VITAMIN A STATUS AND INFLAMMATION DURING THE FIRST WEEK OF LIFE IN EXTREMELY PREMATURE INFANTS AT RISK FOR BRONCHOPULMONARY DYSPLASIA

DISSERTATION

Presented in Partial Fulfillment of the Requirements for

The Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Anne M. Mentro, M.S.

*****

The Ohio State University
2004

Dissertation Committee:

Professor Deborah Steward, Adviser
Professor Terry Lennie
Professor Anne Smith
Professor Nancy Ryan-Wenger

Approved by

Adviser
College of Nursing
ABSTRACT

Bronchopulmonary dysplasia (BPD) is a chronic lung disease of prematurity affecting infants born at low gestational ages. The exact pathophysiology of BPD is unknown, but inflammation and oxidative stress immediately after birth are thought to be contributing factors. Vitamin A (retinol) is a potent antioxidant with anti-inflammatory properties. Although previous studies have revealed deficient plasma retinol concentrations and higher degrees of inflammation in premature infants, no studies have explored the collective relationship between these variables as they relate to oxygen dependence at 36 weeks postconceptional age. The purpose of this study was to explore relationships among plasma retinol, urinary interleukin-1 receptor antagonist (IL-1ra), and nutritional intake during the first days of life in premature infants high risk for BPD. Forty infants born at less than or equal to 30 weeks gestational age were recruited following birth. At day 1 and day 7 of life, 0.5 mL of whole blood, 1 mL of urine, and nutritional intake data were collected. Plasma retinol and IL-1ra concentrations were analyzed with HPLC and ELISA, respectively.

Plasma retinol concentrations remained stable over the first week despite increasing vitamin A intakes. Most infants had plasma values indicative of vitamin A deficiency. Plasma retinol values on day 1 and day 7 were positively related to IL-1ra
concentrations on day 7 of life ($p < 0.05$ for both retinol values), suggesting that infants with greater inflammation had greater mobilization of retinol into the plasma. IL-1ra concentrations were very high throughout the first week and were indicative of substantial inflammation. For infants who required oxygen at 36 weeks, day 1 IL-1ra concentrations were significantly higher ($p < 0.05$) than those of infants without oxygen needs. Oxygen dependent infants also had lower mean vitamin A intakes ($p = 0.05$). These findings confirm that premature infants are vitamin A deficient and experience significant inflammation throughout the first week of life. Inflammatory markers produced within hours after birth may provide an early measure of BPD in extremely premature infants and increasing vitamin A intake may alleviate some of the early inflammation associated the disorder.
ACKNOWLEDGMENTS

I wish to thank my adviser, Dr. Deborah Steward, for her unrelenting support, availability, and for encouraging me to reach my full potential. I am grateful to Deb for patience throughout the dissertation process and for her repeated revisions of my work.

I thank Dr. Terry Lennie for the demonstration of cytokine assay techniques and for his patience as I learned to work with urine samples. I also thank Terry for the intellectual discussions of cytokine determination in premature infants and for his willingness to commute from Lexington.

I also thank Dr. Anne Smith for her valuable insight into the nutritional management of premature infants. I am also grateful to Anne for her willingness to collaborate on future projects and for her unflaltering interest in advancing my professional career.

I am grateful to Dr. Nancy Ryan-Wenger for her insight into statistical techniques and research methodology. I also thank Nancy for her constant support and willingness to collaborate with me on additional projects.
I also wish to thank the nursing staff at The Ohio State Medical Center and Riverside Hospital for assisting with patient identification and sample collection. I also thank the laboratory technicians, particularly Marliese Dion and Julie Chitchumroonchokchoi, who made the sample analysis possible.

The training required for this research project was supported in full by an Individual National Research Service Award grant from the National Institutes of Health, National Institute of Nursing Research (5F31NR07850). This research was also supported by the American Nurses Foundation Germaine S. Krysan Scholar Award and by the Distinguished University Fellowship of The Ohio State University.
VITA

March 4, 1977……………………………… Born, Kingsport, Tennessee

1999……………………………………….. B.S. Nursing, The University of Tennessee
           Chattanooga, Tennessee

1999-2000…………………………………. Registered Nurse,
           T.C. Thompson Children’s Hospital
           Chattanooga, Tennessee

2002-2004…………………………………. Registered Nurse,
           Children’s Hospital
           Columbus, Ohio

2003……………………………………….. M.S. Nursing, The Ohio State University
           Columbus, Ohio

