discussion

Linear Visualization

Figure 1 shows the linear visualization of neonates 1 and 2 one hour before eyedrops were administered and one hour after the eye exam was complete.

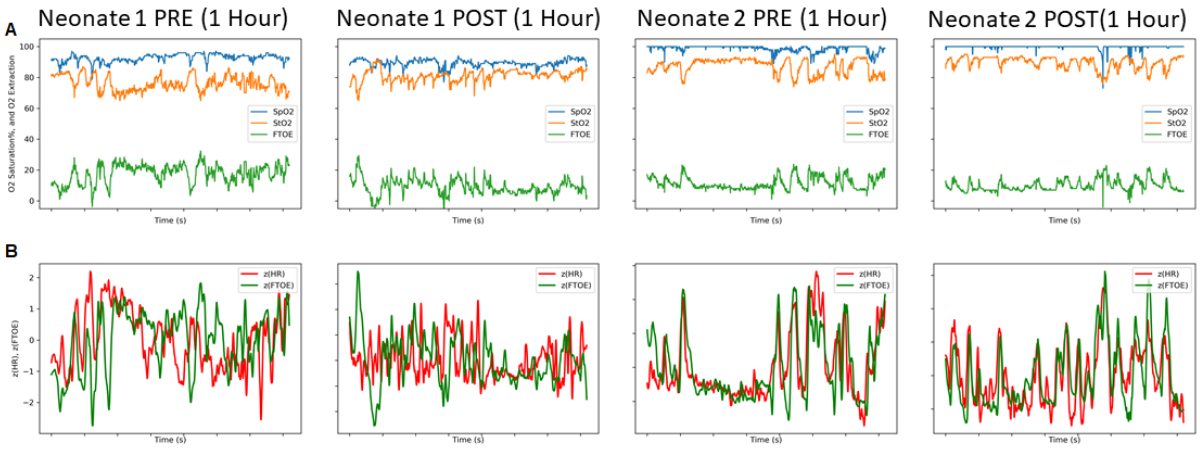

Figure 1. Linear Analysis Visualization for one-hour time epochs of (a) SpO_2, StO_2, and FTOE and (b) z-score normalized and smooth HR and FTOE.

In the z-score normalized and smoothed HR and FTOE plot, Neonate 1 almost shows an asynchronous relationship between HR and FTOE both before and after the ROP exam. Neonate 2, meanwhile, has a close similarity between FTOE and HR for both time epochs. Interpreting the results based on the subject, we may see a higher crosstalk between HR and FTOE in Neonate 2 because Neonate 2 is breathing spontaneously in room air, while Neonate 1 requires ventilatory support via high flow nasal cannula.

Sample Entropy

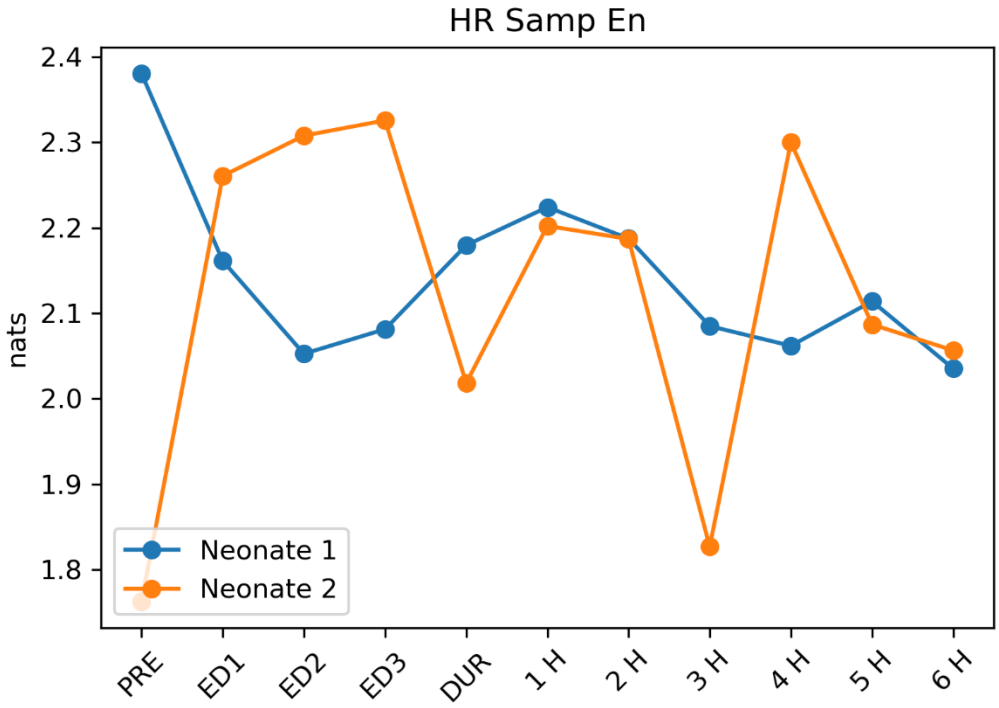

Figure 2. Heart rate Sample Entropy over time

Figure 2 shows a line plot of the subjects' HR, with unit nats to denote that the logarithm base used was *e*. Neonate 1 had a higher HR SampEn before the eye drops and eye exam began, and it decreased in response to the eye exam. Neonate 2 had a lower HR SampEn before the eye exam, and it increased in response to the eye drops and eye exam. This suggests that Neonate 1's heart rate decreased in complexity and increased in regularity in response to stress, and Neonate 2's heart rate increased in complexity and decreased in regularity in response to stress.

