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Title: Clinical Predictors of Necrotizing Enterocolitis in Premature Infants

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Abstract: Abstract

**BACKGROUND:** Necrotizing enterocolitis (NEC) has emerged as the most common neonatal gastrointestinal emergency, is the most common cause of death in neonates undergoing surgery, and accounts for yearly additional hospital charges in excess of \$6.5 million. Prematurity is the only common variable identified in case-controlled studies exploring this disease.

**OBJECTIVES:** The aim of this study was to improve the understanding of the relationship between factors related to intestinal inflammation and ischemia and the enteral feeding regimen in the context of the premature gut, thereby identifying antecedents of NEC.

**METHOD:** This retrospective, case-controlled study involved data collected from the medical records of 247 premature infants. Diagnosis of NEC, as defined by Bell Stages IIA-IIIB, was required for study group assignment (n=84). Multivariate analysis techniques were used to predict the relationships between selected variables on the outcome of NEC.

**RESULTS:** Premature infants were 14 times more likely to develop NEC if the infant required increased respiratory support to maintain oxygenation during the early neonatal period and 4.5 times more likely to develop NEC if the infant did not receive nutritionally fortified enteral feedings of breast milk. When both factors were present, the odds of NEC increased 28.6 times when compared to infants without these factors.

DISCUSSION: The study findings extend our knowledge of antecedents to NEC beyond prematurity, highlighting the role that respiratory support and nutritional fortification of enteral feedings play in the pathogenesis of this disease. Early identification of antecedents to NEC will improve critical care management of the neonate and in turn, decrease the incidence of this devastating gastrointestinal disease. The study findings will guide further inquiry in neonatal nutrition, physiologic and metabolic functioning, and acute clinical management of the neonate.

June 1, 2007

Molly C. Dougherty, PhD, RN, FAAN  
Editor  
*Nursing Research*  
School of Nursing  
CB#7460 Carrington Hall  
University of North Carolina  
Chapel Hill, NC 27599-7460

Dear Dr. Dougherty:

Please find enclosed the manuscript entitled “Clinical Predictors of Necrotizing Enterocolitis in Premature Infants”, authored by Katherine E. Gregory, RN, PhD. This manuscript reports findings from the retrospective medical review of 247 premature infants cared for in the neonatal intensive care unit at Tufts-New England Medical Center in Boston, MA. The results highlight the relationship between factors related to intestinal inflammation and ischemia, and nutritional fortification in enteral feedings on necrotizing enterocolitis (NEC) in premature infants.

Prior to any research activities, Institutional Review Board Approvals were obtained from Tufts-New England Medical Center and Boston College. Because this research involved personal information obtained from individual medical records, all HIPAA guidelines, as well as hospital policy pertaining to medical record retrieval and review were strictly adhered to by the investigator.

NEC in premature infants is a problem of relevance to researchers as evidenced by the recent Request for Applications: New Approaches for the Prevention and Treatment of Necrotizing Enterocolitis (R01), (RFA-HD-07-018). This RFA was posted by National Institute of Child Health and Human Development, National Institute of Allergy and Infectious Diseases, and National Institute of Diabetes and Digestive and Kidney Diseases. The research reported in this manuscript will make an important contribution to the literature on NEC as it was conducted from a nursing perspective using theoretically derived clinical variables to predict NEC in a population of premature infants.

Thank you for your review and consideration of this manuscript for publication in *Nursing Research*.

Sincerely,

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Running head: CLINICAL PREDICTORS OF NEC

Clinical Predictors of Necrotizing Enterocolitis in Premature Infants

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14 Clinical Predictors of Necrotizing Enterocolitis in Premature Infants

## Abstract

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3 gastrointestinal emergency, is the most common cause of death in neonates undergoing surgery,  
4 and accounts for yearly additional hospital charges in excess of \$6.5 million. Prematurity is the  
5 only common variable identified in case-controlled studies exploring this disease.

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11 required for study group assignment (n=84). Multivariate analysis techniques were used to  
12 predict the relationships between selected variables on the outcome of NEC.

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14 increased respiratory support to maintain oxygenation during the early neonatal period and 4.5  
15 times more likely to develop NEC if the infant did not receive nutritionally fortified enteral  
16 feedings of breast milk. When both factors were present, the odds of NEC increased 28.6 times  
17 when compared to infants without these factors.

18 DISCUSSION: The study findings extend our knowledge of antecedents to NEC beyond  
19 prematurity, highlighting the role that respiratory support and nutritional fortification of enteral  
20 feedings play in the pathogenesis of this disease. Early identification of antecedents to NEC will  
21 improve critical care management of the neonate and in turn, decrease the incidence of this  
22 devastating gastrointestinal disease. The study findings will guide further inquiry in neonatal  
23 nutrition, physiologic and metabolic functioning, and acute clinical management of the neonate.

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2 Key Words: Necrotizing enterocolitis, premature infant, neonatal intensive care

## Clinical Predictors of Necrotizing Enterocolitis in Premature Infants

1           Necrotizing enterocolitis (NEC), characterized by ischemic necrosis of the bowel, has  
2 emerged as the most common neonatal gastrointestinal emergency in many countries of the  
3 world. NEC affects approximately 5% of infants requiring neonatal intensive care with the  
4 incidence estimated as high as 15% if early neonatal deaths caused by cardiac and respiratory  
5 disease are excluded (Chandler & Hebra 2000; Lee & Polin, 2003). NEC is the most common  
6 cause of death in neonates undergoing surgery and results in as many as 3,000 deaths per year  
7 (Lee & Polin, 2003; Neu, 1996). Infants diagnosed with NEC require longer, more resource  
8 intensive hospitalization. The yearly additional hospital charges for NEC are estimated in excess  
9 of \$6.5 million, or approximately \$216,666 per survivor (Bisquera et al., 2002).

11           NEC is a disease of premature infant survivorship. The sophisticated technology and  
12 pharmaceuticals implemented in neonatal intensive care over the past two decades have  
13 revolutionized care, resulting in improved outcomes for premature infants. Survival among  
14 infants born premature has increased from 15% to 75% over the past decade. This is due in large  
15 part to the decrease in incidence of acute and chronic respiratory diseases, which historically  
16 were the limiting factors in premature infant survival and clinical outcomes. However, with  
17 gains in premature infant survival have come significant increases in morbidity related to  
18 immaturity of gastrointestinal function (Berseth, 1996). Infants who develop gastrointestinal  
19 diseases such as NEC are not only at increased risk of infection and death, but also of long term  
20 health consequences that include bowel stricture, fistula, abscess, recurrent NEC, short-gut  
21 syndrome, malabsorption, and total parenteral nutrition (TPN) related complications (Bisquera,  
22 Cooper, & Berseth, 2002; Lee & Polin, 2003). These issues seriously compromise premature  
23 infant growth and development. In short, identifying the clinical predictors of this catastrophic

1 gastrointestinal disease and more important, identification of prevention strategies for NEC  
2 remains one of the major unsolved problems in neonatal care.