PUBLICATIONS


**FIELDS OF STUDY**

Major field: Nursing
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>iv</td>
</tr>
<tr>
<td>Vita</td>
<td>vi</td>
</tr>
<tr>
<td>List of Tables</td>
<td>xi</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xii</td>
</tr>
<tr>
<td>Chapters:</td>
<td></td>
</tr>
<tr>
<td>1. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>The pathophysiology of bronchopulmonary dysplasia</td>
<td>2</td>
</tr>
<tr>
<td>BPD and the role of oxidative stress</td>
<td>4</td>
</tr>
<tr>
<td>BPD and vitamin A status</td>
<td>7</td>
</tr>
<tr>
<td>BPD and the role of inflammation</td>
<td>9</td>
</tr>
<tr>
<td>BPD and the IL-1β:IL-1ra imbalance</td>
<td>11</td>
</tr>
<tr>
<td>The relationship between oxidative stress and inflammation in BPD</td>
<td>13</td>
</tr>
<tr>
<td>Research questions</td>
<td>14</td>
</tr>
<tr>
<td>Methodology</td>
<td>16</td>
</tr>
<tr>
<td>Procedure</td>
<td>19</td>
</tr>
<tr>
<td>Data analysis</td>
<td>25</td>
</tr>
<tr>
<td>Description of results</td>
<td>25</td>
</tr>
</tbody>
</table>
2. Vitamin A and bronchopulmonary dysplasia: research, issues, and clinical practice
   Antioxidant properties of vitamin A
   Vitamin A deficiency in premature infants
   Clinical trials of vitamin A supplementation for premature infants
   Vitamin A dosages and delivery routes
   Vitamin A and dexamethasone
   Vitamin A deficiency and nutritional intake
   Clinical implications
   Conclusions

3. Vitamin A status and inflammation in the extremely premature infant during the first week of life
   Research questions
   Materials and methods
   Procedure
   Data analysis
   Results
   Correlations between study variables
   Differences in plasma retinol and IL-1ra over the First postnatal week
   Differences in study variables between groups
   Predictive ability of study variables at 36 weeks postconceptional age
Discussion................................................................. 59
  Plasma retinol....................................................... 59
  Vitamin A intake.................................................. 61
  Urinary IL-1ra...................................................... 64
  Retinol and IL-1ra................................................ 69
  IL-1ra and caloric intake...................................... 71
Conclusion............................................................ 73

4. Methodological issues associated with nutrition research in the
   Neonatal Intensive Care Unit........................................ 74

   Anthropometric Measurement.................................. 75
     Weight assessment.............................................. 76
     Length assessment............................................ 79
     Head circumference assessment............................. 80
     Growth chart usage.......................................... 82
   The collection of biological specimens..................... 83
     Blood collection............................................... 84
     Urine and fecal collection.................................. 87
   Ethical and other methodological considerations........... 89
     Informed consent and psychological stress............... 90
     Sampling issues............................................... 92
     Environmental issues....................................... 93
Conclusion........................................................... 94
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>50</td>
</tr>
<tr>
<td>3.2</td>
<td>51</td>
</tr>
<tr>
<td>3.3</td>
<td>53</td>
</tr>
<tr>
<td>3.4</td>
<td>56</td>
</tr>
</tbody>
</table>