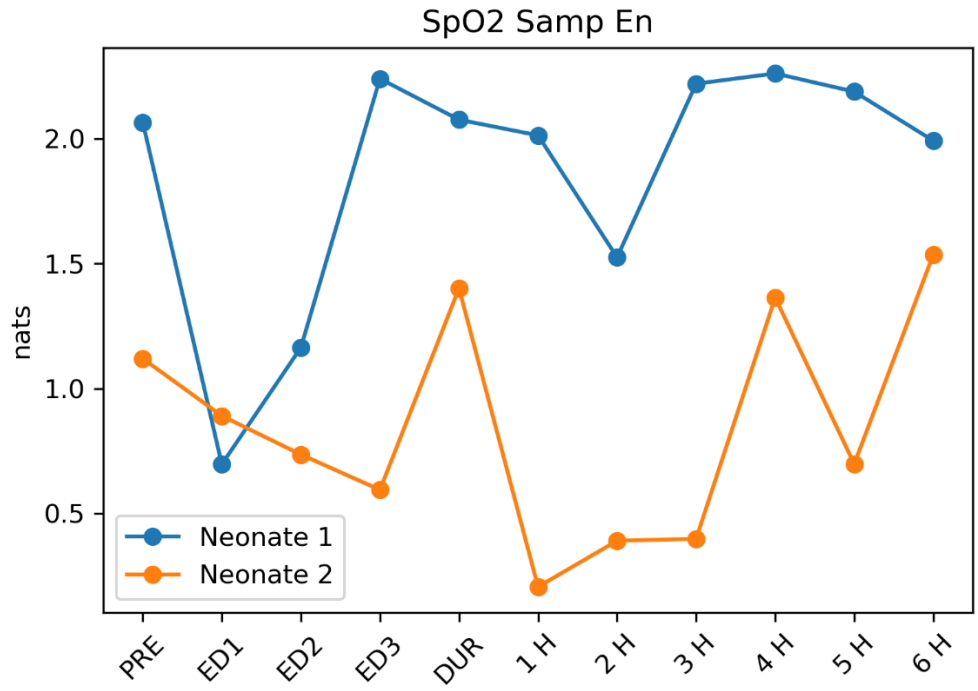

Figure 3. SpO_2 Sample Entropy over time

Figure 3 shows a line plot of the subjects' SpO_2, with unit nats to denote that the logarithm base used was *e*. Neonate 1's SpO_2 decreased in response to the first eye drop set, and continually increased past baseline in the third eye drop set. This suggests that the mechanisms behind the complexity and regularity of SpO_2 Sample Entropy may be masked by ventilatory support. This may be because FiO2 was increased to compensate for lowered SpO_2. Neonate 2's SpO_2 continually decreased until the eye exam began, and had its three lowest sample entropy values the first three hours after the eye exam. This suggests that respiratory regulatory mechanisms may have not recovered adequately until 4 hours after the eye exam.

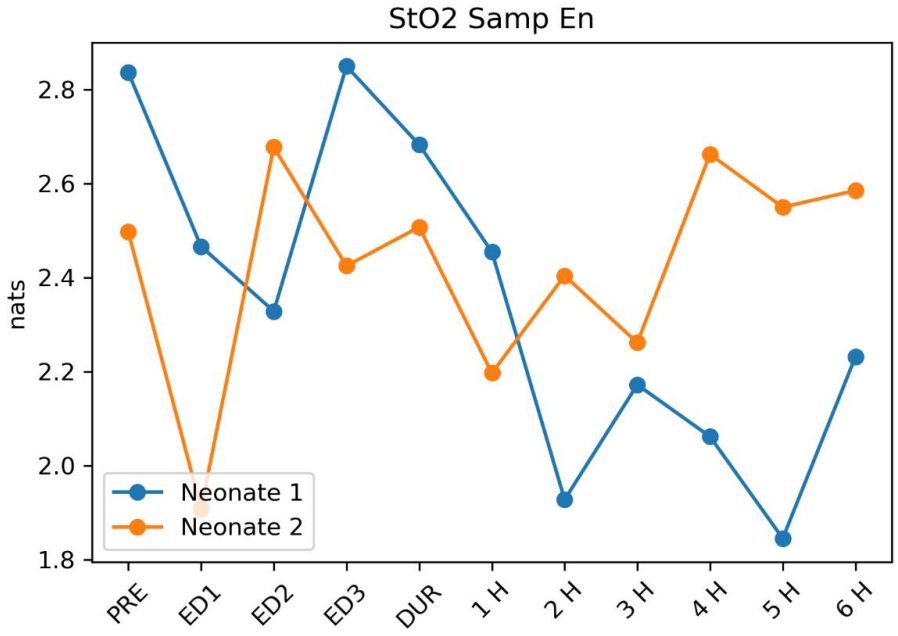

Figure 4. StO_2 Sample Entropy in described epochs

Figure 4 shows a line plot of the subjects' StO_2, with unit nats to denote that the logarithm base used was *e*. Neonate 1's StO_2 sample entropy decreased from baseline until the third eye drop was administered and had its lowest values two hours after eye exam. Neonate 2's StO_2 sample entropy decreased after eyedrop administration, but promptly recovered after the second eyedrop administration. Interestingly, the entropy values are above baseline values from 4-6 hours after the ROP exam.