3         The specific aim of this research, as derived from the conceptual framework in Figure 1,  
4 was to explore the relationship between variables related to intestinal inflammation and ischemia  
5 during the early neonatal period and factors related to the enteral feeding regimen on the  
6 incidence of NEC in the premature infant. The study proposes that hypoxia, hypotension and  
7 hypothermia, as well as decreased superior mesenteric artery flow during the early neonatal  
8 period decrease blood flow to the premature intestine causing the bowel to be at especially high  
9 risk for ischemic injury. The mechanism of intestinal injury under these circumstances may be  
10 related to decreased or absent intrinsic ability of the neonatal intestine to regulate blood flow and  
11 oxygenation. Since oxygen extraction and mucosal blood flow appear to be near maximal during  
12 feeding, the newborn intestine may be unable to maintain oxygen uptake in the presence of  
13 superimposed cardiovascular stress. For this reason, physiologic stability (i.e. structural  
14 integrity, conservation of energy, and hemodynamic functioning) in the frail infant must  
15 underscore research initiatives aimed at better understanding the incidence of NEC in the  
16 premature infant patient population.

## 17 Background

18         Modern sophistication of neonatal intensive care including surfactant therapy, improved  
19 methods of mechanical ventilation and the availability of highly skilled neonatal teams working  
20 in technologically enabled NICUs has resulted in a growing population of very low birth weight  
21 (VLBW) infants. Epidemiologic and case controlled studies indicate that the incidence of NEC  
22 varies inversely with an infant's birth weight and gestational age (Chandler & Hebra, 2000;  
23 Rowe et al., 1994; Stoll, 1994). Infants weighing between 500 and 1500 grams at birth and born

1 prior to 28 weeks gestation constitute the majority of NEC patients and have thus been identified  
2 as highest risk of developing the disease (Chandler & Hebra, 2000; Kosloske, 1997; Rowe et al.,  
3 1994). Mortality secondary to NEC in the premature infant population weighing less than 1,000  
4 grams ranges from 35% to 50% (Chandler & Hebra, 2000). In sum, the morbidity and mortality  
5 secondary to NEC will become an increasingly significant neonatal problem as the number of  
6 VLBW infants continues to rise.

7 Numerous physiologic variables are associated with the development of NEC; however,  
8 only prematurity has been consistently identified as a common factor in case-controlled studies  
9 exploring this serious gastrointestinal disease (Chandler, & Hebra, 2000; Crissinger, 1999;  
10 Gamsu & Kempley, 1997; Kliegman, 2003). Intestinal barrier immaturity and dysfunctional  
11 gastrointestinal functioning common to the premature infant are the suspected underlying defects  
12 that play a significant role in the pathogenesis of NEC (Crissinger, 1999). However, it is likely  
13 that NEC involves multiple factors in the setting of a stressed gut with immature protective  
14 mechanisms (Lee & Polin, 2003). Intestinal ischemia, infectious bacteria, and introduction of  
15 substrate (infant formula or breast milk) to the immature neonatal gastrointestinal tract have long  
16 been the focus of investigators seeking to better understand NEC (Lawrence, Bates, & Gaul,  
17 1982; Santulli, Shullinger, & Heird, 1975). This study tested the relationship of intestinal  
18 inflammation and subsequent ischemia in concert with factors related to the enteral feeding  
19 regimen on the development of NEC.

#### 20 *Intestinal Inflammation and Ischemia*

21 Hypoxic-ischemic injury resulting from inflammation of the newborn bowel has long  
22 been identified as an antecedent in the pathogenesis of NEC (Gamsu & Kempley, 1997;  
23 Kosloske, 1997; Lee & Polin, 2003; Lloyd, 1969). Early researchers proposed that infants who

1 were physiologically stressed exhibited reflex circulatory shunting to selectively perfuse the  
2 brain and heart at the expense of other “nonvital” organs (Gamsu & Kempley, 1997; Lloyd,  
3 1969). This hemodynamic reflex as it is understood serves to conserve blood flow to essential  
4 organs and decrease perfusion to the gut, leading to low cardiac output and selective splanchnic  
5 ischemia (Lloyd, 1969; McElhinney et al., 2000). While decreased blood flow to the gut  
6 resulting in inflammation and ischemia is accepted as an important component in the  
7 development of NEC, the point at which changes in intestinal tissue occur in the pathogenesis of  
8 NEC has been the topic of recent debate and the focus of innovative studies (Chan, et al., 2002;  
9 DiLorenzo & Krantis, 2001). As a result of research studying the roles of inflammation and  
10 intestinal ischemic injury in the cascade of events leading to NEC, researchers have proposed a  
11 more sophisticated physiologic model underpinning this disease in the preterm infant.

12         Laboratory research using animal models has demonstrated that a lack of perfusion to the  
13 bowel is a critical antecedent to inflammation, ischemia and tissue necrosis (Crissinger, Burney,  
14 Velasquez, & Gonzalez, 1994; Crissinger & Granger, 1989; Crissinger, Tso, & Burney, 1992).  
15 Furthermore, histopathologic data has consistently indicated that inflammation and ischemia of  
16 the intestinal tissue occur as part of the disease process underlying NEC (Ballance, Dahms,  
17 Shenker, & Kliegman, 1990). However, in contrast to early researchers’ hypotheses that  
18 intestinal ischemia was one of the initial events that occurred predisposing the bowel to NEC, the  
19 current theory proposed by scientists is that intestinal ischemic injury is the final step in a  
20 cascade of events that result from the premature infant’s immature intestinal function. It is  
21 believed that the mucosal injury, which then progresses to NEC, is due to a number of interacting  
22 risk factors at play in the preterm intestine (Crissinger, 1999). Risk factors, most of which are  
23 related to intestinal barrier immaturity, include decreased mucus production, increased

1 susceptibility to disruption of the epithelial cell layer, decreased repair capacity, decreased tissue  
2 antioxidant activity, immature regulation of intestinal blood flow and oxygenation, increased  
3 susceptibility to inflammatory mediators, dysfunctional immune response, and abnormal gut  
4 motility (Crissinger, 1999).