#### 3.1 Birthweights and gestational ages of enrolled infants

#### 3.2 Oxygen, ventilation, and nutritional requirements at day 1 and day 7 of life

#### 3.3 Correlations between major study variables

#### 3.3 Plasma retinol and urinary IL-1ra concentrations at day 1 and day 7 of life

#### 3.4 Mean daily caloric and vitamin A intakes during the first week of life
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Proposed model of variable relationships</td>
<td>15</td>
</tr>
<tr>
<td>3.1</td>
<td>Mean daily vitamin A intake (IU) during the first week of life</td>
<td>57</td>
</tr>
<tr>
<td>3.2</td>
<td>Mean daily caloric intake (kilocalories per kilogram) during the first week of life</td>
<td>58</td>
</tr>
</tbody>
</table>
APPENDICES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. The Ohio State University consent to investigational treatment or procedure</td>
<td>92</td>
</tr>
<tr>
<td>B. Children’s Hospital agreement to participate in research</td>
<td>97</td>
</tr>
</tbody>
</table>
Bronchopulmonary dysplasia (BPD) is a chronic lung disease of infancy that is a significant health problem for premature infants. Although surfactant administration and advances in neonatal care have improved the survival rates of premature infants (Soll & Morley, 2001; Stevenson et al., 1998), BPD continues to account for much of the morbidity and mortality associated with extremely premature birth (National Institutes of Health: National Heart, Lung, and Blood Institute, 1998). In the United States, approximately 14,000 premature infants weighing less than 1500 grams at birth develop BPD each year (Lemons et al., 2001; Martin et al., 2003). For premature infants of 24-26 weeks gestation, incidence rates of approximately 85% have been reported (Egreteau et al., 2001; McElrath et al., 2001; National Institutes of Health: National Heart, Lung, and Blood Institute, 1998). Although the overall incidence of BPD has remained relatively stable at approximately 19-29% since the early-to-mid 1990’s (Lemons et al., 2001; Manktelow, Draper, Annamalai, & Field, 2001; Stevenson et al., 1998), the increasing viability of extremely premature infants is contributing to substantial BPD in those survivors (Hack & Fanaroff, 1999; McElrath et al., 2001). Consequently, BPD is one of the most severe chronic pulmonary disorders of infancy, accounting for nearly $2.4
billion of health care expenditures in 1998, second only to the costs for treating asthma (National Institutes of Health: National Heart, Lung, and Blood Institute, 1998).

The Pathophysiology of BPD

The pathophysiology of BPD is complex and multifaceted, and the exact mechanisms associated with lung injury in premature infants are not completely understood. The development of BPD is primarily attributed to the use of mechanical ventilation and/or oxygen administration for the treatment of neonatal respiratory distress syndrome, leading to a persistent deterioration of pulmonary function over time (American Thoracic Society, 2003; Jobe & Bancalari, 2001). The classic form of BPD, first described in 1967 by Northway and associates, involved severe epithelial lesions and extensive fibrosis of the lungs with alternating sites of atelectasis and overinflation (Bonikos, Bensch, Northway, & Edwards, 1976; Northway, Rosan, & Porter, 1967). Infants with BPD in this era did not have the benefit of exogenous surfactant and were often exposed to very high oxygen concentrations, volutrauma, and barotrauma from mechanical ventilation (Bancalari, 2001). In contrast, the “new” form of BPD, which developed following the routine administration of surfactant for neonatal respiratory distress syndrome, is characterized by delayed or absent alveolar development without severe fibroproliferation (Albertine et al., 1999; Maniscalco, Watkins, O’Reilly, & Shea, 2002; Thibeault, Truog, & Ekekezle, 2003). Infants with the “new” BPD are exposed to significantly less volutrauma, barotrauma, and inspired oxygen concentrations than infants with the classic form (Charafeddine, D’Angio, & Phelps, 1999). However,
pulmonary defects are still apparent, including decreased vascular endothelial growth
factor (Bhatt et al., 2001; Lassus et al., 1999), abnormal distributions of elastic tissue
(Bland et al., 2000; Thibeault, Mabry, Ekekezie, & Truog, 2000), and pulmonary
basement membrane damage coupled with defects in membrane remodeling (Aghai et al.,
2002; Ohki et al., 2001). Although these defects are not as severe as those observed in
infants with classic BPD, they ultimately lead to disruptions in pulmonary function that
may persist well into childhood (Kennedy et al., 2000).

Given the changes in the histopathological presentation of BPD over the last 30
years, diagnosis of the disorder remains controversial. Although the clinical presentation
of the “new” BPD is relatively consistent among institutions, questions have been raised
as to when the diagnosis should be made (Davis et al., 2002). Historically, when oxygen
dependency coupled with abnormal changes on the chest radiograph persisted at 28 days
of life, BPD was diagnosed (Bancalari, Abdenour, Feller, & Gannon, 1979; National
Institutes of Health: National Heart, Lung, and Blood Institute, 1979). By contrast,
current clinical guidelines recommend delaying the diagnosis of BPD until 36 weeks
postconceptional age (American Thoracic Society, 2003). This change is due in part to
the influx of premature infants born at 30 weeks gestation or less who are incapable of
independent lung function by 28 days of age (Kotecha, 2000). Improved prediction of
pulmonary outcomes in later childhood have also been noted when diagnosis is delayed
until this time (American Thoracic Society, 2003; Davis et al., 2002; Jobe & Bancalari,
2001; Shennan et al., 1988). When the 36-week criterion is used, in general, fewer
infants are diagnosed with BPD than at 28 days (Egreteau et al., 2001; Korhonen et al.,
1999; Manktelow, Draper, Annamalai, & Field, 2001). However, for the most premature infants born at less than 30 weeks gestational age, there is little variation in the incidence of BPD when diagnosed at 36 weeks postconceptional age versus 28 days of life (Egreteau et al., 2001). For these extremely premature infants, it may be that the initial insult of BPD develops during the first hours to days of life and persists well until term corrected age.