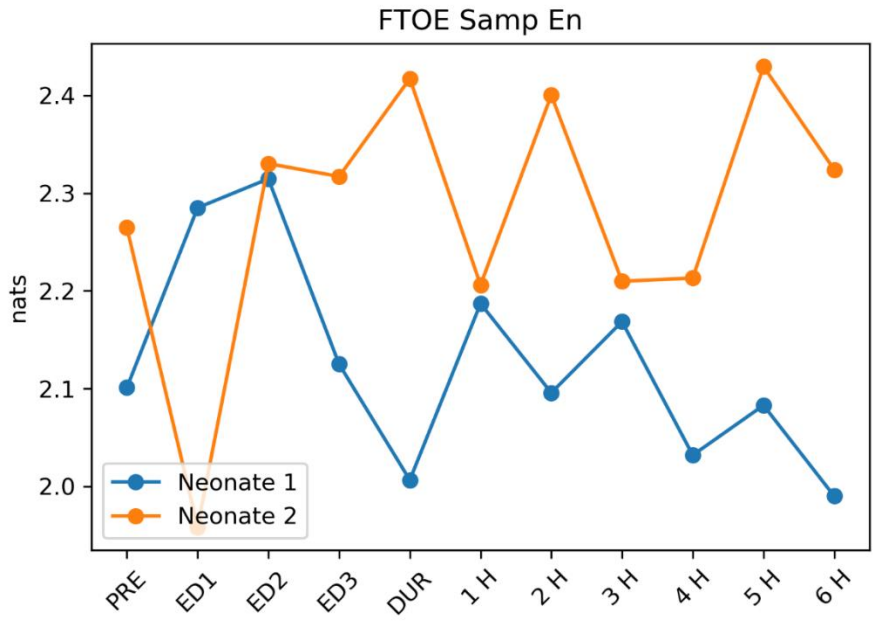

Figure 5. FTOE Sample Entropy in described epochs

Figure 5 shows a line plot of the subjects' FTOE, with unit nats to denote that the logarithm base used was *e*. Neonate 1 had an increase in FTOE sample entropy in response to the first and second eyedrop administration, but promptly fell during the eye exam. Neonate 2 had a decrease in FTOE sample entropy in response to the eye drop administration, but it promptly increased during the second eye drop administration.

Shannon Entropy

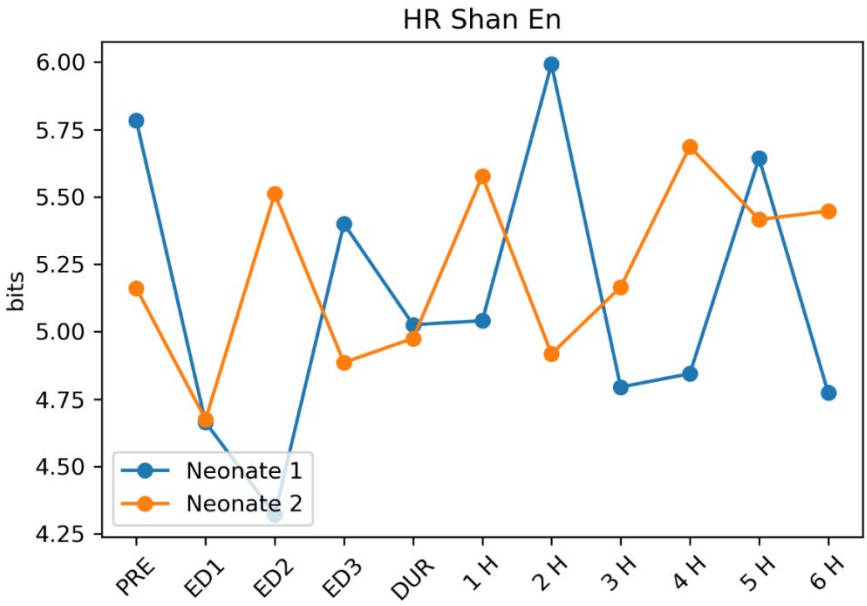

Figure 6. HR Shannon Entropy in described epochs

Figure 6 describes the HR Shannon Entropy for both subjects. The units are denoted as bits to indicate that the logarithm base used for entropy calculation was 2. Neonate 1's HR Shannon entropy decreased in response to the eye drops and the eye exam, while Neonate 2's HR Shannon entropy decreased after the first eye drop and increased compared to baseline after the second eye drop. Of note is the decrease in Shannon entropy in both neonates in response to eye drop administration.