5 In sum, ischemic injury to the bowel is perhaps a secondary event that is mediated by  
6 biochemical processes within the vascular workings of the preterm gut. Regardless of the  
7 specific biochemical and molecular pathways underlying ischemic injury observed in the preterm  
8 intestine and characterizing NEC, adequate blood flow to the bowel remains an essential  
9 component in ensuring integrity of intestinal function. However, little has been published on the  
10 relationship between specific clinical events that may act as precursors to decreased blood flow  
11 precipitating inflammation, intestinal ischemia, and NEC. Such events include significant  
12 hypoxia, hypotension, and hypothermia, all of which have been associated with disruption in  
13 intestinal blood supply (Alexander, 1975; Chandler & Hebra, 2000; Gamsu & Kempley, 1997).

14 In this study, clinical events measured as precursors to hypoxia, inflammation, and  
15 ischemia included: all resuscitation efforts at birth and during the acute phase of neonatal care to  
16 correct for hypoxia, hypotension, and hypothermia; apnea, bradycardia or significant oxygen  
17 desaturation requiring increased respiratory or hemodynamic support interventions; and  
18 interventions known to decrease superior mesenteric artery blood flow including indomethacin  
19 administration and umbilical vessel catheterization. These variables are defined in Table 1.

#### 20 *Enteral Feeding Regimen*

21 Although NEC has been diagnosed in infants who have never been fed, 90-95% of cases  
22 occur in infants with a history of recent feeding volume advancement or reinitiation of enteral  
23 feedings (Stoll, 1994). The introduction of enteral substrate such as infant formula or breast milk

1 into the neonatal intestinal lumen presumably causes a disruption of mucosal integrity, blood  
2 flow and motility playing a key role in the pathogenesis of NEC (Lee & Polin, 2003). The  
3 overgrowth of pathogens in both the small and large intestine are thought to be promoted by  
4 unabsorbed luminal nutrient, which acts as a substrate for bacterial growth (Kliegman, 2003).  
5 These enteric bacteria may ferment, producing the intraluminal gas which characterizes  
6 pneumatosis intestinalis, a key diagnostic radiological feature of NEC. The various gases  
7 produce distension and increased intraluminal pressure, which are likely to play a role in  
8 decreased mucosal blood flow (Kien, 1990; Kliegman, 2003). This provides evidence linking  
9 bacterial fermentation of unabsorbed carbohydrate to NEC and strongly suggests that enteral  
10 alimentation is critical in the cascade of events leading to NEC. In sum, the preterm infant's  
11 immature gastrointestinal functions (digestion, gut motility, defense mechanisms, vascular and  
12 microvascular circulation, and mucosal integrity) contribute in large part to the role enteral  
13 feeding plays on the incidence of NEC (Kliegman, 2003; Williams, 1997).

14         The premature infant's gastrointestinal tract has many developmental and mechanical  
15 features that make use of it difficult or impossible during the early neonatal period.  
16 Nevertheless, evidence suggests that small amounts of early enteral nutrient delivery enhances  
17 infant growth and maturation of the gastrointestinal system, and postponing enteral feeding does  
18 not prevent NEC (Berseth, 1992, 1995, 1996; McClure & Newell, 2000; Schanler, Shulman,  
19 Lau, O'Brien Smith, & Heitkemper, 1999). Early case controlled studies indicate no significant  
20 difference in age of first enteral feeding between infants who developed NEC and those who did  
21 not develop NEC (deCurtis et al., 1987; Stoll, Kanto, Glass, Nahmias, & Brann, 1980). A more  
22 recent case controlled study did demonstrate that NEC infants were fed significantly earlier than  
23 non-NEC infants (5.1 versus 7.7 days of life at first feed) but also indicated that some late-fed

1 infants developed NEC at a later point during the neonatal period (McKeown et al., 1992). Thus,  
2 any early benefit seemingly attributable to withholding initiation of enteral feeding may simply  
3 reflect postponement of the disease.

4         The time of initial enteral feeding, initial volume and rate of advancement of feedings  
5 over the course of the neonatal period, as well as the type of enteral feeding are important factors  
6 in determining the degree of mucosal insult that may occur in the preterm gut. Much of the  
7 research in this area is conflicting and no consensus has been reached regarding the most  
8 effective neonatal enteral feeding regimen. The association between several neonatal enteral  
9 feeding variables and their significance in the pathogenesis of NEC warrants their inclusion in  
10 studies exploring this disease.

#### 11 Conceptual Framework

12         Figure 1 illustrates the conceptual framework developed for the purposes of this study.  
13 The framework is based in part on Myra Levine's Conservation Principles (Levine, 1989). It  
14 serves as a theoretical underpinning for the interrelationships between factors pertaining to  
15 enteral feeding progression and intestinal inflammation and ischemia thought to precipitate NEC  
16 in the context of the premature gut. This framework does not seek to identify NEC as a disease  
17 related to enteral feeding or intestinal ischemia alone. Rather, NEC is seen as a neonatal  
18 problem of adaptation and conservation that stems from a multitude of factors that unfold and  
19 interrelate when the preterm infant is compromised as a result of being born too soon. In the  
20 model, the bold arrows around the figure represent conservation of energy and structural  
21 integrity of the preterm infant. Growth and development are not compromised. The physiologic  
22 stability of the preterm infant intestine remains intact. The dotted lines leading from the  
23 constructs of enteral feeding progression and intestinal inflammation and ischemia represent

1 disruption in physiologic stability and growth and development of the premature infant, leading  
2 to NEC in the presence of the immature gastrointestinal system.

3         This model is based on the idea that when one physiologic system of the body is stressed,  
4 other physiologic systems capable of conserving energy will do so. In the premature infant,  
5 physiologic conservation during respiratory and cardiovascular induced stress has the potential to  
6 compromise the integrity of the bowel, ensuring that the infant's heart, lungs and brain remain  
7 hemodynamically intact. NEC therefore may be an unfortunate side effect of a well designed  
8 system of physiologic conservation. Nursing research is needed to understand how multiple  
9 simultaneous physiologic stressors such as respiratory and cardiovascular instability, interruption  
10 in systemic blood flow, and stresses on the gastrointestinal system impact physiologic stability,  
11 growth, development, and subsequent disease pathogenesis. This knowledge will result in the  
12 identification of antecedents to disease and has the potential to create a framework for  
13 development of prevention strategies.

#### 14 Methods

##### 15 *Data Collection and Study Sample*

16         This retrospective, case-controlled study involved data collected from the medical  
17 records of 247 preterm infants born at an urban, academic medical center in the Northeastern  
18 United States. Study sample inclusion criteria were infant birth weight less than 1500 grams and  
19 gestational age less than 34 weeks. Diagnosis of NEC, as defined by Bell Stages IIA-IIIB (Bell,  
20 et al., 1978), was required for assignment to the study group (n=84). Two non-NEC diagnosed  
21 patients were matched to each NEC diagnosed patient based on two study variables: year of birth  
22 and one of four birth weight cohorts (500-750 grams, 751-1000 grams, 1001-1250 grams, 1251-

1 1500grams). These matching variables were selected to control for any change practice from  
2 one year to the next and to ensure that the groups were similar as predicted by birth weight.