**BPD and the Role of Oxidative Stress**

Recent studies have indicated that oxidative stress from hyperoxia is a significant contributor to the development of BPD in premature infants during the initial days of life (Ogihara et al., 1998; Ogihara et al., 1999; Schock et al., 2001). When supplemental oxygen is administered to premature infants for the management of neonatal respiratory distress syndrome, oxygen molecules combine with hydrogen ions to form either water or reactive oxygen species (ROS). These ROS, which include free radicals such as superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl anion (OH$^-$), react with cellular molecules and promote altered mitochondrial function, oxidation of lipids and proteins, DNA damage, and ultimately cell death (Freeman & Crapo, 1981; Kazzaz et al., 1996; Turrens, Freeman, & Crapo, 1982; Turrens, Freeman, Levitt, & Crapo, 1982; Zhang et al., 2003). The degree and severity of these ROS-induced complications is thought to increase with increasing concentrations of inspired oxygen. In studies of neonatal and adult rats, exposure to high oxygen concentrations of 85-95% during the first week of life resulted in dysplastic lung changes similar to those observed in infants
with BPD, including a decreased number of alveoli, irregularly large alveoli with
incomplete septation, decreased proliferation of alveolar cells, lung fibrosis, and
decreased vascular endothelial growth factor (Klekamp, Jarzecka, & Perkett, 1999;
Further studies have also indicated a disruption in pulmonary surfactant gene expression
in premature animals exposed to similar degrees of hyperoxia following birth (D’Angio
et al., 1997; Minoo et al., 1991; White, Greene, Allen, & Shannon, 2001). While the
precise mechanism involved in this hyperoxia-mediated pulmonary dysfunction is
unclear, these findings substantiate the important role of oxygen administration and ROS
formation in the development of BPD.

Although some degree of ROS-induced cellular injury is apparent in all healthy
individuals, circulating plasma and cellular antioxidant concentrations are generally
sufficient to scavenge free radicals and prevent structural cellular defects. However,
when an imbalance between pro-oxidant and antioxidant forces is apparent, “oxidative
stress” occurs. It is thought that oxidative stress is more pronounced during the newborn
period, with gradual diminishing throughout childhood and early adulthood. Studies
have revealed reduced antioxidant concentrations in newborns when compared to adults
and children (Erden-Inal, Sunal, & Kanbak, 2002; Metsvaht et al., 1999; Oostenbrug et
al., 1998), and significantly reduced antioxidant concentrations in premature versus full
term infants (Phylactos, Leaf, Costeloe, & Crawford, 1985; Robles, Palomino, & Robles,
2001). Because antioxidant stores increase in the infant up to the end of normal gestation
(Frank & Sosenko, 1987a; Frank & Sosenko 1987b; Sosenko & Frank, 1987), premature
birth leads to marked deficiency of necessary antioxidants. Even though these antioxidant concentrations gradually increase in healthy premature infants over time, a recent study revealed significant delays in the increase of ascorbate, urate and gluthathione in infants with BPD when compared to premature controls (Vyas et al., 2001). Further research has also revealed significantly decreased total radical trapping capacity and decreased iron-binding antioxidant capacity in premature infants with BPD over the first 28 postnatal days (Moison, Haasnoot, Van Zoeren-Grobben, & Berger, 1998). Although all premature infants are predisposed to antioxidant deficiencies due to physiological immaturity, these deficiencies may be more pronounced in premature infants who later develop BPD. Consequently, the premature infant at risk for BPD may be more susceptible to free radical damage and oxidative stress immediately following birth. This notion has been supported by studies of biomarkers of oxidative stress-induced tissue breakdown, such as malondialdehyde, plasma aliphatic aldehydes, exhaled pentane, and protein carbonylation products, which are increased in premature infants with BPD during the first week of life when compared to healthy controls (Banks et al., 1998; Buss, Darlow, & Winterbourne, 2000; Nycyk, Drury, & Cooke, 1998; Ogihara et al., 1999; Varsila, Pesonen, & Andersson, 1995). Although additional studies are warranted regarding oxidation products in premature infants with BPD, these findings suggest that measures to decrease ROS formation may be beneficial for premature infants at high risk for the development BPD.
BPD and Vitamin A Status