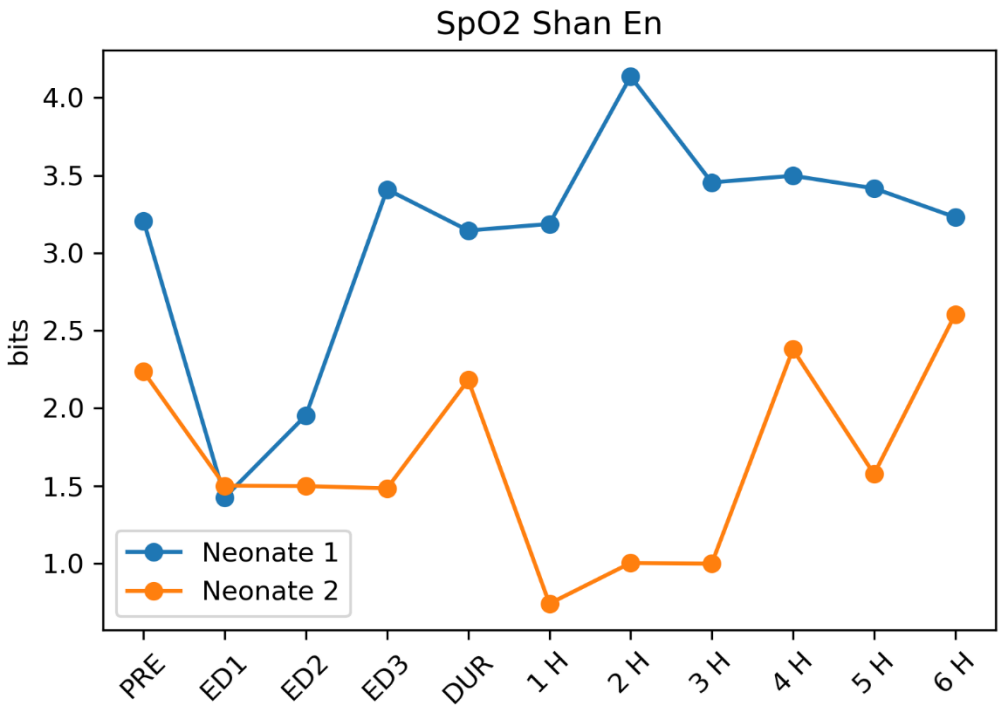

Figure 7. SpO_2 Shannon Entropy in described epochs

Figure 7 describes the SpO_2 Shannon Entropy for both subjects. Shannon entropy was increased in Neonate 1 compared to Neonate 2 in most of the time epochs. Ventilatory support could play role in the addition of uncertainty when considering the entropy of SpO_2. For example, if the bedside nurse increased FiO_2 in response to increase in desaturation events, then uncertainty would be added to the time series data.

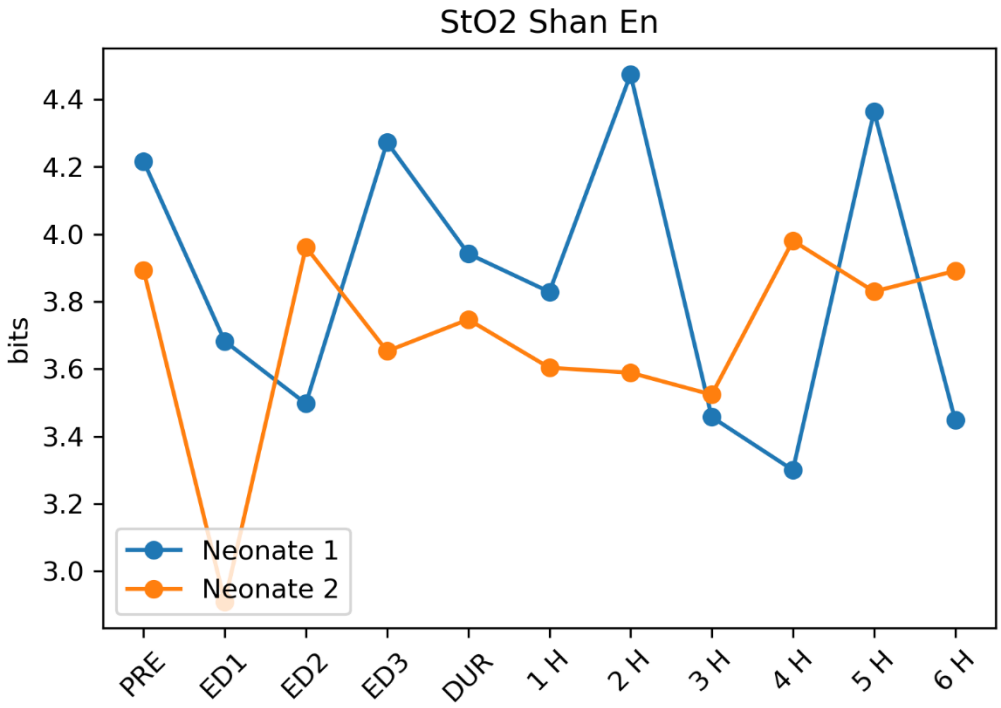

Figure 8. StO_2 Shannon Entropy in described epochs

Figure 8 describes the StO_2 Shannon Entropy in both subjects. In Neonate 1, StO_2 Shannon entropy decreased in response to eye drops set 1 and set 2 and reached baseline levels at the third set of eye drops. Meanwhile, Neonate 2's StO_2 Shannon entropy decreased in response to eyedrops set 1 and reached baseline levels at eyedrop set 2.

