HIGH PROTEIN PRETERM FORMULA: EFFECT ON GROWTH AND OUTCOMES IN PRETERM INFANTS ADMITTED TO THE NEONATAL INTENSIVE CARE UNIT

By

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CHAPTER I

INTRODUCTION

This study will evaluate the impact of the transition from a standard preterm formula to a higher protein preterm formula on infant growth and outcomes, specifically: protein status, morbidity, mortality and length of stay in the neonatal intensive care unit (NICU) at St. John Medical Center (SJMC) in Tulsa, Oklahoma. The primary goal in caring for the increasing numbers of preterm infants is to promote growth equal to that of a similarly aged infant in utero (AAP, 2009). Proper nutrition in early stages of life promotes the accrual of lean body mass, particularly in organs such as the brain, minimizing detrimental cognitive outcomes (Abernethy, Cooke & Foulder-Hughes, 2004; Ehrenkranz et al., 2006; Ehrenkranz, 2000; Smart, 1990). Studies have shown a 40% reduction in total brain volume in preterm infants (Abernethy et al., 2004; Mewes et al., 2006), which can last into adolescence (Isaacs et al., 2008). Animal models indicate reduced neuron length and dendritic connections associated with undernutrition (Smart, 1990). Many preterm infants do not match growth of their term counterparts even when born appropriate for gestational age. However, preterm infants do have the ability for catch-up growth, including in relation to the brain, when provided with adequate protein and energy (Isaacs et al., 2008).

Enfamil Premature 24 Cal formula was used by the St. John Medical Center NICU as their standard preterm formula until it was recently redeveloped in response to research on the protein needs of neonates (Tsang, Uauy, Koletzko, Zlotkin, 2005; Klein, 2002). In November 2010, Enfamil Premature 24 Cal High Protein was introduced to the unit changing the standard preterm formula from 3 grams of protein per 100 kcal to 3.5 grams to meet the infant's nutritional needs without the use of modular supplements. The unit made a full transition to the new product by April 2011. As this new high protein preterm formula is based on guidelines provided by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Committee on Nutrition (2010), the Food and Drug Administration approved the production and distribution of this product without any clinical trials given that there were only changes to the amount of macronutrients, with all ingredients remaining the same (Personal communication, Mary Engelland, MEd, RD, CSP, CD Mead Johnson Nutrition, Global Medical Affairs). The new formulation continued to fall within the guidelines of the Infant Formula Act requiring the minimum level of protein of 1.8 g per 100 kcal and the maximum level of 4.5 g per 100 kcal (Federal Food, Drug, and Cosmetic Act, 1980). The purpose of this study was to investigate if theoretical outcomes associated with providing higher concentrations of protein to preterm infants were true for infants in the unit at Saint John. Specifically; growth, protein status, and selected basic outcomes such as, length of stay and morbidity were compared between the two formula groups.

The hypothesis of this study is that those infants in the high protein preterm formula group will show better overall growth with no adverse outcomes. The specific objectives of the study are:

I. To evaluate the effect of high protein preterm formula related to the promotion of improved growth in the preterm infants admitted to the Saint John Medical Center NICU. Expected outcomes will be: improved growth and a decrease in infants with

extrauterine growth restriction (EUGR) as measured by differences in growth plotted on the Fenton growth chart from birth to discharge.

II. To evaluate changes in biochemical markers of protein status in preterm infants on different feeding regimens. With an increase in protein provided, increased levels of blood urea nitrogen (BUN) were expected. The BUN should remain stable and indicate if the infant is receiving adequate amounts of protein once the infant is on a stable fluid regimen and receiving full feeds, defined as an infant receiving 120-150 ml/kg from feeds.

III. Morbidity outcomes such as length of stay and incidence of necrotizing enterocolitis were evaluated. Mortality was evaluated.

The design of the study was a retrospective chart review utilizing a convenience sample at St. John Medical Center's NICU. Qualifying cases six (6) months prior to the unit's transition to high protein preterm formula in December 2010 and six (6) months after transition to the unit's use of high protein preterm formula, with no more than 60 in either group, were considered. Singleton infants with a birth weight between 600 and 2500 grams, and gestation at birth greater than 24 weeks and less than 37 weeks, admitted to the NICU during study the period were considered in this study.

Assistance was needed to identify the subjects for the study from the data collection personnel in the St. John Medical Center's NICU. All data were collected using the electronic medical record of the identified cases. The St. John Medical Center Institutional Review Board and Oklahoma State University Institutional Review Board approved the study's methods and procedures.

Summary:

Meeting the specialized nutritional needs of preterm infants is important not just for

adequate growth, but also for optimal brain development (Abernethy et al., 2004; Ehrenkranz et al., 2006; Ehrenkranz, 2000; Smart, 1990). With the use of the high protein preterm formula, neonates are closer to meeting their estimated protein goals. The aim of this study was to evaluate if this new formulation increases the growth of infants in the NICU at Saint John Medical Center without any adverse outcomes such as an increase in necrotizing enterocolitis, elevated BUN levels, increased sepsis rates, or an increase in length of stay. Improving growth outcomes in this patient population is important, but only if this is achieved while not causing increased incidences of morbidity and mortality.

CHAPTER II

REVIEW OF LITERATURE

In the United States more and more infants are being born prematurely requiring admittance to neonatal intensive care units, which are then charged with providing specialized care to meet the needs of this fragile population. Preterm infants are at increased risk for feeding problems due to an immature gastrointestinal system, and have higher risk for infections due to an underdeveloped immune system. Practitioners have to balance feeding strategies to decrease the risk of disease while promoting adequate growth. Many factors are considered in choosing both the composition of feeds and the timing and advancement of feeds, including gestational age, birth weight and acuity (the level of severity of illness) of the infant. The ultimate goal is to promote growth similar to growth of a comparably aged fetus growing in utero (AAP, 2009).

Section 1 – Prematurity in the United States

Prematurity in the United States is a growing problem. According to the 2009 National Vital Statistics Report, 12.2% of infants were born pre-term or at less than 37 weeks gestation. The majority of these infants are born between 34-36 weeks of gestation, but the 16% of preterm infants born at less than 32 weeks are at an increased risk for adverse health outcomes. Overall,

There has been a 36% increase over the past 30 years in preterm births (March of Dimes, 2012). While rates of preterm birth are increasing so are the survival rates. Infant mortality has greatly decreased from ~20 deaths per 1000 births in 1950, to a rate of 6.5 deaths per 1000 births; a statistic which has remained fairly stable over the past 10 years (Mathews & MacDorman, 2011). The incidence of low-birth-weight infants is currently about 1 out of every 12 live births (Martin et al., 2009). An infant born less than 2500 grams would be classified as low-birth-weight and while survival rates for these infants are increasing, they are at greater risk for mental retardation, learning disabilities, cerebral palsy and vision and hearing loss compared to their term counterparts (Cloherty, Eichenwald & Stark, 2008). The survival rate disproportionally favors the older pre-term infants- going from ~99% among infants born 34-36 weeks gestation to ~80% in those infants born less than 28 weeks. In the infant population, 54% of the deaths occur in the 2% of infants born at less than 32 weeks, with low- birth-weight being one of the three leading causes of infant death (Mathews & MacDorman, 2011).

Section 2 – Nutrition issues associated with prematurity and the importance of adequate nutritional care

As more and more preterm and low-birth-weight infants survive (Mathews & MacDorman, 2011; March of Dimes, 2012), neonatal intensive care units are charged with caring for this population and addressing their specialized needs. These specialized needs include both feeding difficulties and altered and generally increased nutrient requirements.

Sub Section 2.1- Feeding Difficulties:

Preterm infants are at an increased risk for feeding difficulties due to inadequate feeding skills (Sankar, Agrawal, Mishra, Deorari & Paul, 2008). Many are not able to feed orally and require alternative feeding methods. Most preterm infants start on parenteral nutrition and make a gradual transition to enteral gastric tube feedings. The ultimate goal is to provide feedings

exclusively from the bottle or directly from the breast. These infants are also at an increased risk for significant illnesses in the first few weeks of life, which may take precedence over providing enteral nutrition. Nutrient accumulation occurs in the later part of the third trimester, therefore; those infants born prior to 37 weeks are unable to build these stores in utero leaving them with low body stores at birth. These infants require higher supplementation of nutrients and have increased needs to support catch-up growth, including protein, overall energy, calcium, and phosphorous among other nutrients. Lastly, due to the immaturity of the gut, they are more likely to experience feeding intolerance. This increases the need for additional nutritional support, monitoring, and treatment associated with feeding problems. (Sankar et al., 2008)

Some of the factors influencing feeding decisions are how premature the infant is due to the concomitant immaturity of the gastrointestinal system and time needed to develop prior to feeding. At 32-34 weeks the suck swallow breath coordination is developed. Prior to that time, infants will need to be fed via orogastric or nasogastric feedings. There is also dysmotility of the GI system with decreased production of lactulose, bile salts, pancreatic lipase, GI hormones, and peptides. These infants have an underdeveloped defense against pathogens and careful consideration must be taken to decrease possible causes of infection (AAP 2009).

Feeding has implications beyond growth including the development and general health of these infants. According to the Committee on Nutrition from the American Academy of Pediatrics (AAP) (2009), the goals of enteral feeding regimens are to decrease and subsequently discontinue parenteral nutrition support to minimize associated complications such as cholestasis, sepsis, and osteopenia. Using the gut by providing enteral nutrition also enhances absorption of nutrients including: fatty acids, DHA, EPA, calcium, phosphorus, and protein, and can allow for the benefits of trophic feeding. The American Academy of Pediatrics (2009) defines trophic feeds as providing feedings of formula or breast milk at 1-25 ml/kg/d. The intention is to provide small volume feedings to prime the gut not to provide a major source of nutrition. This practice used in

conjunction with parenteral nutrition (PN) for nutritional support, aids in preventing gut atrophy with prolonged NPO status, and helps to establish tolerance prior to providing full feeds. The ultimate goal is to transition to and establish tolerance for the type of feeding the infant will be sent home on of either formula or expressed breast milk (AAP, 2009).