Vitamin A circulates as retinol in the plasma and is a crucial antioxidant for premature infants because it functions as a chain-breaking antioxidant that scavenges lipoperoxyl radicals, suppresses membrane peroxidation, and inhibits ROS (Palacios, Piergiacomi, & Catala, 1996; Schwarz et al., 1997; Sharma, Lewandoski, & Zimmerman, 1990). Retinol also fosters pulmonary epithelial differentiation and lung maturation (Perrotta et al., 2003). When vitamin A deficiency is present, lung tissue is significantly damaged, evidenced by cilia loss, depressed surfactant synthesis, and replacement of the columnar mucus-secreting epithelium with stratified squamous keratinizing epithelium in the trachea and bronchi (Baybutt, Hu, & Molteni, 2000; Jetten et al., 1987; Stahlman, Gray, Chytil, & Sundell, 1988; Paquette et al., 1996). These alterations are similar to the histopathologic changes that have been noted in premature infants with BPD.

There is growing evidence to suggest that premature infants are deficient in vitamin A, which may predispose them to the development of BPD. Premature infants not only have significantly decreased plasma retinol concentrations compared to full-term infants, but also possess only half the liver stores of retinol compared to full-term controls (Carlson, Peeples, Werkman, & Koo, 1995; Chan, Greenough, Cheeseman, & Gamsu, 1993a; Shenai, Chytil, & Stahlman, 1985b). Consequently, premature infants are predisposed to retinol deficiency following premature birth. Furthermore, plasma retinol concentrations continue to decline in these infants throughout the first 2 postnatal weeks (Tammela, Aitola, & Ikonen, 1999), reaching a nadir at approximately 5 weeks of life (Mupanemunda et al., 1994). Increases to full-term plasma retinol levels are not noted
until 4-6 weeks corrected age (Brandt et al., 1978). These retinol deficiencies are likely to predispose premature infants to acute lung injury during the first weeks of life, which may have lasting consequences for pulmonary function.

For premature infants who later develop BPD, concentrations of plasma retinol following birth are even more depressed than those observed in healthy premature controls (Chan, Greenough, Cheeseman, & Gamsu, 1993b; Inder et al., 1998; Shenai, Chytil, & Stahlman, 1985a). For these infants, plasma retinol concentrations are significantly decreased both during the first week of life and throughout the first 28 postnatal days (Chan et al., 1993b; Inder et al., 1998; Shenai, Chytil, & Stahlman, 1985a). In one study, plasma retinol levels below 100 µg/L were noted in approximately 42% of infants who later developed BPD, compared to only 7% of infants who did not develop BPD (Inder et al., 1998). In a similar study, 85% of the premature infants with plasma retinol concentrations less than 100 µg/L on day 10 of life later developed BPD (Verma, McCulloch, Worrell, & Vidyasagar, 1996). Given that plasma retinol concentrations below 100 µg/L are indicative of both severe vitamin A deficiency and depleted liver stores (World Health Organization, 1997), there appears to be some correlation between vitamin A status and BPD. Although clinical trials of vitamin A supplementation for premature infants have produced conflicting results, a recent meta-analysis of pooled data revealed a reduction in death and/or oxygen requirements at 36 weeks postconceptional age in infants less than 1000 grams who received vitamin A supplementation (Darlow & Graham, 2002). These findings suggest that vitamin A status may be an important factor in the development of BPD.
BPD and the Role of Inflammation