3 Study data were collected by the investigator from individual medical records after  
4 appropriate approvals from the Institutional Review Boards were granted. All other regulations  
5 specific to medical record review required by hospital policy were strictly adhered to by the  
6 investigator. Data collected on the specific study variables were organized under three main  
7 constructs: biodemographic factors, intestinal inflammation and ischemia, and enteral feeding  
8 regimen. The study variables are further defined in Table 1. The majority of the variables were  
9 measured dichotomously. Threshold values were determined for variables that were continuous  
10 in nature (i.e. Apgar scores).

#### 11 *Data Analysis*

12 Descriptive statistics were calculated for each variable. Variables that were highly  
13 correlated to one another were collapsed accordingly. A Bonferroni correction was made to  
14 correct for a Type I error. The alpha of significance for variable selection was set at  $p < 0.002$   
15 ( $.05/28 = 0.00178$ ). Multivariate analysis techniques including logistic regression with maximal  
16 likelihood estimation were used to predict the relationships between theoretically derived study  
17 variables on the outcome of NEC in the premature infant. Odds ratios and estimates of relative  
18 risk were calculated to predict which factors best differentiated between infants who developed  
19 NEC and those who did not. A 95% confidence interval was used for this study.

#### 20 Results

##### 21 *Study Sample*

22 In this sample of 247 preterm infants, 92.7% (n=229) were born prior to 32 weeks  
23 gestation and 74.9% (n=185) had a birth weight less than 1250 grams. 51.8% (n=128) of the

1 sample were male in gender and 71.3% (n=176) were white-non Hispanic in race. Of the 84  
2 subjects diagnosed with NEC, the average age at diagnosis was 16.27 days of life. Surgical  
3 management of NEC was required for 12.6% (n=31) of the study subjects or 38% of the total  
4 NEC patient group.

5 Descriptive data on the study sample are reported in Table 2, with  $p$  values of the chi-  
6 square analysis conducted to make group comparisons prior to selection of variables to be  
7 included in the logistic regression model. Variables of significance between the NEC and non-  
8 NEC groups included increased respiratory support ( $p=.000$ ) and increased hemodynamic  
9 support/neonatal code ( $p=.000$ ) during the early NICU course, and three enteral feeding  
10 variables: presence of fortification in breast milk ( $p=.000$ ), presence of fortification in infant  
11 formula ( $p=.001$ ), and change from unfortified enteral feeding to fortified enteral feeding  
12 ( $p=.000$ ). These five variables were selected for the logistic regression model.

13 A correlation matrix was assessed prior to conducting the logistic regression analysis. While  
14 several of the correlations were significant at the 0.01 level (2-tailed), the majority of the  $r$  values  
15 fell below 0.49 representing little if any relationship to one another.

#### 16 *Predictors of NEC*

17 The variables of significance were entered in two blocks in congruence with the  
18 conceptual framework. Increase in respiratory support and increase in perfusion support  
19 required to maintain hemodynamic perfusion of the neonate, both physiologic stability factors  
20 representing inflammation and ischemia, were entered in the first block. The first block was  
21 significant ( $p=.000$ ) and accounted for 16.8% (Cox & Snell  $R$  Square) to 23.2% (Nagelkerke  $R$   
22 Square) of the variance. The Hosmer and Lemeshow Goodness of Fit test indicated that the data  
23 fit the model ( $p= 1.000$ ). Variables related to enteral feeding were entered in the second block.

1 Fortification of breast milk, fortification of infant formula, and a change in fortification added to  
2 enteral feedings were entered in the second block, which was significant ( $p=.000$ ). The final  
3 model was significant ( $p=.000$ ) and accounted for 29.4% (Cox & Snell  $R$  Square) to 40.6%  
4 (Nagelkerke  $R$  Square) of the variance.

5 The specificity for correct classification was higher for non-NEC cases (88.7%) than was  
6 the sensitivity for predicting NEC cases (62.7%). The overall correct group classification was  
7 79.8%, with the model performing better at correctly classifying patients not diagnosed with  
8 NEC than patients who were diagnosed with NEC.

9 Two variables made significant contributions to predicting which premature infants  
10 developed NEC. When an infant required an increase in respiratory support in the NICU, the  
11 odds of developing NEC increased 13.9 times (RR= 5.01, CI= 4.815-40.218). When an infant's  
12 enteral feeding regimen did not include nutritional fortification of breast milk, the odds of  
13 developing NEC increased 4.47 times (RR= 3.13, CI= 1.128-17.713). These results are reported  
14 in Table 3.

15 The findings provide evidence that preterm infants who require an increase in respiratory  
16 support to maintain oxygenation during the neonatal period are almost 14 times more likely to  
17 develop NEC than preterm infants who do not have this physiologic need. In reviewing the  
18 analysis related to enteral feedings, preterm infants who do not have nutritional fortification  
19 added to the breast milk that they receive are 4 to 5 times more likely to develop NEC than  
20 preterm infants who do have fortification added to the breast milk that they receive. When both  
21 of these factors were present, the odds of developing NEC increased to 28.61, when compared to  
22 premature infants who did not have these either of these risk factors.

23 Discussion

1           The study findings underscore significant relationships between hypoxic events requiring  
2 increased respiratory support during the early neonatal period and the role that nutritional  
3 fortification as part of the enteral feeding regimen plays in development of NEC.

#### 4 *Intestinal Inflammation and Ischemia*

5           The single variable of significance identified within the construct of intestinal  
6 inflammation and ischemia was the premature infant's experience of hypoxia and need for  
7 increased respiratory support during the early neonatal period. As previously stated and reported  
8 in Table 1, hypoxia was defined and measured as any increase in the respiratory support  
9 measures put in place to maintain adequate oxygenation during the NICU course. Transitioning  
10 an infant to continuous positive airway pressure (CPAP) via nasal prongs from respiratory  
11 support in the form of oxygen delivered via a nasal canula was measured as an increase in  
12 respiratory support. Transitioning the infant from CPAP to intermittent mechanical ventilation  
13 (IMV) via endotracheal tube, again, as a result of worsening clinical performance and abnormal  
14 blood gases, qualified as an increase in respiratory support. This predictor was important in the  
15 context of the theoretical underpinnings of this research, which assert that any change in the  
16 physiologic functioning of the infant that disrupts physiologic stability and growth and  
17 development place the infant at risk for comorbid conditions such as NEC.