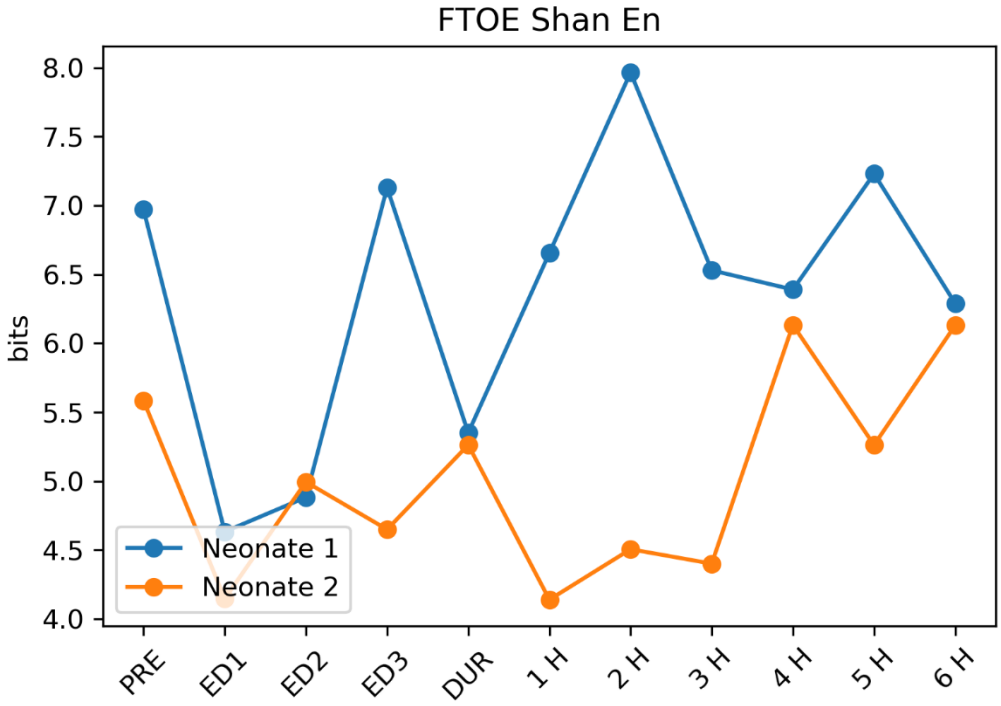

Figure 9. FTOE Shannon Entropy in described epochs

Figure 9 describes the FTOE Shannon Entropy in both subjects. FTOE Shannon entropy was markedly increased in Neonate 1 especially in eye drops set 3 and two hours after the eye exam. Because a change in SpO_2 directly affects FTOE levels, this increase in Shannon entropy is expected.

Mutual Information

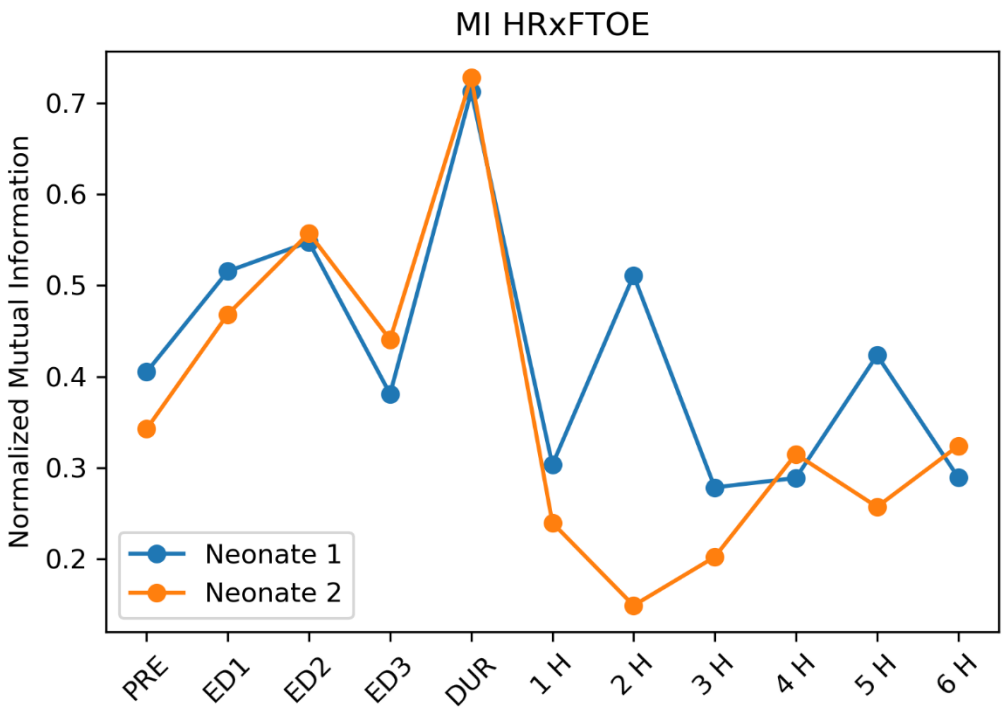

Figure 10. Mutual Information between Heart Rate and FTOE

Figure 10 is a line plot of normalized mutual information between heart rate and FTOE. Mutual information between heart rate and FTOE is increased in Neonate 1 and reaches its maximum during the eye exam. Interestingly, we also see this maximum during eye exam for Neonate 2. This suggests that the crosstalk between heart rate, SpO_2, and StO_2 is increased during the eye exam itself. Two hours after the eye exam occurs, Neonate 1 exhibits an increase in mutual information while Neonate 2 exhibits a decrease in mutual information when compared to the previous time point. This suggests a difference between both subjects in how FTOE and HR are regulated, especially after the ROP exam.