Like oxidative stress, inflammation also plays a critical role in the development of BPD in premature infants. When a premature infant experiences respiratory distress during the initial hours of life, the subsequent endotracheal intubation and ventilation that ensue, whether it be conventional or high frequency ventilation, produce an insult to the lungs leading to a pulmonary inflammatory response (Jaarsma et al., 2001; Thome, Gotze-Speer, Speer, & Pohlandt, 1998; Yoder, Siler-Khodr, Winter, & Coalsion, 2000). This response is characterized by the release of pro-inflammatory cytokines from mononuclear phagocytes, which signal the movement of leukocytes to the interstitial and alveolar spaces (Clement et al., 1988; Groneck et al., 1994; Munshi, Niu, Siddiq, & Parton, 1997). This response occurs within the first 24 hours of life (Contreras et al., 1996; Jackson et al., 1987; Nupponen et al., 2002) and is apparent as early as 2 hours following the initiation of intubation and mechanical ventilation (Jaarsma et al., 2001; Naik et al., 2001). As activated macrophages, neutrophils, and lymphocytes collect in the interstitium and alveoli, there is further production of pro-inflammatory cytokines (Coalson, Winter, Siler-Khodr, & Yoder, 1999; Jonsson et al., 1997) as well as the generation of macrophage-derived ROS (Buss et al., 2003; Jankov et al., 2003; Narasaraju et al., 2003). This ultimately leads to a continuing cycle of inflammation-induced lung injury through the degradation of collagen, elastin, and fibronectin in the lungs (Coalson, Winter, Siler-Khodr, & Yoder, 1999; Sluis, Darlow, Vissers, & Winterbourn, 1994; Strieter & Kunkel, 1994).
In healthy adults, the triggering of the pro-inflammatory cascade leads to the production of anti-inflammatory cytokines that inhibit the production and function of the pro-inflammatory cytokines (de Wall Malefyt et al., 1991; Fiorentino et al., 1991; Seckinger et al., 1987). However, the activation and production of these anti-inflammatory cytokines appears to be reduced in premature infants when compared to adults (Jones et al., 1996; Schultz et al., 2004). Although controversy exists as to whether neonatal secretion of pro-inflammatory cytokines is decreased or equivalent when compared to adult secretory levels (Bessler et al., 1993; Dembinski et al., 2003; Peters, Bertram, Gahr, & Speer, 1993; Weatherstone & Rich, 1989), the overall inhibition of the pro-inflammatory cytokines is markedly less than that of adults (Schultz et al., 2004). Consequently, the premature infant displays a significant imbalance between pro-inflammatory and anti-inflammatory responses following pulmonary insult, which may ultimately predispose the infant to BPD (Jones et al., 1996).

In healthy premature infants recovering from respiratory distress syndrome, the presence of pro-inflammatory cytokines appears to be related to the duration of mechanical ventilation (Schultz, Tautz, Reiss, & Moller, 2003). As respiratory distress syndrome resolves, markers of inflammation decrease over the first 7-10 days of life in conjunction with decreased neutrophil activation (Contreras et al., 1996; Merritt et al., 1981; Merrit et al., 1983; Nupponen et al., 2002; Todd et al., 1998). However, in premature infants who subsequently develop BPD, activated neutrophils and inflammatory markers remain elevated during this time (Contreras et al., 1996; Jonsson et al., 1997; Munshi, Niu, Siddiq, & Parton, 1997; Ogden, Murphy, Saunders, & Johnson,
Although the presence of these markers in premature infants with BPD appears to peak around day 10 to 14 of life (Rozycki, 1994; Todd et al., 1998), elevations are still apparent at 21 to 28 of life in infants who continue to receive mechanical ventilation for respiratory distress (Kotecha et al., 1996; Kwong et al., 1998). Ultimately, the persistence of these cytokines contributes to epithelial fibrosis and decreased pulmonary function in premature infants who develop BPD (Ozdemir, Brown, & Morgan, 1997).

**BPD and the Interleukin-1\(\beta\):Interleukin-1 Receptor Antagonist Imbalance**

Interleukin-1\(\beta\) (IL-1\(\beta\)) is the primary cytokine associated with inflammation of the lung in extremely premature infants. Following a pulmonary insult such as intubation, ventilation, and/or hyperoxia, IL-1\(\beta\) is produced within the lungs by mononuclear phagocytes (Dinarello, 1991; Piedboeuf et al., 1998) and further augments the inflammatory reaction through the stimulation of interleukin-6, interleukin-8, T-cells, tumor necrosis factor, and T-cell-derived interleukin-2 (Dinarello, 1996; Dinarello & Wolff, 1993). High levels of IL-1\(\beta\) have been detected in bronchoalveolar lavage fluid obtained during the first 24 hours of life in premature infants with respiratory distress syndrome (Dammann et al., 2001; Kazzi et al., 2001; Watterberg, Demers, Scott, & Murphy, 1996), and significantly increased concentrations during this time period have been reported in premature infants who later develop BPD (Jonsson et al., 1997; Kotecha et al., 1996; Kwong et al., 1998; Rozycki, 1994). Although the duration of this increase remains unclear, increased concentrations of IL-1\(\beta\) have been reported in premature infants with subsequent BPD throughout the first 28 days of life (Kotecha et al., 1996;
Kwong et al., 1998; Rozycki, 1994; Tullus et al., 1996), suggesting a key role of IL-1β in both the development and promotion of the disorder.