18           Hypoxia as evidenced by increased respiratory support as a predictor variable in this  
19 analysis is problematic in that many preterm infants require an increase in respiratory support for  
20 other clinical reasons, regardless of forthcoming NEC diagnosis. Sepsis, for example, often  
21 accompanies the onset of NEC, and almost always requires an increase in respiratory support to  
22 maintain adequate oxygenation. The question of whether or not the increased respiratory support  
23 required by premature infants to maintain adequate oxygenation was truly a result of decreased

1 physiologic stability in the context of NEC or merely an associated factor of sepsis or other  
2 clinical complication cannot be determined by this study.

### 3 *Enteral Feeding Regimen*

4 Enteral feedings of breast milk without the addition of nutritional fortification was the  
5 second significant predictor identified by the logistic regression model. While a great deal has  
6 been published about the protective effects of breast milk on the preterm infant gut (Lucas &  
7 Cole, 1990; Williams, 1997), not as much is known about the nutritional fortification that is  
8 routinely added to the enteral feedings of preterm infants to promote growth. The findings of  
9 this study suggest that nutritional fortification, when added to breast milk, might have a small  
10 protective effect against NEC. While NEC was 4.5 times higher in preterm infants fed breast  
11 milk without fortification as compared to preterm infants fed breast milk with fortification, there  
12 are several issues associated with this variable that warrant discussion.

13 Nutritional fortification was a dichotomous measure captured as any form of fortification  
14 added to breast milk or infant formula to increase the caloric density of the enteral feeding  
15 substrate. Fortification included elements such as commercially prepared human milk fortifier  
16 (HMF), medium chain triglyceride (MCT) oil, high concentrate glucose solutions (Polyose),  
17 and protein powders (Beneprotein). This study did not differentiate between the various types or  
18 amounts of fortification added to the enteral feedings. The measure also did not capture the time  
19 at which fortification was added to the enteral feeding (i.e. infant age at which fortification was  
20 added or day on enteral feeding regimen). Further study of these more specific factors related to  
21 the types of nutritional fortification added to enteral feedings administered to preterm infants is  
22 an area of neonatal research that requires further inquiry.

1           The enteral feeding regimen can vary widely in implementation from one infant to another.  
2   Since nutritional fortification is typically only added to the enteral feeding regimen after full  
3   feeding volumes have been achieved (120-150 cc/kg/day of fluid intake supplied solely by  
4   enteral feedings), this predictor might have more to do with the age and health of infant receiving  
5   enteral feedings, as well as their success with the enteral feeding regimen. Infants fed breast  
6   milk with nutritional fortification are more likely to have tolerated early enteral feeding and be  
7   demonstrating consistent weight gain and growth. Infants who have not progressed along the  
8   enteral feeding regimen far enough to be weaned from IV fluids and fed full volume feeds with  
9   fortification are more likely to be younger, sicker, and consequently, at higher risk of NEC. In  
10   sum, one might expect NEC to be higher in infants who receive breast milk without fortification  
11   and lower in infants who receive breast milk with fortification simply because it is a reflection of  
12   the infant's maturity, success with the enteral feeding regimen, and overall health. Thus, the  
13   findings of this study support a potentially new theory that risk of NEC is lower in infants who  
14   receive all of their daily nutrition volume from enteral feedings and have progressed along the  
15   enteral feeding regimen with enough success to a point where nutritional fortification is added to  
16   their breast milk.

17           Enteral feeding regimens have become more complicated in the NICU, with several  
18   attributes, and many poorly understood factors. The type and amount of substrate administered,  
19   and the time at which feedings are initiated and advanced not only have implications on the  
20   growth and development of the preterm infant, but also play a role in the development of NEC.  
21   The findings from this study related to enteral feeding regimens are unique in that infants who  
22   progressed to full volume feedings of breast milk fortified with nutritional supplementation were  
23   at lower risk of NEC. Upon dissemination, this finding will suggest to neonatal clinicians that the

1 time of highest risk for NEC is prior to achievement of full feeding volumes. Clinical protocols  
2 aimed at monitoring for signs of feeding intolerance and poor growth should be designed and  
3 implemented as part of NEC surveillance and prevention throughout the enteral feeding regimen,  
4 but especially during the time of advancement to full feeding volume.

#### 5 *Limitations*

6 In addition to the limitations pertaining to the specific predictors discussed above, the  
7 study findings are limited by the number of subjects included in each of the comparison groups.  
8 Had this been a multi-site study, access to a greater number of patient records would have been  
9 possible, and in turn, a larger sample of data obtained. Replication of this study across multiple  
10 NICU sites would not only increase the sample size of the comparison groups, but also the  
11 representative nature of the findings.

12 Inherent in any retrospective study design using existing medical record data are  
13 unknown errors based on medical record documentation. While the medical record is rich with  
14 patient information and clinical case findings, flawed results are to be expected when the medical  
15 record is the sole source of study data. Incomplete and inaccurate documentation of the patient's  
16 clinical events and critical care progress is the unfortunate reality of contemporary health care  
17 that functions without electronic medical records and too few patient care resources.

18 Researchers seeking to use information documented in the medical record as study data should  
19 consider a prospective research design that allows for review of the medical record and data  
20 collection while the patient is being cared for by the clinical team documenting on the patient  
21 status. This design would ensure that study data obtained from the medical record was verified  
22 with the clinicians caring for the patient at the current time of hospitalization.

23 Conclusion

1           Premature birth and low birth weight are serious infant health issues for all demographic  
2 groups, affecting males and females from a diversity of racial and ethnic backgrounds. NEC is  
3 one of the many devastating comorbidities associated with prematurity. As a result of the  
4 findings reported, neonatal clinicians will have a heightened awareness for NEC when an infant  
5 requires an increase in respiratory support or is fed breast milk without nutritional fortification  
6 prior to achieving full volume enteral feedings. These findings are timely as the incidence of  
7 NEC has not declined along with other neonatal conditions alleviated by advances in critical care  
8 technology, pharmaceuticals, and improved clinical management of the high risk antepartum  
9 patient. Multidisciplinary research aimed at decreasing the incidence of this disease and thereby  
10 improving the overall long-term growth and development outcomes of premature infants will  
11 prove to be among the most challenging work ahead for neonatal researchers. The findings of  
12 this research make a small, but significant contribution to this end.