Those infants at particular risk for feeding difficulties are those that are less than 32 weeks gestation and/or very low birth weight infants. These two groups are at an increased risk for necrotizing enterocolitis (NEC) (Cloherty et al., 2008). During admission to NICUs these infants often experience sub-optimal growth due to restrictions necessitated by the medical management of acute illness, and the slow advancement of feeds in an attempt to minimize NEC risk (AAP, 2009). Necrotizing enterocolitis will be discussed in further detail in section 5. The balancing act of providing adequate nutrition while admitted to the NICU, while not increasing rates of morbidity and mortality is often difficult yet critical for development (Patole & De Klerk, 2005). Establishing what nutrients need to be provided and in what quantities to promote growth and development is essential for adequate care during an infants' admission to the NICU.

Sub Section 2.2 Nutrient requirements:

Proper nutrition in early stages of life promotes growth and accrual of lean body mass, and can promote development in organs such as the brain, minimizing detrimental cognitive outcomes (Abernethy et al., 2004; Ehrenkranz et al., 2006; Ehrenkranz, 2000; Smart, 1990). Studies have shown a 40% reduction in total brain volume in preterm infants (Abernethy et al., Cooke & Foulder-Hughes, 2004; Mewes et al., 2006), which can last into adolescence (Isaacs et al., 2008). Animal models indicate reduced neuron length and dendritic connections associated with undernutrition (Smart, 1990). These studies implying those infants not receiving proper nutrition will likely have poorer neurocognitive development at later stages of development. Many preterm infants do not match growth of their term counterparts even when born appropriate

for gestational age (AGA). However, preterm infants do have the ability for catch-up growth, including in the brain, when provided with adequate protein and energy (Isaacs et al., 2008). The aim is to transition from parenteral nutrition to a feeding that promotes adequate growth, while minimizing the risk of complications.

Energy is needed for the synthesis of new tissue, varying depending on the type of tissue being synthesized and stored, and is dependent on the ratio of protein to energy (Kashyap & Schulze, 2006). If energy intake is inadequate, protein will be used as an energy source with decreased nitrogen balance. When energy intake is increased, protein is spared and there is an increase in nitrogen retention. When protein intake is low, increasing overall calorie intake will spare protein, but when there is both increased energy and protein intake weight gain is promoted. With increased energy and adequate protein provided, there is an increase in fat deposition and positive weight gain (Tsang, Uauy, Koletzko & Zlotkin, 2005).

Many premature infants, especially those born at very low birth weights, experience postnatal growth failure (Ziegler, 2007). According to the Life Sciences Research Office (Klein, 2002) the optimal protein: energy ratio is 2.5-3.6g/100 kcal. When Tsang et al. (2005) compared growth in infants fed varying levels of calories/protein ratios, they found that the infants fed 90 kcal/kg and 3 g protein/kg accrued the least amount of protein, and those fed 137 kcal/kg and 3.5 g protein/kg accrued the same amount of protein as those infants fed 104 kcal/kg and 3.8 g protein/kg, but those infants in the higher calorie group added nearly twice as much fat. This shows there is optimal growth when adequate protein is provided, but when excessive energy is provided it is stored mostly as fat. Traditional preterm formula has 3 g of protein/100 kcal, and high protein formula varies from 3.3 to 3.5 g of protein/100 kcal.

Inadequate nutritional intake in preterm infants has been shown to impair brain development in animal models if the deficiencies occur during critical developmental periods

(Arslanoglu, Moro & Ziegler, 2006). In human studies, impaired neurocognitive development has been seen with preterm infants not receiving adequate nutrition in the early stages of life. (Lucas et al., 1990; Lucas, Morley & Cole, 1998). These effects were studied up to 8 years of age with improved outcomes seen throughout (Gale, O'Callaghan, Bredow & Martyn, 2006).

The metabolic parameter most often used to evaluate protein status in preterm infants is blood urea nitrogen (BUN). With normal renal function a low BUN indicates inadequate protein intake. Very high BUN levels suggest possible excessive protein intake (Arslanoglu et al., 2006). Monitoring BUN can help to detect inadequate protein intake and be used to identify when excessive protein is being provided (Arslanoglu et al., 2006). While the normal range for BUN in preterm infants is not well defined, according to Moyer-Mileur, 2000, an acceptable range is 3-25 mg/dL for preterm infants ≤ 1 week old. No range is provided for those preterm infants ≥ 1 week old, but the term range for infants ≥1 month old is 5-18 mg/dl. Other studies that have used BUN as a protein indicator set the ideal range for BUN from 9-14mg/dl, with 20mg/dl considered as high (Arslanoglu et al., 2006).

Providing excessive protein could lead to metabolic stress from protein overload and at this time biochemical markers for protein status are likely not sensitive enough to identify protein toxicity (Tsang et al., 2005). In 1969, a study showed that when preterm infants were provided with >6 grams of protein/kg/d they exhibited signs of azotemia and pyrexia (Goldman, Freudenthal, Holland, & Karelitz, 1969). Excessive protein intake has shown increases in ammonia, blood amino acids, and late metabolic acidosis (Raiha, Heinonen, Rassin & Gaull, 1976)

The AAP (2009) established nutrient requirements to promote growth at the same rate and composition of the fetus of the same gestational age without inducing nutrient deficiency or toxicity. Tsang (2005) promotes the use of increased energy and protein to decrease postnatal

growth retardation and support catch up growth. ESPGHAN (2010) did not provide guidelines for infants greater than 1800 grams and only provided data on protein for infants <1000 grams. ESPGHAN (2010) suggests growth should be promoted similar to intrauterine growth, and by increasing energy they aimed to increase fat accumulation.

Sub Section 2.3 Nutritional goals

While there is no one resource for estimating nutritional goals for preterm infants three organizations have provided nutrient ranges. In the Tsang et al. (2005), guidelines are defined using birth weight. Those infants classified as extremely low birth weight (ELBW) or less than 1000 grams at birth have the highest nutrient goals, followed by those infants classified as very low birth weight (VLBW) or less than 1500 grams at birth. ESPGHAN (2010) also made recommendations based on birth weight, while the AAP's recommendations (2009) are for all preterm infants. Guidelines are outlined in Table 1.

Table 1: Nutritional goals

Nutrient	AAP 2009	Tsang 2005	ESPGHAN 2010
Energy kcal/kg	105-130	110-130 VLBW 130-150 ELBW	110-135
Protein g/kg	3.5-4	3.4-4.2 VLBW 3.8-4.4 ELBW	3.5-4 infants weighing 1-1.8 kg 4.0-4.5 infants weighing <1 kg

Sub Section 2.4 Breastmilk and need for preterm formula:

Research abounds on the benefits of providing breast milk to infants. According to a review article by Askin and Diehl-Jones in 2005, evidence shows that providing breast milk to term infants can reduce a variety of health problems including: feeding intolerances, the severity of respiratory infections, diarrhea, urinary track infections, and meningitis. Other research finds that human milk can be protective against type I diabetes, celiac disease, Crohn's disease, allergies, and sudden infant death syndrome (SIDS). Additionally long term effects as improving

IQ, decreased inflammation, and increased protection against pathogens have been noted (Askin & Diehl-Jones, 2005).

According to the same article by Askin and Diehl-Jones (2005), these findings can also be applied to preterm and very-low-birth-weight. In addition to the previously listed benefits, providing breast milk to this group is even more important as preterm human milk has increased concentrations of immunoglobulins, and anti-infective components, resulting in an enhanced protection from infection including sepsis and meningitis. The nutrients in breast milk are also better absorbed than those in formula including fat, zinc, and iron. Gut function and maturation is enhanced by the growth factors present in breast milk in addition to a decreased renal solute load in breast milk benefitting the immature kidneys. Lastly, feeding expressed breast milk (EBM) can decrease the incidence of necrotizing enterocolitis, which is not only a potentially deadly condition, but can be very expensive to treat (Askin & Diehl-Jones, 2005).

Nearly all infants benefit from the use of their mothers milk prior to any other type of feeding. The conclusions of Schanler, Shulman and Lau in a 1999 study, showed that human milk improved health by decreasing NEC and late onset sepsis, improved feeding tolerance by decreasing gastric residual volume >2 ml/kg/d, or 50% of 3 hour feeding, and also decreased the amount of times feedings were held. Infants on EBM reached full enteral feeds earlier, and were discharged from the hospital faster. One downfall they found to using breast milk was slower growth in both length and weight (Schanler et al., 1999).

While it is well established that breast milk is best, that is not an option for all moms and infants. Most preterm infants do not yet have the skills to successfully feed directly from the breast. Without stimulation, it's often difficult for mothers to maintain their milk supply. The release of oxytocin in a lactating mother increases milk volume and aids in milk let-down (Anderson et al., 2003a; Anderson, Moore, Hepworth, & Bergman, 2003b). Due to the acuity of

most infants it's not possible for moms to hold their infants as desired therefore decreasing skin-to-skin contact. Without this contact, both volume of milk production and duration of breastfeeding decline (Anderson et al., 2003a; Anderson et al., 2003b) making it more difficult for mothers to provide breast milk.