Recent reviews have suggested that the severity of inflammatory illnesses may be a result of an imbalance between pro-inflammatory and anti-inflammatory mediators (Dinarello, 1996; Dinarello & Wolff, 1993). When IL-1β is produced in response to a pulmonary insult, a common ancestral gene also signals the production of interleukin-1 receptor antagonist (IL-1ra), which antagonizes the effects of IL-1β through competitive binding of its receptors (Dinarello, 1996; Dinarello & Wolff, 1993). Consequently, IL-1ra functions as an endogenous down-regulator of the inflammatory cascade (Janson, Hance, & Arend, 1991; Lee et al., 1995; Roberge et al., 1994). Significant decreases of neutrophil emigration, inhibition of pulmonary pro-inflammatory cytokine release, and increased survival times have been reported in animals receiving exogenous IL-1ra following exposure to IL-1β (Abraham & Allbee, 1994; Leff et al., 1994; Ohlsson et al., 1990; Ulich et al., 1991). IL-1ra administration has further resulted in decreases in protein leakage, pulmonary hypertension, collagen deposition, and myeloperoxidase activity when compared to untreated controls (Dejun et al., 1996; Leff et al., 1994; Piguet, Vesin, Grau, & Thompson, 1993; Voelkel, Tuder, Bridges, & Arend, 1994). For premature infants who develop BPD, IL-1ra concentrations may be insufficient to counter the effects of IL-1β during the inflammatory response. Although plasma concentrations of IL-1ra are significantly increased during the first week of life in premature infants with respiratory distress syndrome (Geiger et al., 1996), these concentrations remain relatively unchanged throughout the first month of life, leading to a significant increase in the ratio
of plasma IL-1β to IL-1ra during the first postnatal week (Rindfleisch et al., 1996). Thus, premature infants at risk for BPD may have ineffective IL-1ra down regulation (Rindfleisch et al., 1996), leading to a significant imbalance of IL-1β and IL-1ra, resulting in poorly opposed inflammation with deleterious effects on pulmonary function.

The Relationship Between Oxidative Stress and Inflammation in BPD

Although oxidative stress and inflammation are generally referred to as separately occurring events in the premature infant at risk for BPD, they are in fact closely related. Substantial research has indicated that both are primary factors involved in the development of BPD through the activation of neutrophils following acute lung injury. Whereas activated neutrophils produce pro-inflammatory cytokines following intubation and ventilation (Groneck et al., 1994; Munshi, Niu, Siddiq, & Parton, 1997), they also produce free radicals in response to hyperoxia (Jankov et al., 2003; Narasaraju et al., 2003) and invading organisms (Winterbourn et al., 2000). The production of these cytokines and free radicals ultimately leads to an inflammatory reaction in premature infants with BPD regardless of the initial cause (Groeneck & Speer, 1995).

Despite the wealth of research literature on premature infants with BPD, little is known regarding the association between inflammation and antioxidants such as vitamin A in extremely premature infants at risk for BPD. Furthermore, few studies have examined the contribution of early nutritional intake to the inflammatory response and vitamin A status of the extremely premature infant during the initial days of life. The purpose of this study is to determine the associations between: 1) plasma retinol, a potent
antioxidant; 2) urinary IL-1ra, an inhibitor of pro-inflammatory IL-1β and marker of IL-1β production; and 3) nutritional intake, including the mean kilocalories per kilogram per day (kcal/kg/day) and mean amount of vitamin A consumed during the first week of life. IL-1ra concentrations were determined in the urine of participating infants due to the rapid secretion of IL-1ra into the urine (Poutsiaka, Clark, Vannier, & Dinarello, 1991) and the high correlation of urinary IL-1ra concentrations with cellular IL-1ra secretion (Lynch, Dinarello, & Cannon, 1994). All nutritional intake data were recorded from the infants’ medical charts.

The proposed model of variable relationships is demonstrated in Figure 1. It was anticipated that the findings of this study would provide a better understanding of the mechanisms associated with pulmonary injury in this population that would aid the development of feeding recommendations for extremely premature infants.

Research Questions

The specific aim of this study was to examine the relationships between a selected plasma antioxidant (retinol), a selected marker of inflammation (urinary IL-1ra), nutritional intake (mean kcal/kg and vitamin A consumed), and oxygen dependence at 36 weeks postconceptional age in a sample of extremely premature infants at high risk for BPD. These relationships were explored at birth and again on day 7 of life in infants born at less than or equal to 30 weeks gestational age with respiratory distress syndrome. The specific research questions that were examined in this study were as follows:
Figure 1.1: Proposed model of variable relationships. Variable relationships that were examined in this study are indicated by a dashed (---) line.
1. What correlations exist between plasma retinol concentrations, urinary IL-1ra concentrations, mean caloric intake, mean vitamin A intake, and oxygen dependence at 36 weeks postconceptional age?

2. Are there significant differences in plasma retinol concentrations and urinary IL-1ra concentrations between birth and 7 days of life?