## References

- Alexander, G. (1975). Body temperature control in mammalian young. *British Medical Bulletin*, 31, 62-68.
- Ballance, W. A., Dahms, B. B., Shenker, N., & Kliegman, R. M. (1990). Pathology of neonatal necrotizing enterocolitis: A ten-year experience. *The Journal of Pediatrics*, 117(1), S6-S13.
- Bell, M.J., Ternberg, J.L., Feigin, R.D., Keating, J.P., Marshall, R., Barton, L., & Brotherton, T. (1978). Neonatal necrotizing enterocolitis: Therapeutic decisions based upon clinical staging. *Annals of Surgery*, 871, 1-7.
- Berseth, C. L. (1992). Effect of early feeding on maturation of the preterm infant's small intestine. *Journal of Pediatrics*, 120, 947-953.
- Berseth, C. L. (1995). Minimal Enteral Feedings. *Clinics in Perinatology*, 22(1), 195-205.
- Berseth, C. L. (1996). Gastrointestinal motility in the neonate. *Clinics in Perinatology*, 23(2), 179-190.
- Berseth, C. L., Bisquera, J. A., & Paje, V. U. (2003). Prolonging small feeding volumes early in life decreases the incidence of necrotizing enterocolitis in very low birth weight infants. *Pediatrics*, 111(3), 529-534.
- Bisquera, J. A., Cooper, T. R., & Berseth, C. L. (2002). Impact of necrotizing enterocolitis on length of stay and hospital charges in very low birthweight infants. *Pediatrics*, 109(3), 423-428.
- Chan, K. L., Hui, C.W.C., Fung, P.C.W., Wo, J.Y.H., Tipoe, G., Tam, P.K.H. (2002). Revisiting ischemia and reperfusion injury as a possible cause of necrotizing enterocolitis: Role of nitric oxide and superoxide dismutase. *Journal of Pediatric Surgery*, 37(6), 828-834.

- Chandler, J. C., & Hebra, A. (2000). Necrotizing enterocolitis in infants with very low birth weight. *Seminars in Pediatric Surgery, 9*(2), 63-72.
- Crissinger, K. D. (1999). Understanding necrotizing enterocolitis- promising directions. *Pathophysiology, 5*, 247-256.
- Crissinger, K. D., Burney, D. L., Velasquez, O. R., & Gonzalez, E. (1994). An animal model of necrotizing enterocolitis induced by infant formula and ischemia in developing piglets. *Gastroenterology, 106*, 1215-1222.
- Crissinger, K. D., & Granger, D. (1989). Mucosal injury induced by ischemia and reperfusion in the piglet intestine: Influences of age and feeding. *Gastroenterology, 97*, 920.
- Crissinger, K. D., Tso, P., & Burney, D. L. (1992). The role of lipids in ischemia/reperfusion-induced changes in mucosal permeability in developing piglets. *Gastroenterology, 102*(1693).
- deCurtis, M., Panoe, C., Vetrano, G., Romano, G., Paludetto, R., & Ciccimarra, F. (1987). A case control study of necrotizing enterocolitis occurring over 8 years in a neonatal intensive care unit. *European Journal of Pediatrics, 146*, 398-400.
- DiLorenzo, M., & Krantis, A. (2001). Altered nitric oxide production in the premature gut may increase susceptibility to intestinal damage in necrotizing enterocolitis. *Journal of Pediatric Surgery, 36*(5), 700-705.
- Gamsu, H. R., & Kempley, S. T. (1997). Enteral hypoxia/ischaemia and necrotizing enterocolitis. *Seminars in Neonatology, 2*, 245-254.
- Kien, L. (1990). Colonic fermentation of carbohydrate in the premature infant: Possible relevance to necrotizing enterocolitis. *Journal of Pediatrics, 117*, S52-S58.

- Kliegman, R. M. (2003). The relationship of neonatal feeding practices and the pathogenesis and prevention of necrotizing enterocolitis. *Pediatrics, 111*(3), 671-672.
- Kosloske, A. M. (1997). The epidemiology and pathogenesis of necrotizing enterocolitis. *Seminars in Neonatology, 2*, 231-238.
- Lawrence, G., Bates, J., & Gaul, A. (1982). Pathogenesis of neonatal necrotizing enterocolitis. *Lancet, 1*(137-139).
- Lee, J. S., & Polin, R. A. (2003). Treatment and prevention of necrotizing enterocolitis. *Seminars in Neonatology, 8*, 449-459.
- Levine, M. (1989). The conservation principals of nursing: Twenty years later. In J. Riehl-Sisca (Ed.), *Conceptual models for nursing practice* (3<sup>rd</sup> ed.) (p. 325-337). Norwalk, CT: Appelton & Lange.
- Lloyd, J. R. (1969). The etiology of gastrointestinal perforations in the newborn. *Journal of Pediatric Surgery, 4*, 77-84.
- Lucas, A., & Cole, T. J. (1990). Breast milk and neonatal necrotizing enterocolitis. *Lancet, 336*, 1519-1523.
- McClure, R. J., & Newell, S. J. (2000). Randomized controlled study of clinical outcome following trophic feeding. *Arch Dis Child Fetal Neonatal Ed, 82*, F29-F33.
- McElhinney, D. B., Hedrick, H. L., Bush, D. M., Pereria, G. R., Stafford, P. W., Gaynor, J. W., et al. (2000). Necrotizing enterocolitis in neonates with congenital heart disease: Risk factors and outcomes. *Pediatrics, 106*, 1080-1087.
- McKeown, R. E., Marsh, D., Amarnath, U., Garrison, C. Z., Addy, C. L., Thompson, S. J., et al. (1992). Role of delayed feeding and of feeding increments in necrotizing enterocolitis. *Journal of Pediatrics, 121*, 764-770.

- Neu, J. (1996). Necrotizing Enterocolitis: The search for a unifying pathogenic theory leading to prevention. *Pediatric Clinics of North America*, 43(2), 409-432.
- Rowe, M. I., Reblock, K. K., Kurkchubasche, A. G., & Healey, P. J. (1994). Necrotizing enterocolitis in the extremely low birth weight infant. *Journal of Pediatric Surgery*, 29(8), 987-991.
- Santulli, T. V., Shullinger, J. N., & Heird, W. C. (1975). Acute necrotizing enterocolitis in infancy: a review of 64 cases. *Pediatrics*, 55, 376-387.
- Schanler, R. J., Shulman, R. J., Lau, C., O'Brien Smith, E., & Heitkemper, M. M. (1999). Feeding strategies for premature infants: Randomized trial of gastrointestinal priming and tube-feeding method. *Pediatrics*, 103(2), 434-439.
- Stoll, B. J. (1994). Epidemiology of necrotizing enterocolitis. *Clinics in Perinatology*, 21(2), 205-218.
- Stoll, B. J., Kanto, W. P., Glass, R. I., Nahmias, A. J., & Brann, A. W. (1980). Epidemiology of necrotizing enterocolitis: A case controlled study. *Journal of Pediatrics*, 96, 447-451.
- Williams, A. F. (1997). Role of feeding in the pathogenesis of necrotizing enterocolitis. *Seminars in Neonatology*, 2, 263-271.