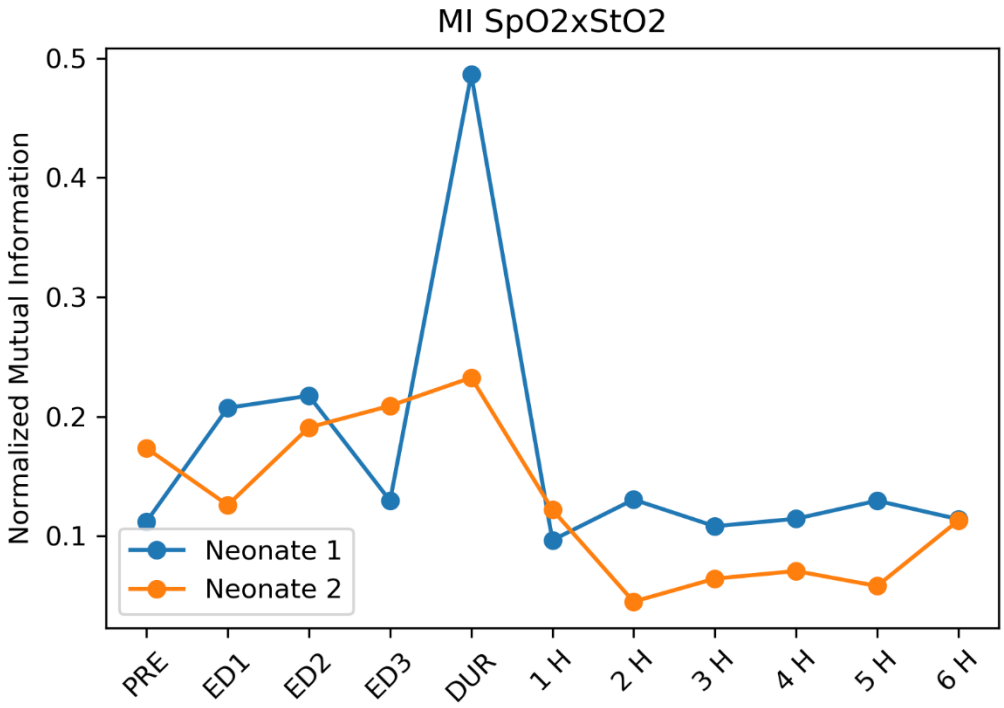

Figure 11. Mutual Information between SpO_2 and StO_2 in described time epochs

Figure 11 is a line plot of mutual information between SpO_2 and StO_2. Of note is the sharp increase in mutual information in Neonate 1 during the eye exam. Neonate 2 also sees a muted increase. Perhaps a reason for this large increase during the eye exam is the overcompensation of the ventilator to deliver oxygen in response to stress to prevent hypoxia.