There are few contraindications to breast-feeding, which include infants with galactosemia, mothers with active tuberculosis, women who test positive for human immunodefiency virus (HIV), and some maternal medications (Cloherty et al., 2008). Mothers who have a positive drug screen would also be disqualified from providing breastmilk to their infants (Anderson, Wood, Keller & Hay, 2006). Other mothers are physiologically unable, or simply choose not to breastfeed. Because of these situations there is a need for preterm formula to be on hand to provide nutrition to preterm infants. Even when breastmilk is available and despite its benefits, human milk is often unable to provide enough energy and protein to promote optimal growth. Fortification is needed to meet infants elevated estimated needs (Tsang et al., 2005).

The Infant Formula Act of 1980 provided guidelines and regulations for the manufacturing of formula in the United States. It was amended in 1986 to provide the government with more power to ensure the requirements for quality were being met. This set of laws did not account for preterm needs. In 2002, the Life Sciences Research Office of the American Society for Nutritional Sciences (Klein, 2002) released a report after the FDA requested recommendations for preterm infants. The preterm formula developed and used in most NICU's was based on a 24 kcal/oz ratio with 3 g protein/100 kcal. In response to research on the protein needs of neonates, (Tsang et al., 2005; Klein, 2002) a new higher protein formula was developed and released in November 2010 changing the traditional preterm infant formula from 3 g protein/100 kcal to 3.5 grams to meet the nutritional needs without the use of modular supplements. While this new high protein preterm formula is based on guidelines provided by the

ESPGHAN Committee on Nutrition (2010), the Food and Drug Administration (FDA) approved the production and distribution of this product without any trials, as there were only changes to the amount of macronutrients, not type of ingredients (Personal communication, Mary Engelland, MEd, RD, CSP, CD Mead Johnson Nutrition, Global Medical Affairs). The new formulation continued to fall within the guidelines of the Infant Formula Act requiring the minimum level of protein of 1.8 g per 100 calories and the maximum level of 4.5 g per 100 calories (Federal Food, Drug, and Cosmetic Act, 1980).

While breast milk is the standard when it comes to feeding all infants including preterm infants, it isn't nutrient dense enough in most situations to promote adequate growth (Tsang et al., 2005). Table 2 compares breast milk to formulas designed to meet preterm infants nutritional needs.

Table 2: Nutrition provided in specialized formulas compared to standards

Nutrient per kg/d	AAP 2009	EBM**	EBM+ 4 pkt HMF/ 100ml**	Premature Formula*	High protein Preterm 3.3 g/100 kcal**	High protein Preterm 3.5 g/100 kcal*	Discharge Formula*	Term Formula*
Volume (ml)		150	150	150	150	150	150	150
Energy (kcal)	105-130	100	120	120	120	120	110	100
Protein (g)	3.5-4	1.4	2.9-3	3.6	3.96	4.2	3.4	2.4

*Mead Johnson Nutrition Pediatric Products Handbook 2011
** Abbott Pediatric Nutrition Product Guide 2011

Section 3: Goals for adequate growth

Growth is measured by gains in weight, length, and head circumference. The goal is to promote growth at the same rate as in an infant of the same gestational age in utero (AAP, 2009). Nutrient requirements for preterm infants are much higher than those of term infants to promote adequate growth. How to establish exactly what those requirements are has long been debated, and several approaches have been used to attempt to define these requirements. The distinguishing factor is to what extent to account for catch-up growth (Zielger, 2007). Several

large studies comparing actual growth in preterm infants found average weight gain ranged from 14.9g/kg/d reported by Lubchenco, Hansman, Dressler and Boyd in 1963 to 20 gm/kg/d reported by Alexander, Himes, Kaufman, Mor & Kogan in1996, with Ehrenkranz et al. 1999 showing 14-16 gm/kg/d and Klein in 2002 reporting 16-17 g/kg/d. Guidelines provided in the Nutritional Care for High Risk Newborns (1994) state premature infants should be gaining 10-30 grams/day with an 0.8-1.1 cm/week gain in both length and head circumference (Catrine, 2000). In 2007, Ziegler reported guidelines for weight gain based on the infants' birth weights using data from Kramer et al, 2001 (Griffin, 2007). The lower the infants body weight is, the larger gains in gm/kg/d; for an infant weighing 500-700 grams, goal weight gain is 21 gm/kg/d, ranging to infants current weight of 1800-2000 grams with a goal weight gain of 14 gm/kg/d (Ziegler, 2007). Being able to provide adequate nutrition is essential in prevention of postnatal growth failure and also promotes optimal neurodevelopmental outcomes (Zielger, 2007).

The most commonly used chart for plotting preterm infants growth is the Fenton growth chart (Fenton, 2003). It is based on a Babson and Benda fetal growth chart published in 1976 which was widely used for tracking preterm infants' growth on a graph, prior to the updated Fenton. Several weaknesses of the Babson and Benda chart were the graph was based on a small sample size with limited data, and the graph started at 26 weeks gestation limiting it's usefulness for younger infants. It also had 500 gram increments making plotting infants' growth more difficult. A meta-analysis of literature on the growth of preterm and post term infant was completed looking at data from 1980-2002. Four data sets were identified for use including fetal weight gain data from Kramer et al. 2001, which included 676,605 infants, 22-43 weeks gestation, born in Canada from 1994-1966. Two studies were included for data on fetal length and head circumference; one (Niklasson, Ericson, Fryer, Karlberg, Lawrence & Karlberg, 1991) was based on 376,000 Swedish infants, 28-40 weeks gestation, born from 1977-1981, and another by Beeby, Bhutap, and Taylor in 1996 which was based 22-40 week gestation infants born

between 1982-1995 with information on 29,090 infants' head circumference and 26,973 infants' lengths. Lastly, information the CDC had collected from 1963-1994 was used. CDC had data on 2,200 to 38,000 subjects per year including infants with varying racial and ethnic backgrounds, and included information on infants who were both breastfed and formula fed. This data was cross sectional and longitudinal. (Fenton, 2003). The advantages of this new preterm growth chart were a large sample size, more up-to-date data, a range of gestation ages from 22-50 weeks, and smaller (100 gram) increments to ease plotting (Fenton, 2003). Due to the Fenton's strengths. it's one of the most widely used charts for tracking weight gain.

Infants are plotted on this chart not just to evaluate individual growth, but also to evaluate if the infant falls below the 10 percentile indicating growth restriction (Cloherty et al., 2008). If an infant is born at less than the 10 percentile, they are classified as small for gestational age (SGA) or intrauterine growth restriced (IUGR) (Cloherty et al., 2008). If an infant falls below the 10 percentile after birth, they are considered to be exrauterine growth restriced (EUGR) (Clark, Thomas & Peabody, 2003).

To prevent growth failure, Table 3 shows accepted overall guidelines and goals for preterm infant growth. With these gains, adequate growth should be achieved to promote gains similar to those seen in utero (Baylor College of Medicine Section of Neonatology, 2009).

Table 3: Growth rate guidelines

Growth	Average
Weight Infants <2 kg	10-20 g/kg/d
Weight Infants >2 kg	20-30 g/d
Head Circumference	0.8-1 cm/wk
Length	0.8-1.1 cm/wk

Modified from Baylor College of Medicine Section of Neonatology (2009) Guidelines for Acute Care of the Neonate.

Meeting growth guidelines depends on providing adequate nutrition, but nutritional goals can vary depending on the acuity of the infant or the infant's particular disease state that affects

growth, nutritional and fluid needs. Knowing initial acuity and gestational age can aid clinicians in determining appropriate course of treatment (Cloherty et al., 2008).

Section 4 – Determining acuity and gestational age

The Apgar system of evaluating infants at birth was proposed by Virginia Apgar in 1953 to focus on the initial clinical state of the infant. It is most useful in establishing if resuscitation should be performed or not. The assessment focuses on heart rate, respiratory effort, reflex irritability, muscle tone and color, and each symptom is assigned a number 0, 1, or 2 depending on the presence, absence and degree of each of these indicators. In the original report, Apgar (1953) found an inverse relationship between the Apgar score and neonatal death. Infants with poor scores, from 0-2 had mortality rates of 9-14%, scores from 3-7 mortality of 1.1-2% and scores of 8-10 had the lowest mortality rates of 0.13-1%. These finding were later reinforced with a follow up study in 1960 (Apgar & James, 1962).

Classifying neonates based on gestation age is more meaningful than classifying them based on birth weight, as they are more likely to possess qualities of infants similar in gestational age opposed to other infants of similar weight (Cloherty et al., 2008). Gestational age is also the most significant predictor of survival in this population (Ballard et al., 1991). Gestational age is typically estimated based on the first day of the last menstrual period. Confirmation by ultrasound can be made using the fetal crown-rump length during the first trimester. Infants can be classified as preterm if they are less than 37 weeks of gestation, and term if they are 37 to less than 42 weeks gestation. When using weight to classify, infants are said to be normal weight if they are greater than 2500 grams at birth, low birth weight (LBW) if they are less than 2500 grams, very low birth weight (VLBW) if they are less than 1500 grams, and extremely low birth weight (ELBW) if they are less than 1000 grams.

The New Ballard Score was developed to aid health care professionals in evaluating gestational age, especially for infants born to mothers with unreliable menstrual history or no prenatal care. Ballard is the most commonly used score for estimating gestational age, and was expanded in 1991 to be more accurate in evaluating those infants with a gestational age of less than 28 weeks (Ballard et al., 1991). The scoring system was expanded by close examination of neurological and physical characteristics of extremely premature infants, even those considered to be nonviable. These evaluations were compared to those of term infants noting differences in flexibility, which allowed the expansion of the neuromuscular maturing scale to include -1. Premature infants were noted to have sticky, transparent skin, and to be free of any lanugo, with imperceptible breast markings and little differentiation of the genitals. These indications allowed the expansion to the score of -1 for the physical items. When the New Ballard Score was tested, researchers found that gestational age was overestimated by 2-4 days in infants less than 37 weeks, while gestational age was accurate for infants between 32-37 weeks (Ballard et al., 1991). Having an accurate gestational age directs the care of this population by identifying expected feeding behavior. Many feeding decisions are based on an infants presumed maturity to identify those at greater risk for morbidity and mortality.