Table 1. Study Variables: Biodemographic Factors, Intestinal Inflammation and Ischemia, and Enteral Feeding Regimen

Study Variables	Data obtained from medical record as measure of specific factors
<b>Biodemographic Factors:</b>	
▪ Birth weight	▪ Documented birth weight measured in grams.
▪ Gestational age	▪ Gestational age measured in weeks and days, confirmed by maternal dates and/or Ballard Gestational Age Estimation. Documented in the medical record.
▪ Gender	▪ Male or female sex, confirmed by genetic analysis if ambiguous genitalia present.
▪ Race	▪ Assignment to one of the following groups based on maternal ethnicity/race: <ul style="list-style-type: none"> <li>○ White, non-Hispanic</li> <li>○ All other race groups</li> </ul>
▪ Age of NEC onset	▪ Day of life when NEC diagnosis made and medical and/or surgical treatment began. NEC diagnosis confirmed by Bell Staging criteria IIA-IIIB.
▪ Prenatal steroid administration	▪ Documentation in medical record of administration of corticosteroid dose (Betamethasone/Celestone) to mother prior to birth (coded in VON data)
• Maternal infectious disease	▪ Documentation in medical record of positive screen for maternal infectious disease (i.e. Group Beta Strep)

Table 1. Study Variables: Biodemographic Factors, Intestinal Inflammation and Ischemia, and Enteral Feeding Regimen

Study Variables	Data obtained from medical record as measure of specific factors
<p><b>Factors Related to Intestinal Inflammation and Ischemia:</b></p>	
<ul style="list-style-type: none"> <li>▪ Resuscitation efforts at birth</li> </ul>	<ul style="list-style-type: none"> <li>▪ Any and all stabilization and/or resuscitation interventions that occur at the time of birth, including administration of:               <ul style="list-style-type: none"> <li>○ Oxygen</li> <li>○ Bag/Mask ventilation</li> <li>○ Endotracheal tube (ETT) ventilation</li> <li>○ Epinephrine</li> <li>○ Cardiac compressions</li> </ul> </li> </ul>
<ul style="list-style-type: none"> <li>▪ Physiologic instability during early neonatal care:               <ul style="list-style-type: none"> <li>○ Hypoxia</li> <li>○ Hypotension</li> <li>○ Hypothermia</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>▪ Apnea, bradycardia, oxygen desaturations or abnormal blood gases <b>requiring increased respiratory support</b> to maintain infant’s oxygenation. <b>Increased respiratory support</b> required includes any additional measures implemented to maintain the infant’s oxygenation (i.e. change from nasal cannula to CPAP oxygenation; change from CPAP to ET tube positive pressure ventilation).</li> <li>▪ Severe apnea, bradycardia, oxygen desaturations or abnormal blood gases <b>requiring administration of fluid resuscitation (albumin, bicarb), positive pressure ventilation via ETT, chest compressions or epinephrine in the NICU to maintain infant’s oxygenation and perfusion. Increased hemodynamic support</b> includes any clinical intervention measures taken to support the infant’s hemodynamic status under conditions of failing oxygenation and perfusion status (i.e. fluid resuscitation, positive pressure ventilation, chest compressions, epinephrine administration, full neonatal code)</li> <li>▪ Hypotensive episodes requiring vasopressor medications to maintain adequate blood pressure and perfusion based on gestational age</li> <li>▪ Hypothermic/cold stress episodes requiring additional external heat source</li> </ul>
<ul style="list-style-type: none"> <li>▪ Decreased SMA flow:               <ul style="list-style-type: none"> <li>▪ Indomethacin administration</li> </ul> </li> <li>▪ Umbilical vessel catheter</li> </ul>	<ul style="list-style-type: none"> <li>▪ Documented dose of Indomethacin</li> <li>• Placement and infusion of intravenous fluids via umbilical vessels during neonatal intensive care. Includes placement of UAC or UVC.</li> </ul>

Table 1. Study Variables: Biodemographic Factors, Intestinal Inflammation and Ischemia, and Enteral Feeding Regimen

Study Variables	Data obtained from medical record as measure of specific factors
<p><b>Factors Related to the Enteral Feeding Regimen:</b></p>	
<ul style="list-style-type: none"> <li>▪ Time at which enteral feedings initiated during infant's neonatal course.</li> </ul>	<ul style="list-style-type: none"> <li>▪ Dichotomous measurement based on these aspects of the feeding regimen.               <ul style="list-style-type: none"> <li>○ Initiation of enteral feeding during first 48 hours of life (early)</li> <li>○ Greater than 20ml/kg/day advancement during a 24 hour period</li> <li>○ Enteral feeding of breast milk</li> <li>○ Enteral feeding of fortified breast milk</li> <li>○ Enteral feeding of formula</li> <li>○ Enteral feeding of formula with fortification</li> <li>○ Change in infant diet from breast milk to formula</li> <li>○ Change in infant diet from unfortified milk/formula to fortified milk/formula</li> </ul> </li> </ul>
<ul style="list-style-type: none"> <li>▪ Volume and rate of advancement of enteral feeding</li> </ul>	
<ul style="list-style-type: none"> <li>▪ Type of enteral feeding: may include breast milk, nutritionally fortified breast milk, infant formula, nutritionally fortified infant formula</li> </ul>	

Table 2. Study Sample Descriptive Data, NEC vs. Non-NEC samples

Study Variable	Total (n=247)	NEC Sample (n=84)	Non-NEC Sample (n=163)	<i>p</i> *
Male gender - # (%)	128 (52%)	52 (61.9%)	76 (46.6%)	.016
Race - # (%)				.057
White non-Hispanic	176 (71.3%)	54 (64.3%)	122 (74.8%)	
All other race groups	91 (28.7%)	30 (35.7%)	41 (25.1%)	
Gestational Age - # (%)				.390
<26.0 weeks	53 (22%)	19 (22.6%)	30 (18.4%)	
26.1-28.0 weeks	72 (29%)	28 (33.3%)	46 (28.2%)	
28.1-30.0 weeks	68 (28%)	23 (27.4%)	47 (28.8%)	
30.1-32.0 weeks	36 (15%)	8 (9.5%)	28 (17.1%)	
>32.1 weeks	18 (7%)	6 (7.1%)	12 (7.3%)	
Birth weight - # (%)				.959
500-750 grams	40 (16%)	15 (17.9%)	25 (15.3%)	
751-1000 grams	77 (31%)	25 (29.7%)	51 (31.3%)	
1001-1250 grams	68 (28%)	23 (27.3%)	46 (28.2%)	
1251-1500 grams	62 (25%)	21 (25%)	41 (25.2%)	
Maternal Data - # (%)				
Prenatal steroids administered	215 (87%)	69 (82.1%)	146 (89.6%)	.023
GBS positive ***	44 (18%)	14 (16.7%)	30 (18.4%)	.821
Apgar Score, 1 minute				.479
0-3	55 (22.4%)	15 (18.1%)	40 (24.5%)	
4-6	78 (31.7%)	29 (34.9%)	49 (30%)	
7-10	113 (45.9%)	39 (46.9%)	74 (45.4%)	
Apgar Score, 5 minute				.746
0-3	6 (2.4%)	2 (2.4%)	4 (2.5%)	
4-6	45 (18.3%)	13 (15.7%)	32 (19.6%)	
7-10	195 (79.3%)	68 (81.9%)	127 (77.9%)	
Resuscitation in Delivery Room				
Oxygen	242 (98%)	82 (97.4%)	160 (98.2%)	.554
Bag/Mask	161 (65.2%)	56 (66.7%)	105 (64.4%)	.418
ET Tube Intubation	164 (66.4%)	55 (65.5%)	109 (66.9%)	.467
Epinephrine	7 (2.8%)	2 (2.4%)	5 (3.1%)	.555
Chest Compression	18 (7.3%)	6 (7.1%)	12 (7.4%)	.586