Power Spectral Density and Poincaré Plot Analysis

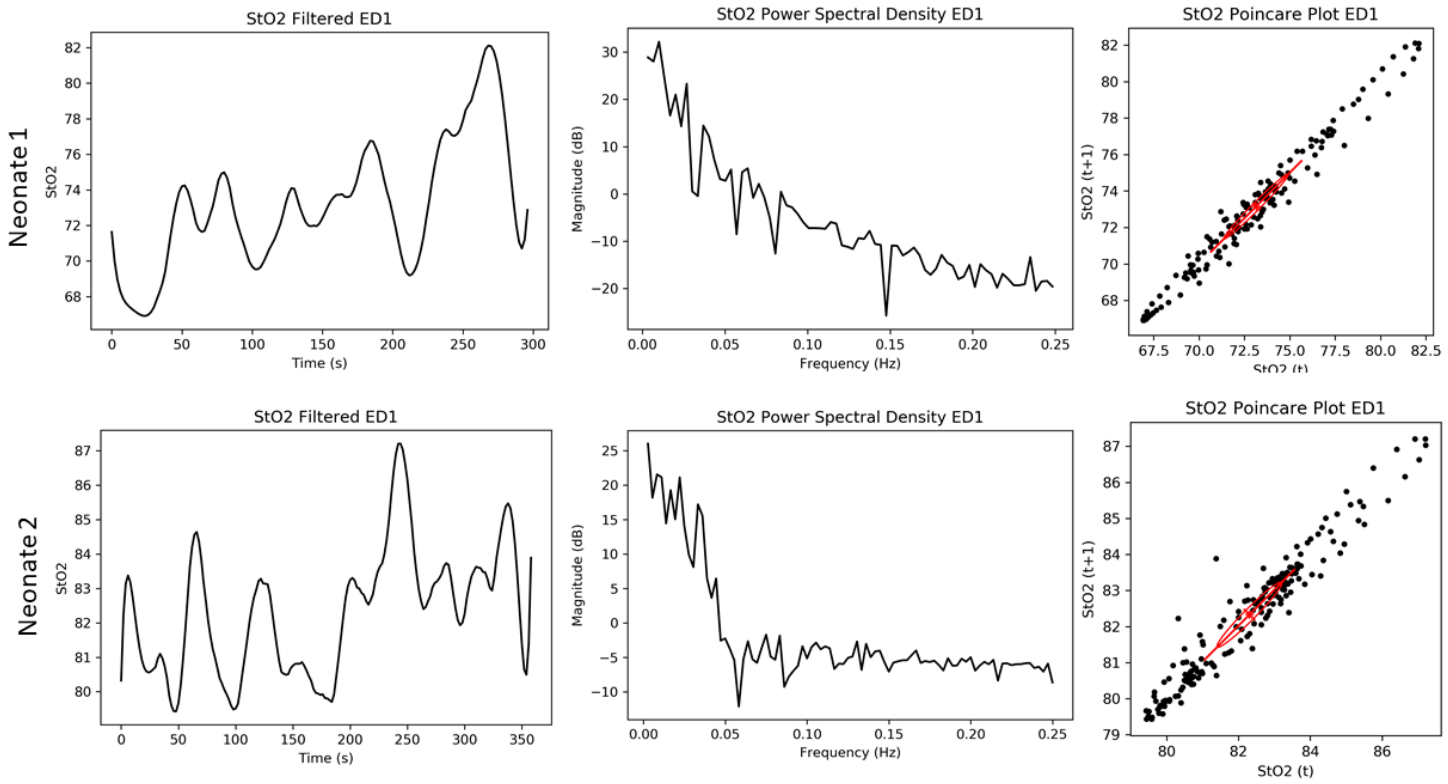

Figure 12. Filtered StO_2, Power Spectral Density, and Poincaré Plot during first eye drop administration of both neonates

Figure 12 shows the plots of StO_2 after Savitzky-Golay filter smoothing and its resulting power spectral density and Poincaré plot. The power spectral density plot shows that the frequencies with the highest magnitude were low frequency signals. This may reflect the pulsatile oxygen flow present in slow wave contractions of the smooth muscles of the intestine [22]. The low wave contractions of smooth muscle cells are driven by interstitial cells of Cajal and neuronal input from the enteric nervous system. Because the parasympathetic nervous system uses acetylcholine as a neurotransmitter to communicate with the enteric nervous system [23], we can expect to see changes in slow wave contractions in response to anticholinergic mydriatic eye drops. Figure 13 shows longitudinal analysis of the maximum dB at 0.25 Hz of StO_2 in different time epochs.

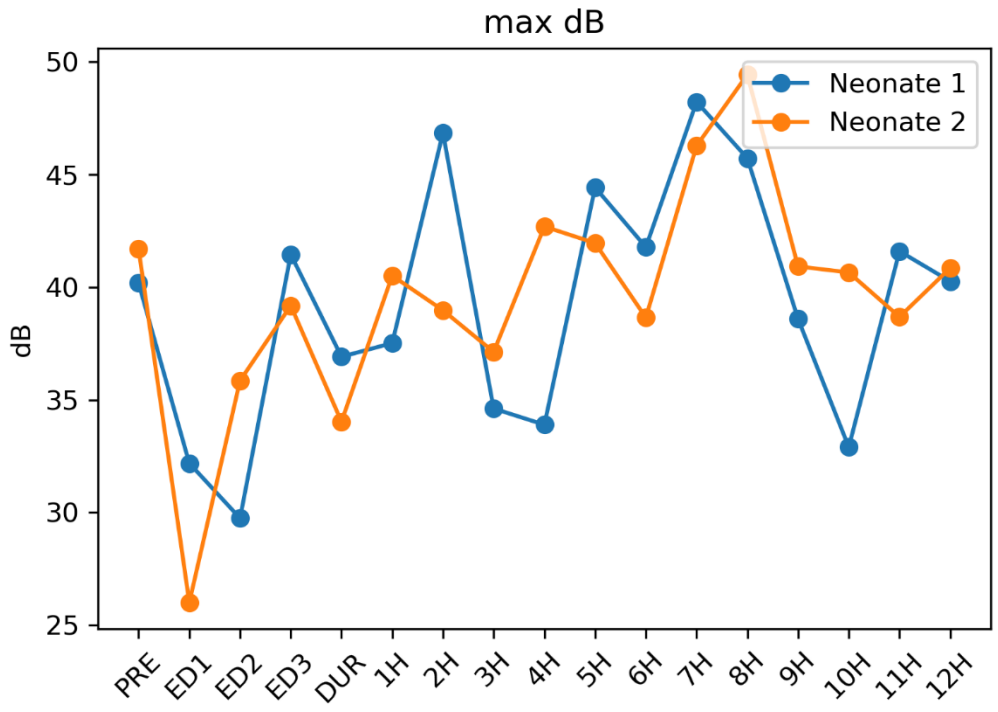

Figure 13. Maximum decibels of StO_2 at 0.25 Hz at different time epochs.

After initial eye drop administration, both neonates exhibited a decrease in magnitude of StO_2 at 0.25 Hz. This may reflect decreased slow wave contractions and ischemic damage to the tissue. Interestingly, neonate 1 exhibits a sharp increase at hour 7 and neonate 8 exhibits a sharp increase at hour 8, especially when compared to baseline. This could reflect reperfusion injury as slow wave contractions increase in magnitude to compensate for the earlier ischemic insult.

Table 2. Poincaré plot SD1, SD2, and SD1/SD2 for both neonates

Time Epoch	Baby 1 SD1	Baby 2 SD1	Baby 1 SD2	Baby 2 SD2	Baby 1 SD1/SD2	Baby 2 SD1/SD2
PRE	0.321296	0.257898	6.795455	6.900946	0.047281	0.037371
ED1	0.420309	0.314682	5.059079	2.535646	0.08308	0.124103
ED2	0.451529	0.373892	4.077222	6.816233	0.110744	0.054853
ED3	0.362471	0.238849	6.747351	5.975439	0.05372	0.039972
DUR	0.361663	0.385006	6.933321	5.636962	0.052163	0.0683
1H	0.259716	0.219028	5.198794	5.432468	0.049957	0.040318
2H	0.354585	0.223541	8.607192	4.240046	0.041196	0.052721
3H	0.222349	0.205336	4.002012	3.909321	0.055559	0.052525
4H	0.178262	0.251662	3.633623	5.430672	0.049059	0.046341
5H	0.329097	0.208948	7.533661	5.171373	0.043684	0.040405
6H	0.198249	0.269618	4.197666	5.489082	0.047228	0.049119
7H	0.296437	0.22698	8.899293	6.807959	0.03331	0.03334
8H	0.282812	0.180334	7.562157	8.272754	0.037398	0.021799
9H	0.21642	0.314497	4.99942	6.546178	0.043289	0.048043
10H	0.189112	0.224639	3.635034	5.291736	0.052025	0.042451
11H	0.269763	0.189443	5.987574	4.156689	0.045054	0.045575
12H	0.325635	0.283996	6.773398	6.436399	0.048076	0.044123