Section 5 – Nutrition and neonatal morbidity

While most infants admitted to the NICU do not face additional morbidities besides the effects of prematurity, they are at increased risk for disease that alters growth and nutrition. Some of these diseases are necrotizing enterocolitis, which is an inflammatory bowel disease, sepsis (or an infection of the blood) and broncopulmonary dysplasia and chronic lung disease.

Sub Section 5.1 Necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is a bowel disease characterized by acute intestinal necrosis syndrome of unknown etiology (Cloherty et al., 2008). It is the most common neonatal

intra-abdominal emergency occurring in 1 to 3/1000 live births, requiring surgery in 20-40% of cases, and leading to mortality in 10-30% of cases (Barclay, Stenson, Simpson, Weaver & Willson, 2007). The primary risk factor for NEC is prematurity with greater than 90% of cases occurring in infants less than 36 weeks of gestation (Henry & Moss, 2009) with the mean age of occurrence between 30-32 weeks (Cloherty et al., 2008). As gestational age decreases, mortality increases. Other risk factors include low birth weight (with less than 10% of cases occurring in infants weighing more than 2000 grams (Guthrie et al., 2003)), the initiation of enteral feedings, or a hypoxic or hemodynamic insult (Cloherty et al., 2008). NEC most commonly presents with bloody stools, abdominal distention, increased gastric residuals, abdominal tenderness or redness, a lack of bowel sounds, or a dusky discoloration of the abdominal wall. It has three stages based on severity using Bell's staging of NEC (Bell et al., 1978). In a study by Caplan, Simon, and Jilling (2005), infants diagnosed with NEC had an increased length of stay in the NICU, a higher likelihood of having short gut syndrome, and abnormal neurodevelopment.

Strategies suggested to prevent NEC include an increased use of human milk versus formula, using a lower milk volume and advancing the feedings slower, and using products with a low osmotic load (Bhatia, 2010). Low osmotic load products have been shown to decrease transit time, therefore helping to avoid substrate fermentation related to the infants' slow motility and poor digestion. The use of pre- and probiotics may also be beneficial even though research is limited on the safety of this practice.

Table 4: Bell stages of necrotizing enterocolitis

Stage	Systemic Signs	Intestinal Signs	Radiologic Signs	Treatment
1: Suspected	Temperature instability, apnea, bradycardia	Elevated pregavage residuals, mild abdominal distension, occult blood in stool	Normal or mild ileus	NPO, antibiotics for 3 days
2: Definite	Same as stage 1, plus mild metabolic acidosis, mild thrombocytopenia	Same as 1, plus absent bowel sounds, definite abdominal tenderness, abdominal cellulitis, right lower quadrant mass	Ileus, pneumatosis intestinalis plus portal vein gas, with or without ascites	NPO, antibiotics for 7-14 days depending on severity
3: Advanced	Bowel intact: Same as stage 2, plus hypotension, bradycardia, respiratory acidosis, metabolic acidosis, disseminated intravascular coagulation, neutropenia Bowel perforated: Same as above	Bowel intact: Same as stage 2, plus signs of generalized peritonitis, marked tenderness and distension of abdomen. Bowel perforated: Same as above	Bowel intact: Same as stage 2 plus definite ascites Bowel perforated: Same as above plus pneumoperitoneum	Bowel intact: NPO, antibiotics for 14 days, fluid resuscitation, inotropic support, ventilator therapy, paracentesis Bowel perforated: Same as above, plus surgery

Modified from Bell et al. 1978

Sub Section 5.2 Late onset sepsis

Neonatal Late Onset Sepsis is defined as a bacterial infection occurring from 8-90 days of life, and is diagnosed by positive blood culture for gram-positive, gram-negative, or fungal infection (Cloherty et al., 2008). Up to 21% of infants admitted to the NICU develop sepsis with mortality in 18% of those infants diagnosed. Some of the risk factors for sepsis are infants

weighing less than 1500 grams, those with the presence of central lines, the prolonged use of TPN, delayed enteral feeding, mechanical ventilation, bronchopulmonary dysplasia, patent ductus arteriosus, and a diagnosis of NEC. Due to the increased risk of sepsis, one of the primary nutritional care objectives is to reduce time on parenteral nutrition support while concurrently not increasing the risk for necrotizing enterocolitis.

Sub Section 5.3 – Broncopulmonary dysplasia and chronic lung disease

Broncopulmonary dysplasia (BPD), also known as chronic lung disease of prematurity (CLD), is defined as an infant born less than 32 weeks gestation who remains on oxygen support for the first 28 days of life. Infants born less than 1250 grams are the most susceptible, with a decreased risk in African-Americans and girls (Cloherty et al., 2008). There are approximately 3,000 to 7,000 cases of BPD/CLD per year. Mortality is estimated in 10-20% of infants during the first year of life, often due to infection. Early growth failure can result from an increase in energy needs due to increased work of breathing and inadequate intake for growth. Energy needs continue to be increased even after clinical symptoms have resolved (Bhakta & Stark, 2006). Growth failure is more severe in lower birth weight infants and can continue in 33-66% of these infants up to 2 years corrected age. The degree of growth failure is influenced by the duration and severity of CLD with weight being the most affected and head circumference being the least affected (Cloherty et al., 2008).

Section 6- Summary

The number of infants born preterm in the United States continues to rise (March of Dimes, 2012). These infants are not yet fully developed and have increased feeding difficulties which can lead to poor growth. Premature infants are also at increased risk for morbidity, which can significantly affect growth. The importance of adequate feeding to promote growth is essential for long-term development. This study evaluates a particular strategy to improve these

outcomes by increasing the protein provided in the diet while the infants are admitted to the NICU.

CHAPTER III

METHODOLOGY

Section 1 – Background and design

The design of this study is a retrospective chart review utilizing a convenience sample from the St. John Medical Center (SJMC) NICU. Data used for the study was collected from the electronic medical record (EMR). This study qualified for a waiver of consent as this project is retrospective and no identifying data were collected The St. John Medical Center Institutional Review Board and the Oklahoma State University Institutional Review Board approved the study's methods and procedures (see Appendix 2).

Section 2 – Sample

All singleton infants with birth weight between 600 and 2500 grams and gestational age between 24 and <37 weeks were eligible if admitted to St. John's NICU between the dates of June 1, 2010 to September 31, 2011, excluding the months of December 2010 to March 2011. The excluded months were to allow time for the unit to transition fully from traditional preterm formula (TPF) to high protein preterm formula (HPF).

NICU research personnel provided the PI with a list of infants admitted to unit during study period for screening. Infants were included if they received feedings of breast milk, TPF or HPF. Infants excluded were those with major congenital abnormalities, and/or chromosomal aberrations. All others were included in the study. There were 78 qualifying cases in the six months prior to the unit's transition to HPF in December 2010 and 54 qualifying infants in the six (6) months after transition to sole use of HPF. The maximum number in each group was set at 60. In the traditional preterm formula group all 35 infants born at less than 2000 grams were included. The first 25 infants born between 2000 grams and 2500 grams were also included as no pattern could be established in list of infants provided therefore the first 25 were considered to be selected at random. .

Section 3 - Materials

All information was obtained from review of the infant's electronic chart. One spread sheet was used to collect initial information including, gender, race, Apgar score, Ballard score, gestational age, birth weight, length, and head circumference in addition to the infants initial percentage on the Fenton growth chart. Discharge weight, length, and head circumference, and percent on Fenton growth chart at discharge were also collected. Information on morbidity and mortality was obtained using the discharge or death summary along with an official diagnosis by the neonatologist.

A second spreadsheet was used to track multiple points of data on the same infant during their entire stay. This data was collected to monitor growth, changes in type of feeding, tolerance of feeding and protein status. The phase of feeding was recorded indicating if the infant was on full TPN support, in the transitional stage weaning off of TPN as feedings were advanced, or on full feedings defines as feedings of 120-150 ml/kg (AAP, 2009). Data on what type of formula was used and the tolerance of that formula was collected. Once an infant was on full feedings,

information on blood urea nitrogen was collected weekly along with weight, and head circumference. Initially length was to be collected, but limited information was available on length in the majority of the charts reviewed; for example in the traditional preterm formula group only 20 out of the sample of 60 had discharge lengths. Creatinine was also to be collected; however upon review of the medical records laboratory results for creatinine were missing therefore could not be included.

Once all the data was initially collected, the PI cleaned the data set. All values that looked to be potential errors were marked and that chart was review for a second time to confirm accuracy and changes made as appropriate

Section 4 – Evaluation of growth

Growth was measured by comparing birth weight to discharge weight, and birth head circumference to discharge head circumference. To evaluate differences in extrauterine growth restriction each infant was plotted on the Fenton growth chart at birth. If the infant was plotted at $<10^{th}$ %ile, the infant was considered to be small for gestational age (SGA) or intrauterine growth restricted (IUGR). Each infant was plotted again on the Fenton growth chart at discharge, those infants plotting at <10% were classified as extrauterine growth restricted (Clark et al. 2003). Daily weights and head circumferences were also obtained along with the feeding that was being provided to track weight gain associations with formula type.