Table 2. Study Sample Descriptive Data, NEC vs. Non-NEC samples

Study Variable	Total (n=247)	NEC Sample (n=84)	Non-NEC Sample (n=163)	<i>p</i> *
<b>Physiologic Instability During Early NICU Course</b>				
Increase respiratory support	169 (68.4%)	77 (91.7%)	92 (56.4%)	.000*
Increase hemodynamic support/ Neonatal code	53 (21.5%)	32 (38.1%)	21 (12.9%)	.000*
Hypotensive episode	100 (40.5%)	39 (46.4%)	61 (37.4%)	.116
Hypothermic episode	24 (9.7%)	12 (14.3%)	12 (7.4%)	.069
Indomethacin Administration	66 (26.7%)	27 (32.1%)	39 (23.9%)	.110
<b>Umbilical Catheter</b>				
UAC and/or UVC	149 (61.6%)	56 (66.6%)	93 (57%)	.080
<b>Enteral Feeding Factors</b>				
Feeding in 1 <sup>st</sup> 48 hrs Advancement > 20cc/kg/day in 24 hrs	105 (43.2%)	26 (31.0%)	79 (48.5)	.005
<b>Type of Feeding:</b>				
Breast milk (BM)	167 (69.3%)	50 (59.5%)	117 (71.8%)	.032
Fortified BM	95 (38.5%)	14 (16.7%)	81 (49.7%)	.000*
Formula	136 (55.1%)	41 (48.8%)	95 (58.3%)	.095
Fortified Formula	80 (33.1%)	16 (19.0%)	64 (39.3%)	.001*
Change from BM to Formula	61 (25.2%)	15 (17.9%)	46 (28.2%)	.044
Change from unfortified feeding to fortified feeding	138 (55.9%)	27 (32.1%)	111 (68.1%)	.000*

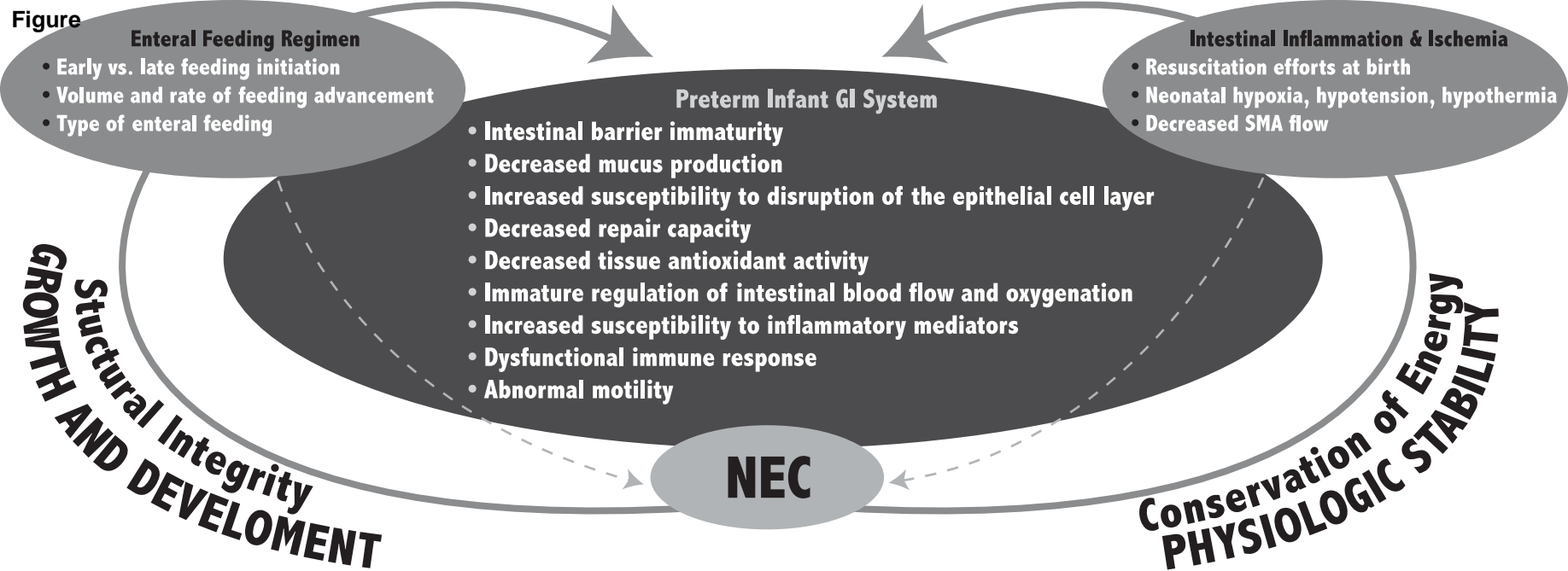
\* Alpha of significance for variable selection for logistic regression model  $p < 0.002$

Table 3. Logistic Regression Model: Clinical Predictors of NEC  
 $R^2 = 40.6\%$  of Variance of NEC Explained

Variable	Beta	S.E.	Odds ratio	Relative Risk	CI (95%)	p
Increase in respiratory support	2.633	.541	13.916	5.05	4.815 - 40.218	.000
Increase in perfusion support	.294	.413	1.342		.597 - 3.014	.477
(LACK OF) Fortification of breast milk *	1.497	.703	4.470	3.13	1.128- 17.713	.033
Fortified infant formula	-.909	.707	.403		.101 - 1.612	.199
Change in fortification	.493	.828	1.637		.323 - 8.291	.552

Constant: -2.316

- Variable recoded (0=yes, 1=no) after a preliminary analysis indicated an inverse relationship in the *B* value



**Figure 1: Conceptual Framework: Pathogenesis of NEC in Premature Infants**