Table 2 shows SD1, SD2, and SD1/SD2 ratio of both neonates at different time epochs. There were no notable patterns in the individual plots of SD1 (short term variability) and SD2 (long term variability) of StO_2. The line plot of the ratio between

SD1/SD2 showed an increase during the first and second eyedrop administration in both neonates, suggesting an increase in randomness and a change in autonomic nervous system signaling [24] in those time epochs (Figure 14).

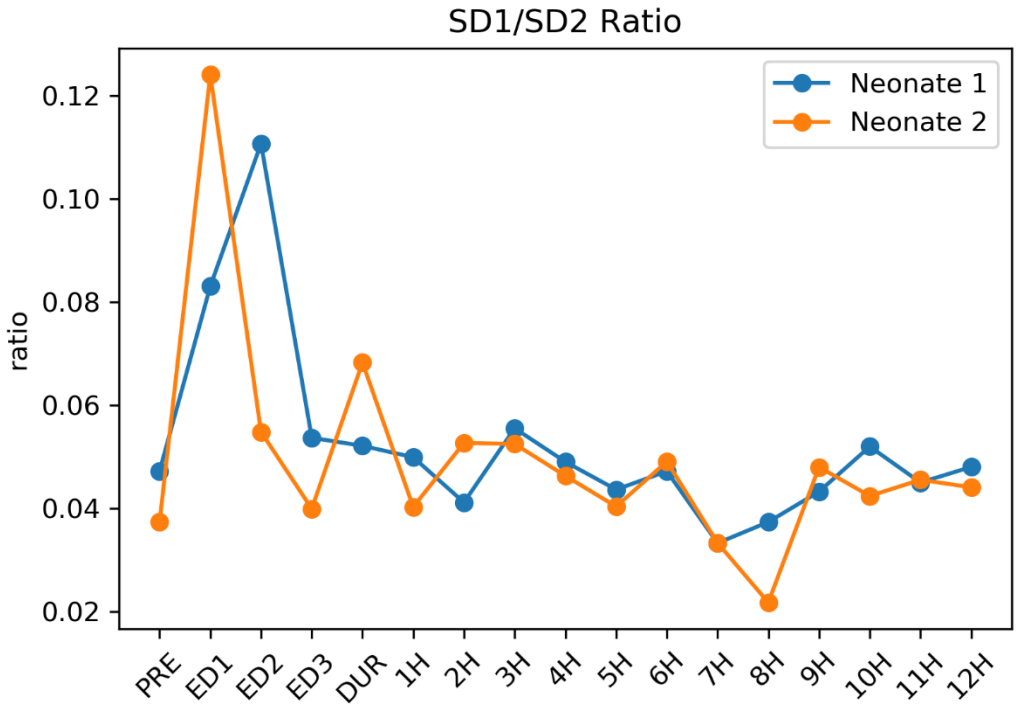

Figure 14. SD1/SD2 ratios at different time epochs

Conclusion

We were able to see the increase in both Shannon and sample entropy of SpO_2 in Neonate 1 due to the added complexity in response to the ventilator. We were able to visualize the nonlinear relationship of HR and FTOE after normalization and smoothing of both variables. This nonlinear relationship was able to be quantified using the metric of mutual information. Furthermore, we were also able to see how mutual information of different systems change in response to stressful events.

We were also able visualize a decrease in maximum StO_2 dB in response to the eye drops and a sharp increase up to 8 hours later, which may reflect long-term ischemic-reperfusion injury in response to altered slow wave contractions in smooth muscle cells in the intestine. Finally, we saw a sharp increase in SD1/SD2 ratio in response to the eyedrop administration, which suggests an alteration in the signaling pathways of the autonomic nervous system and the enteric nervous system.

The physiological interpretation of these findings is not straightforward because all of the individual's properties have an influence on nonlinear metrics. Many factors may impact these nonlinear parameters. Poor sleep-wake cycling, general clinical state of the patient, time and number of feedings, previous "pain memory" in response to the eye exam, state of energy deficit, and general ability to respond to allostatic load could all play a role on these outcomes. Therefore, caution must be exercised when interpreting these results as physiological phenomenon until more validation work is completed. However, the potential of these metrics to vastly inform and improve clinical care is present, especially in the non-vocal population of premature infants.

In conclusion, nonlinear analysis systems can be used as a method to quantify autoregulated cardiovascular systems, hemodynamic fluctuations within the mesentery, and the crosstalk between cardiovascular systems and gastric tissue oxygenation usage. Future studies that focus on these nonlinear metrics may provide novel insights into the pathologies of the premature infant, enabling clinical care to progress into a more evidence-based approach than the current standard of care.

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