Section 5 – Evaluation of protein status

The metabolic parameter used to evaluate protein status was blood urea nitrogen (BUN). With normal renal function, blood urea nitrogen will indicate low protein intake and when levels are very high can also indicate excessive protein intake (Moro et al., 1995). Normal range for BUN in preterm infants is not well defined, but in this study 9-20 mg/dL was considered within normal limits (Nutritional Care for High-Risk Newborns, 1994). Infants were screened for

abnormal renal function and data was only collected once the infant reached full feds of 150 ml/kg and fluid intake was stable

Section 6 – Assessment of acuity and gestational age

Two types of scores were used to indicate the infant's health status and gestational age.

Apgar:

Apgar scores were collected to provide initial information on acuity of the infant. The assessment focuses on heart rate, respiratory effort, reflex irritability, muscle tone and color, and is assigned a number 0, 1, or 2 depending on the presence, absence and degree of each of these indicators. Scores range from 0-10 with decreased values associated with a lower survival rate (Apgar, 1953).

Gestational age:

Information to estimate gestational age was collected from the infants chart based on what the mother had reported and based on the mothers prenatal care and estimation from her physician. It was not indicated if this was based on the last day of her menstrual cycle, or based on an estimate by ultrasound of rump-crown length. Infants < 37 weeks are considerer preterm, and all infants included were <37 weeks. Of the 114 infants in the study, 110 had a new Ballard Score assessment within the first 96 hours of life. Scores range from -10 to 50, indicating an estimated gestational age of 20-44 weeks gestation based on neuromuscular and physical maturity (Ballard et al., 1991). Both gestational age and Ballard score were collected in this study.

Section 7 – Morbidity and mortality

Three types of illness particularly common in preterm infants were tracked and data on

survival to discharge were also collected.

Necrotizing enterocolitis and sepsis:

Each qualifying infant's discharge summary was reviewed for official diagnoses of necrotizing enterocolitis and sepsis made by the neonatologist. Only cases of confirmed necrotizing enterocolitis, stage 2 or 3 were included in the study and designated as surgical or non-surgical.

Sepsis was defied as a positive blood culture after day of life 7. All blood cultures were analyzed in the hospital's laboratory. Official diagnosis by the neonatologist in the discharge summary was also used to confirm the finding.

Broncopulmonary dysplasia and chronic lung disease:

Each qualifying infant's discharge summary was reviewed for official diagnoses of broncopulmonary dysplasia (BPD), chronic lung disease (CLD), or both made by the neonatologist. Infants that remained on respiratory support for 28 days or more were diagnosed with broncopulmonary dysplasia or chronic lung disease. These infants are indicated in the study due to alterations in growth due to increased work of breathing.

Morality:

Specifics of the infants' death were collected from the neonatologist death summary report.

Section 8 – Statistical analysis

SPSS version 19.0 was used to analyze the data. Descriptive statistics, including frequencies, means and standard deviation, were computed from gender, race, Apgar Score, Ballard Score, gestational age, mortality, and birth and discharge weight, length, head

circumference, and percent on Fenton growth chart. Independent two sample T-test analysis was done to compare the differences between the two groups of infants. Chi Square tests were used to evaluate NEC, and BPD/CLD. Difference in groups were considered significant at $p < 0.05$.

HLM2, Hierarchical Linear and Nonlinear Modeling, also referred to as multilevel models, were used to evaluate the effect of high protein preterm formula on growth and BUN status. This program is used to show hierarchical relationships by arranging variables into groups. The program then evaluates the influence other groups of variables on each other. In this study level 1 and level 2 units were used. This allowed for evaluation of the relationships of several variables at both levels to be used in a single analysis. It takes into account variations at each level, by estimating the model coefficients at each level and predicts the random effects associate with each sampling unit at each level. Repeated measures for each infant were evaluated. Baseline was established using growth from all other infants in the study that did not receive the traditional preterm formula or the high protein preterm formula. Multiple regression analysis was used to compare expected weight gain and BUN comparing infants who received traditional preterm formula, or high protein preterm formula from expected growth of the baseline infants, who received breastmilk or specialized formulas. Significance was determined as $p < 0.05$.

CHAPTER IV

RESULTS

This study examined the effect on growth and protein status comparing the infants receiving traditional preterm formula to infants receiving a new formula with a higher protein content. The growth was evaluated using weight data, which was collected periodically during each infant's admission. Protein status was evaluated using blood urea nitrogen (BUN) from laboratory values assessed after the infant was on full feedings, or feedings of 120-150 ml/kg. There were two groups in this study, one admitted during the study period prior to St. John Medical Center's NICU transition from the traditional preterm formula (TPF), the other after the introduction and transition to a redeveloped higher protein preterm formula (HPF). Data were collected to determine to what extent the infants in each formula group were similar or different in terms of growth and protein status, with expected improvements for those infants in the high protein preterm formula group with no adverse outcomes.

Section 1 – Demographics of study sample

The infants in this sample were predominately white (84.7%) in the traditional preterm

formula group (TPF) and (75.9%) in the high protein preterm formula group (HPF), with 6.7% and 13% of the sample reporting African American ethnicity in the TPF and HPF groups respectively as you can see in Table 5. There was a mean age of 31.1 ± standard deviation (SD) of 3.2 weeks gestation in the TPF and 31.5 ± SD of 3.2 weeks gestation in the HPF. There were more boys admitted during the study period (51.7%) in the TPF and (64.8%) in the HPF group, which is to be expected as rates of prematurity are higher in boys than girls (Carrascosa, Yeste, Copil, Almar, Salcedo & Gussinye, 2004). Figure 1 shows the majority of infants in the TPF (68%) and in the HPF (64%) were classified as low birth weight (<2500 gm). In the TPF group, at full feeds 15/60 infants received preterm formula exclusively or preterm formula that transitioned to discharge formula prior to discharge. 11/60 received preterm formula and breastmilk and 30/60 received only breastmilk or a specialized formula product and 4/60 infants died prior to reaching full feeds. In the HPF group, at full feeds 12/54 infants received preterm formula exclusively or preterm formula that transitioned to discharge formula prior to discharge. 10/54 received preterm formula and breastmilk and 28/54 received only breastmilk or a specialized formula product, and 4/60 infants died prior to reaching full feeds

Table 5: Demographic features

Variable	**TPF [n(%)]**	**HPF [n(%)]**
Race		
White	50 (84.7)	41 (75.9)
African American	4 (6.7)	7 (13.0)
Hispanic	1 (1.7)	3 (5.6)
Other	4 (6.7)	2 (3.7)
Missing	1 (could not be identified)	1 (could not be identified)
Gender		
Male	31 (51.7)	35 (64.8)
Female	29 (48.3)	19 (35.2)

Figure 1: Classification of weight

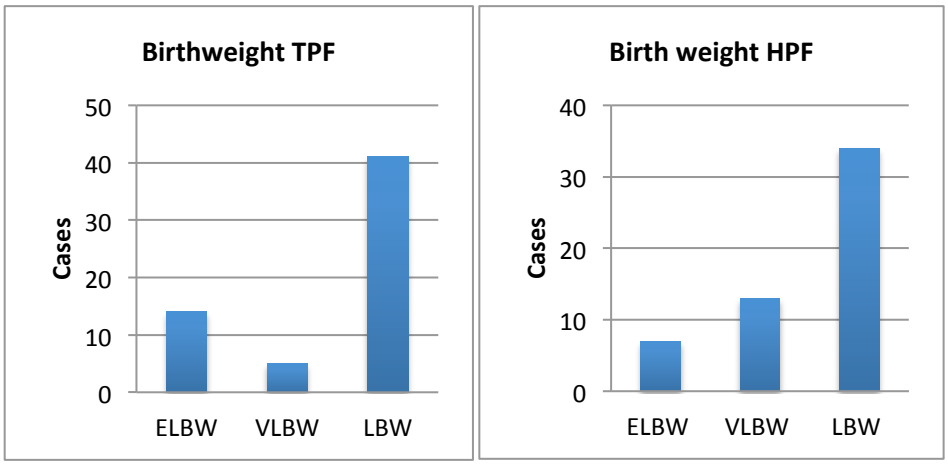


Figure 1: Infants defined as ELBW with birth weights <1000 grams, VLBW with birth weights from 1000-<1500 grams, and LBW with a birth weight from 1500 grams to <2500 grams. The majority of infants in both groups fall within the LBW category.

Extrauterine Growth Restriction:

Growth restriction was established by evaluating where each infant plotted on the Fenton growth chart at birth and then again at discharge. If the infant fell below the 10th percentile at either time, growth failure was identified. As seen in Figure 2, there were increases in growth failure in both groups. Growth restriction increased from 10% to 34% in the TPF group and 13% to 36 % in HPF group. However, there was no statically significant difference between the TPF and the HPF groups in regards to EUGR rates based on chi-squared testes with p = 0.49.

Figure 2: Small for gestational age/ intrauterine growth restriction vs. extrauterine growth restriction

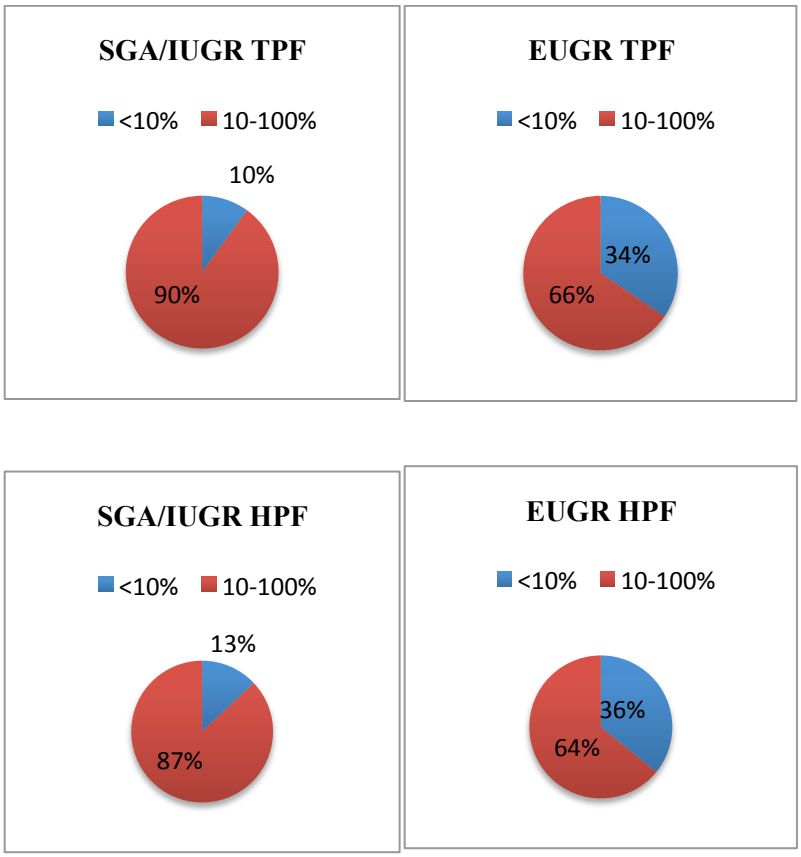


Figure 2: SGA- Small for gestational age, IUGR- intrauterine growth restricted, EUGR- extrauterine growth restricted. In each growth more infants were discharged growth restricted or <10th percentile than were born growth restricted.

Rates of necrotizing enterocolitis were similar between the two groups with 6 cases in each group, shown in Table 7.

Section 2- Relationships between groups

Table 6: Descriptive variables

	TPF		HPF		
Variable	**n**	**(Mean±SD)**	**n**	**(Mean±SD)**	***p* Value**
Apgar 1	59	6.39 ± 1.7	54	6.78 ± 2.0	0.265
Apgar 5	59	8.02 ± 0.8	54	8.11 ± 1.1	0.614
Ballard	60	18.8 ± 7.7	54	20.1 ± 7.1	0.366
GA (wk)	60	31.1 ± 3.2	54	31.5 ± 3.2	0.469
BW (gm)	60	1625 ± 528	54	1703 ± 535	0.433
d/c wt (gm)	58	2777 ± 616	52	2518 ± 464	0.014 *
Fenton % birth	60	41.6 ± 23.3	54	42.8 ± 25	0.786
Fenton % d/c	58	20.2 ± 17.6	52	21.1 ± 20.4	0.798
Length birth (cm)	58	40.5 ± 4.6	51	40.9 ± 4.4	0.615
FOC birth (cm)	60	28.5 ± 3.1	54	28.8 ± 2.8	0.583
FOC d/c(cm)	55	32.9 ± 1.8	52	32.5 ± 1.9	0.252
LOS	60	50.7 ± 38.8	54	39.7 ± 30.8	0.099^

^ $p < .10$. * $p < .05$.

Table 6: GA- gestational age, BW- birth weight, d/c wt- discharge weight, FOC- head circumference, LOS- length of stay.

Independent sample T-test were used to compare the means between the two groups on: Apgar score at 1 and 5 minutes, Ballard score, gestational age, birth length, length of stay, and birth and discharge weight, head circumference, percentile on Fenton growth chart. Analysis did not show statistically significant differences between the two groups, with the exception of discharge weight where the average discharge weight was 2777 ± 616 g in the TPF and a discharge weight of 2518 ± 464 g in the HPF group with a p value of 0.014, as seen in Table 6.

Length of stay was marginally significant $p < 0.1$. Infants in the TPF group were discharged at a higher weight, and also were admitted to the unit for longer. All other variables did not differ, including morbidity, initial acuity indicated by Apgar scores, gestational age reported or assessed with the Ballard. Birth length or head circumference (FOC) at birth or discharge did not differ.

The two groups did not differ in terms of morbidity and mortality, Table 7. Sepsis was marginally significant with p< 0.10. More infants the TPF were diagnosed with sepsis.

Table 7: Morbidity between groups

		TPF		**HPF**		
Variable		**n**	**[n(%)]**	**n**	**[n(%)]**	***p* Value**
Mortality		60	3 (5)	54	3 (5.6)	0.609
BPD/CLD	BPD	60	6 (10)	54	5 (9.3)	0.891
	CLD	60	6 (10)	54	8 (14.8)	
	both	60	1 (1.7)	54	1 (1.9)	
NEC	Stage 1	60	4 (6.7)	54	1 (1.9)	0.209
	Stage 2	60	2 (3.3)	54	5 (9.3)	
Sepsis		60	12 (20)	54	5 (9.3)	0.088^

Using Chi-Squared ^ $p < .10$. * $p < .05$.

Section 3 – Test hypothesis

Growth:

Hierarchical linear modeling was used to evaluate the differences between the infants that received traditional preterm formula, and those that received high protein preterm formula, each compared using the difference between birth weight and discharge weight. Each case was coded for the use of high protein preterm formula, traditional preterm formula, or other feeding product. Coding accounted for if the infant was on formula on one particular day and also for cumulative use of the product. Exploratory analysis was used to identify potential level 2 predictors as necrotizing enterocolitis, sepsis, and gender. The initial Ballard score (an indicator of the infant's maturity), initial Fenton %, and head circumference at birth were used to establish the y intercept and predict weight gain differences. To determine the slope of the line for day of life, NEC had a negative impact on weight gain as did sepsis, and female gender.

Table 8: Predictors of infants' weight gain

Predictor	Coefficient	*t*-value
Intercept	1513.57	86.52***
Ballard	35.99	7.59***
% on Fenton	3.30	4.36***
FOC@Birth	100.50	8.71***
Day of Life	24.48	29.34***
NEC X Day of Life	-2.16	-2.18*
SEPSIS X Day of Life	-2.70	-1.88^
Female X Day of Life	-2.92	-2.68**
Days on HPF	8.39	4.82***
Days on TPF	0.45	0.44

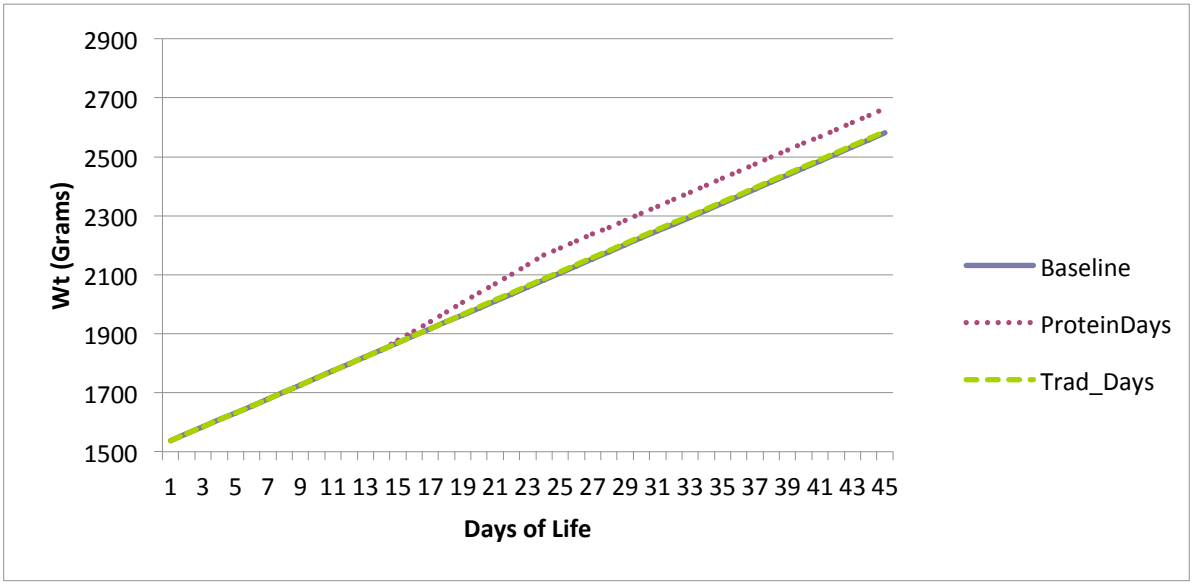
Note: *df*'s = 110, except for *df's* =1505 for last two coefficients.

^ $p < .10$. * $p < .05$. **P < .01. *** P < .001.

As seen in Table 8, the over all effect was an 8.4 gram per day increase in weight for each day the high protein product was used, and a 0.45 gram increase in weight gain for each day the traditional protein product was used compared to infants on breast milk or other formulas (baseline) as seen in the coefficient for the day on HPF and days on TPF respectively.

This graph in Figure 3 was created using the equation created for the analysis of weight gain between the two groups using a representative infant receiving formula starting on day of life (DOL) 15. That infant would then have received the formula for 10 consecutive days at which time they changed to the baseline feeding. It illustrates the expected increase in weight over the course of that infants stay.

Figure 3: Weight gain increases with high protein preterm formula



BUN:

BUN was also evaluated using hierarchical linear modeling. The y-intercept was based on birth weight and the slope was determined by the interaction between DOL day of life and birth weight, which negatively affected the slope. The same coding for formula used to establish the cumulative days on TPF or HPF use in the weight gain model was used in this analysis.

In Table 9, every day the infant received high protein preterm formula there was an increase in the BUN of 0.16 mg/dL above baseline, seen as the coefficient of Days on HPF. Baseline was determined using all infants that were not on preterm formula or the high protein preterm formula. These infants received breast milk, fortified breast milk, and others types of formula during their admission to the unit.

Table 9: Predictors of blood urea nitrogen

Predictor	Coefficient	*t*-value
Intercept	10.317	15.547***
Birth weight	0.003	0.006**
Day of Life	-0.064	-3.201**
BW X Day of Life	-0.0001	-3.631***
Days HPF	0.16	3.949***
Days TPF	-0.029	-1.056

Note: *df*'s = 110, except for *df*'s = 1505 for last two coefficients.

^ $p < .10$. * $p < .05$. **P < .01. *** P < .001.

Table 10: Blood urea nitrogen >20

Variable	**n**	**[n(%)]**	***p* Value**
Baseline	344	11 (3.2)	0.254
TPF	77	0 (0)	
HPF	49	2 (4.1)	

Using Chi-Squared ^ $p < .10$. * $p < .05$.

All BUN values were evaluated once infants were on full feedings. The BUN values were then grouped by what formula the infant was receiving when that lab was taken, TPF, HPF, or other used to establish the baseline. When chi-squared test was used to evaluate if infants on the high protein preterm formula had BUN >20 mg/dL more often than infants receiving the traditional preterm formula or infants receiving breast milk or another formula no difference was found, p = 0.254.

Section 4 – Summary

Overall there were no significant differences between the two study groups with the exception of discharge weight being higher in the TPF group. Although not significant, infants in the TPF group also had a mean longer length of stay by approximately 10 days and were more likely to be diagnosed with late onset sepsis.

Using modeling to compare traditional preterm formula, and high protein preterm formula, to growth and protein status there were significant increases in growth and BUN associated with the number of days the infants were receiving the high protein preterm formula with no adverse outcomes.

CHAPTER V

CONCLUSIONS AND DISCUSSION

Section 1 – Conclusions

Infants in both groups were similar with the majority of infants being classified as white with a mean gestational age of ~31 ± SD of 3.2 weeks. There were no differences in morbidity or mortality with the exception of sepsis being more prevalent in the traditional group. The infants in TPF group were discharged at a higher weight, but were also admitted to the unit for longer periods of time. This could be due to the higher protein formula leading to not just better growth, but also improved health leading to faster discharge. Overall there was the expected improvement in growth of 8.4 grams per day with every day an infant remained on the higher protein product. It appears that the higher protein product lead to both shorter stays and better daily growth, important findings, both for the health of the infants and the costs to the medical care system.

Section 2 – Relationship of findings to the literature

Promoting growth:

Many premature infants experience postnatal growth failure (Ziegler, 2007). Preterm infants are at a disadvantage as they have to grow outside the uterus. The goal of nutritional care for preterm infants is to promote growth at the same rate as an infant of the same gestational age in utero (AAP, 2009). Adequate nutrition to promote growth is essential in prevention of postnatal growth failure and also promotes optimal neurodevelopmental outcomes (Zielger, 2007). Early nutrition not only prevents growth failure it also promotes accrual of lean body mass, and increased mass in organs such as the brain, minimizing detrimental cognitive outcomes (Abernethy et al., 2004; Ehrenkranz et al., 2006; Ehrenkranz, 2000; Smart, 1990). With proper nutrition preterm infants have the ability to prevent extrauterine growth failure, and even obtain catch up growth in weight, length and head circumference indicating the brain too is catching-up (Isaacs et al., 2008). This study indicates that the higher level protein formula promotes achievement of these outcomes with better daily weight gain and shorter length of stays. However the study was not designed to determine the composition of the weight gain Further research will be needed to determine if infants gain lean mass or fat.

Traditional preterm formula or fortified breast milk is not concentrated enough to meet the ESPGHAN 2010 goals of 4-4.5 grams protein per kilogram. High protein preterm formula boost protein intake to 4.2 gram pro/kg to meet the higher estimated need for growth and development. This study shows that this increase in protein did promote weight gain in the infants receiving the high protein formula while there was no difference in weight gain between the other two options.

Preventing morbidity:

Those infants at particular risk for feeding difficulties are those that are less than 32 weeks gestation and/or very low birth weight infants. These two groups are at an increased risk

for necrotizing enterocolitis (NEC) and last onset sepsis (Cloherty et al., 2008). Both of these morbidities can negatively affect growth, so providing a formula that promotes growth, but increases the likelihood of morbidity or mortality is suboptimal. This study evaluated the effect of high protein preterm formula on these conditions and found no difference between groups in terms of NEC and mortality. The infants in the traditional preterm formula group were marginally significantly ($p = 0.088$) septic more often, indicating that the high protein preterm formula did not cause more late onset sepsis, and might even have improved outcomes.

With normal renal function a low BUN indicates inadequate protein intake. Very high BUN levels suggest possible excessive protein intake (Arslanoglu et al., 2006). Monitoring BUN can help to detect inadequate protein intake and be used to identify when excessive protein is being provided (Arslanoglu et al., 2006). Normal range for BUN in preterm infants in not well defined, according to Moyer-Mileur in Nutritional Care for High-Risk Newborns, 2000, 3-25 mg/dl is an acceptable range for preterm infants ≤ 1 week old. Other studies that have used BUN as a protein indicator set the ideal range for BUN from 9-14mg/dl, with > 20mg/dl considered as high (Arslanoglu et al., 2006). Providing excessive protein could lead to metabolic stress from protein overload (Tsang et al., 2005), increase in ammonia, blood amino acids, and an increase in late metabolic acidosis (Raiha, Heinonen, Rassin & Gaull, 1976). While this study did not evaluate these markers BUN was examined. There was a positive increase in BUN when the high protein formula was used however there was not an increased numbers of infants with a high risk in BUN(being > 20 mg/dL) .Thus it likely that the high protein formula is a safe product that promotes improved protein status. However further research may be necessary determine if there is a risk of excessive protein intake.

Section 3: Implications

This study shows that theoretical outcomes for increasing the protein content in preterm

formula hold true for the infants admitted to the NICU at S.t John's Medical Center during the study period. Improving growth in these infants with no adverse outcomes could potentially lead to infants that are discharged from NICU's with just not improved daily weight gain and potentially larger head circumferences indicating improved brain development but also shorted length of stays in the unit. Further study is indicated to assure that providing increased protein to promote growth does not lead to excessive BUN and an increased incidence of kidney injury. However, this study shows the improvement in growth with no adverse outcomes.

Section 4: Research Questions

The hypothesis of this study is that those infants in the high protein preterm formula group will show better overall growth with no adverse outcomes. The specific objectives of the study were:

I. To evaluate the effect of high protein preterm formula related to the promotion of improved growth in the preterm infants admitted to the Saint John Medical Center NICU. Expected outcomes will be: improved growth and a decrease in infants with extrauterine growth restriction as measured by differences in growth plotted on the Fenton growth chart from birth to discharge.

II. To evaluate changes in biochemical markers of protein status in preterm infants on different feeding regimens. With an increase in protein provided, improvements in blood urea nitrogen (BUN) are expected. Once the infant is on a stable fluid regimen and receiving full feeds the BUN should remain stable and indicate if the infant is receiving adequate amounts of protein.

III. Morbidity outcomes such as length of stay and incidence of necrotizing enterocolitis will be evaluated. Mortality will also be evaluated.

Objective I

While growth was improved when the high protein preterm formula was used there was not a reduction in EUGR rates.

Overall the hypothesis that the use of high protein preterm formula would show positive outcomes for weight gain was confirmed.

Objective II

Blood urea nitrogen was the biochemical marker used in this study to predict protein stores. There was an overall improvement in BUN in infant receiving the high protein preterm formula compared to other feeding type groups. While there was an increase in BUN, there was not an increased likelihood that an infant on high protein preterm formula would have BUN levels >20 indicating no adverse outcomes associated with the increased protein content of the formula.

Objective III

No differences between the groups were seen in terms of NEC and broncopulmonary dysplasia. There was an increase in late onset sepsis in group 1 indicating those infants may have been sicker overall, that same group also had a longer length of stay which likely explains why the infants were discharged at a higher weight than the infants in the high protein preterm formula group.

Section 5: Limitations and Further Research

One of the most significant limitations of this study was that it retrospectively evaluated infants during their admission. Alterations had to be made to what indicators were collected as large amounts of information that would have be helpful for this study were not reported in the electronic medical record. The PI was limited to collecting data from what was reported in the chart. Information like discharge length was unavailable and therefore could not be analyzed. In a prospective study the groups could be randomly assigned to the treatment group with high protein

preterm formula or the control of preterm formula. In this study very few infants in either group were solely on one formula. Most transitioned at least once if not several time from breast milk to one preterm formula or specialized formula or another during their admission. For example an infants trophic feeds would be initialed with preterm formula until the mothers breast milk came in, at that time they would transition to a mixture of breast milk and preterm formula. Often times an infant would then be transitioned back to preterm formula if the mothers supply ran low, or she was unable to maintain supply and opted to formula feed. To more clearly see the effect of the high protein formula having a group receive only that formulation would be beneficial. Analysis of body composition would have shown if the increase in weight gain was attributed to accruing lean body mass or fat mass. If the product simply creates "fatter" infants it likely would not me beneficial for broad use in a community NICU with varying ages and sizes of infants. While continuous data was collected on head circumference analysis of that data is outside the scope of this report. This information likely would have provided a clearer picture on brain development in this population. This study had a relatively small sample size, effects likely would have been more pronounced if the groups were larger.

Another limitation is the high likelihood that standard practice by the neonatologist and nurses in this unit changed from one group to the next due to the extended period of the study. Particularly because there is not a standardized feeding regimen in place at St. John NICU. Having such a protocol would have made it easier to define changes in outcomes. Without a standardized time line, there is an increase in variability in timing and feeding advancement from infant to infant, both of which can affect growth and outcomes.

Indications for future research include further investigation of the effects of increasing the amount of protein. This could also include the composition of the protein provided in the formula. Closer evaluation of composition of weight gain is essential for the promotion of a product such as high protein preterm formula. While there were no differences in morbidity or

mortality, investigation with the sole use of products such as the high protein preterm formula compared to a standard such as breast milk could be beneficial.

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APPENDICES I
LIST OF ABREVIATIONS

SJMC- St. John Medical Center

NICU- neonatal intensive care unit

GA- gestational age- first day of the woman's last menstrual cycle to the delivery date, measured in weeks

AGA- appropriate for gestational age- 10-90% for gestational age at birth

SGA- small for gestational age- less than 10% for gestational age at birth

IUGR- intrauterine growth restriction- less than 10% for gestational age with evidence of restriction due to pathological process in womb

EUGR- extrauterine growth restriction- birth weight that is appropriate for gestational age but by the time of hospital discharge weight is less than the 10% for corrected gestational age

Preterm- infants born before 37 completed weeks of gestational age

BW- birth weight

LBW- less than 2500 grams

VLBW- very low birth weight- less than 1500 grams

ELBW- extremely low birth weight- less than 1000 grams

TPF- traditional preterm formula- formula designed especially for preterm infants typically more highly concentrated in calories and protein.

HPF- high protein preterm formula- higher concentration of protein than preterm formula at same volume or concentration

EBM- expressed breast milk

Full feeds- infants are receiving all nutrition from formula or EBM, parenteral nutrition support discontinued, typically receiving feedings of 120-150 ml/kg.

Trophic feeds- nonnutritive feeds used to prime the gut, 1-25 ml/kg

NPO- nothing by mouth

TPN- Total parenteral nutrition- dextrose, amino acids, and fat provided via central line

FOC- Fronto-occipital circumference or head circumference

BUN- blood urea nitrogen, normal levels 3-25 mg/dL

Cr- creatinine

DOL- day of life

LOS- length of stay

D/C- discharge

NEC- necrotizing enterocolitis- an inflammatory necrosis of bowel

Late onset sepsis- blood infection that occurs between days 8 and 90

BPD- broncopulmonary dysplasia- infant less than 32 weeks gestation remaining on oxygen support for more than 28 days

CLD- chronic lung disease- infant remaining on oxygen support for more than 28 days

EMR- electronic medical record

AAP- American Academy of Pediatrics

ESPGHAN- European Society for Pediatric Gastroenterology, Hepatology, and Nutrition

APPENDIX II

IRB APPROVAL LETTERS

DATE:	March 27, 2012
TO:	Shelby Simpson, RD, LD
FROM:	St. John Medical Center IRB
PROJECT TITLE:	[315340-1] High Protein Pre-term Formula: Effect on Growth and Outcomes in Pre-term Infants Admitted to the NICU
SUBMISSION TYPE:	New Project
ACTION:	APPROVED
APPROVAL DATE:	March 27, 2012
EXPIRATION DATE:	March 26, 2013
REVIEW TYPE:	Full Committee Review

Thank you for your submission of New Project materials for this project. The St. John Medical Center IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a project design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Full Committee Review based on the applicable federal regulation.

Please remember that informed consent is a process beginning with a description of the project and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the project via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

Please note that any revision to previously approved materials must be approved by this office prior to initiation. Please use the appropriate revision forms for this procedure.

All UNANTICIPATED PROBLEMS involving risks to subjects or others (UPIRSOs) and SERIOUS and UNEXPECTED adverse events must be reported promptly to this committee. Please use the appropriate reporting forms for this procedure. All FDA and sponsor reporting requirements should also be followed.

All NON-COMPLIANCE issues or COMPLAINTS regarding this project must be reported promptly to this committee.

This project has been determined to be a Minimal Risk project. Based on the risks, this project requires continuing review by this committee on an annual basis. Please use the appropriate forms for this procedure. Your documentation for continuing review must be received with sufficient time for review and continued approval before the expiration date of March 26, 2013.

Please note that all research records must be retained for a minimum of three years after the completion of the project.

If you have any questions, please contact Dedee Boss at 918 744-2187 or dboss@sjmc.org. Please include your project title and reference number in all correspondence with this committee.

This letter has been electronically signed in accordance with all applicable regulations, and a copy is retained within St. John Medical Center IRB's records.

Oklahoma State University Institutional Review Board

Date:	Wednesday, April 04, 2012
IRB Application No	HE1228
Proposal Title:	High Protein Preterm Formula: Effect on Growth and Outcomes in Preterm Infants Admitted to the NICU
Reviewed and Processed as:	Exempt

Status Recommended by Reviewer(s): Approved Protocol Expires: 4/3/2013

Principal Investigator(s):

Shelby Simpson
4112 S. Madison Place
Tulsa, OK 74105

Tay Seacord Kennedy
301 HES
Stillwater, OK 74078

The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.

☒ The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

As Principal Investigator, it is your responsibility to do the following:

1. Conduct this study exactly as it has been approved. Any modifications to the research protocol must be submitted with the appropriate signatures for IRB approval.
2. Submit a request for continuation if the study extends beyond the approval period of one calendar year. This continuation must receive IRB review and approval before the research can continue.
3. Report any adverse events to the IRB Chair promptly. Adverse events are those which are unanticipated and impact the subjects during the course of this research; and
4. Notify the IRB office in writing when your research project is complete.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about the IRB procedures or need any assistance from the Board, please contact Beth McTernan in 219 Cordell North (phone: 405-744-5700, beth.mcternan@okstate.edu).

Sincerely,

Shelia Kennison, Chair
Institutional Review Board

VITA

Shelby Dederick Simpson

Candidate for the Degree of

Master of Science

Thesis: HIGH PROTEIN PRETERM FORMULA: EFFECT ON GROWTH AND OUTCOMES IN PRETERM INFANTS ADMITTED TO THE NEONATAL INTENSIVE CARE UNIT

Major Field: Nutritional Sciences

Biographical:

Education:

Completed the requirements for the Master of Science in Nutritional Science at Oklahoma State University, Stillwater, Oklahoma in July 2012.
Completed the requirements for the Neonatal Nutrition Fellowship at Baylor College of Medicine Houston, Texas, March 2010.
Completed the requirements for the Bachelor of Science in Public Health Nutrition at Kansas State University, Manhattan, Kansas in May 2006.
Completed the requirements for the Bachelor of Science in Dietetics at Kansas State University, Manhattan, Kansas in May, 2007.

Credentials:

Commission on Dietetic Registration, Registered Dietitian from June 2007 to present.
Oklahoma Board of Medical Licensure, Licensed Dietitian from August 2007 to present.

Experience:

Neonatal, Pediatric and Maternal Clinical Dietitian at St. John Medical Center from July 2010 to present.
Nutrition Core, LLC Consulting Dietitian from November 2010 to December 2011.
Neonatal, Pediatric, and Maternal Clinical Dietitian at Hillcrest Medical Center from August 2008 to May 2010.
Clinical Dietitian at Muskogee Regional Medical Center, from August 2007 to August 2008.

Conferences:

Baylor College of Medicine Neonatal Nutrition Conference, 2009, and assistant 2010.
Sodexo Regional RD meeting, 2010 Moderator of NICU breakout group.
Laying the Foundation for Better Nutrition Practices in the NICU at Shady Grove Adventist Hospital in Rockford Maryland, in 2009.

Name: Shelby Dederick Simpson Date of Degree: July, 2012

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: HIGH PROTEIN PRETERM FORMULA: EFFECT ON GROWTH AND OUTCOMES IN PRETERM INFANTS ADMITTED TO THE NEONATAL INTENSIVE CARE UNIT

Pages in Study: 57 Candidate for the Degree of Master of Science

Major Field: Nutritional Sciences

The purpose of this study was to evaluate if increasing the amount of protein provided to preterm infants improved growth and blood urea nitrogen levels without adverse outcomes. Additional expected outcomes were a decrease in infants with extrauterine growth restriction. Adverse outcomes such as length of stay, incidence of necrotizing enterocolitis, sepsis and mortality were also assessed.

The design of the study was a retrospective chart review utilizing a convenience sample from a local hospital's neonatal intensive care unit. The hospital had recently changed from a traditional formula to a higher protein formula. Singleton infants with a birth weight between 600 and 2500 grams and gestation at birth >24 to <37 weeks, admitted to the NICU during the six months prior to the unit's transition to high protein formula in December 2010 and six months after the transition were included. Differences in extrauterine growth restriction and adverse outcomes were assessed by t- tests or chi-square. Hierarchical linear modeling was used to assess the primary hypothesis of differences in weight gain and blood urea nitrogen between groups.

Infants in both the traditional formula group (n= 60) and the high protein formula group (n = 54) were similar with the majority of infants being classified as white with a mean gestational age of ~31 weeks. There was an improvement in growth of 8.4 grams per day with every day an infant remained on the higher protein product. There was also an increase in BUN of 0.16 mg/dL for every day an infant received the higher protein preterm formula. The infants in the traditional preterm formula group were discharged at a higher weight (p=.014), but were also admitted to the unit for 11 days more on average (p= .10). Sepsis was diagnosed more frequently in the traditional preterm formula group (p= .08).

Conclusion: The higher protein formula lead to better daily growth and improvements in blood urea nitrogen, a measure of protein status. The higher protein product did not increase adverse outcomes, but did demonstrate clinically important trends toward decreased incidence of sepsis, and shortened length of stay.

ADVISER'S APPROVAL: Dr. Tay Kennedy