3. Are there differences between infants who do and do not require oxygen at 36 weeks postconceptional age with regard to plasma retinol, urinary IL-1ra, mean caloric intake, and mean vitamin A intake?

4. Can oxygen dependence at 36 weeks postconceptional age be predicted from plasma retinol concentrations, urinary IL-1ra concentrations, mean caloric intake, or vitamin A intake during the first week of life?

Methodology

A prospective, exploratory design was used to examine the following research variables: plasma retinol, urinary IL-1ra, and nutritional intake on day 1 and day 7 of life in premature infants who were at high risk for the development of BPD. Given that BPD is more prevalent in extremely premature infants (Egreteau et al., 2001; Hack & Fanaroff, 1999; McElrath et al., 2001; National Institutes of Health: National Heart, Lung, and Blood Institute, 1998), a convenience sample of premature infants born at less than or equal to 30 weeks gestational age was obtained for the study. Because premature infants in this age group are in the pseudoglandular and saccular phases of lung development and do not yet have pulmonary alveolarization (Kotecha, 2000), they were considered to be at
high risk for BPD. Infants born at 30 weeks gestational age or less were recruited for the study within 24 hours of birth from two Midwestern Level III Neonatal Intensive Care Units. These Neonatal Intensive Care Units were located within moderately sized hospitals that specialize in the care of premature labor and premature infant complications. Both hospitals serve as high-risk birthing centers for a 33-county area, in which 74% of the population served is Caucasian, 23% is African-American, and the remaining 3% is composed of other ethnicities. Inclusion criteria for the study were as follows:

1. Evidence of neonatal respiratory distress syndrome within 24 hours of birth, as indicated by a need for exogenous surfactant administration and/or mechanical ventilation;

2. Gestational age less than or equal to 30 weeks at birth, determined by a maturational assessment of gestational age using the New Ballard Score (Ballard et al., 1991);

3. Appropriate size for gestational age, determined by plotting birth weight, length, and head circumference measurements on growth charts of intrauterine growth and gestational age (Battaglia & Lubchenco, 1967; Lubchenco, Hansman, & Boyd, 1966);

4. Absence of a congenital pulmonary disease process, such as lung hypoplasia, that may contribute to the syndrome of respiratory distress;
5. Absence of a congenital gastrointestinal disease process, such as gastroschisis or inborn errors of metabolism, that may result in altered provision of nutrients and altered nutrient utilization;

6. Absence of maternal chorioamnionitis, which may contribute to an elevated inflammatory response in the infant (Ikegami, Kallapur, & Jobe, 2004; Watterberg, Demers, Scott, & Murphy, 1996; Yoon et al., 1999), as evidenced by clinical assessment of the amniotic fluid by an experienced obstetrician.

Because sepsis in the neonate invokes a strong inflammatory response (Kantar et al., 2000; Laborada et al., 2003; Romagnoli et al., 2001), any infant who developed sepsis during the first week of life, as evidenced by positive blood cultures, was excluded from the study. Infants were also excluded from the study if they required surgical intervention or systemic corticosteroid administration during the first week of life, due to the interference of these measures with the inflammatory response (Bessler et al., 1996; Groneck, Reuss, Gotze-Speer, & Speer, 1993; Waisman et al., 1998).

Power analysis was completed for the significance of a single Pearson product moment correlation to be obtained from n pairs (X,Y) of observations (Cohen, 1988). Although no correlations between plasma retinol and specific markers of inflammation have been reported for premature infants, highly significant differences (e.g., p < 0.01) in IL-1β production have been reported in retinol-supplemented rats when compared to controls (Moriguchi, Werner, & Watson, 1985; Spencer-Green, 1994). Therefore, a corresponding effect size of $r = 0.40$ was selected for this study. This effect size value
falls between Cohen’s (1988) medium and large conventions of $r = 0.30$ and $r = 0.50$, respectively. Utilizing an alpha level of significance of $\alpha_1 = 0.05$ ($\alpha_2 = 0.10$), a total of 37 infants was needed to achieve adequate power of 0.80 (Cohen, 1988). However, given the extreme prematurity of the population of interest, subjects were over-sampled by 10% to correct for subject attrition due to death before seven days of life.

A total of 40 infants were recruited for the study between September 1, 2003 and February 28, 2004. The sample ethnicity, which was representative of the geographical area, included 30 Caucasians, 4 African Americans, and 6 multi-racial infants. The gender of the enrolled infants corresponded precisely to the national demographics of BPD (Lemons et al., 2001; National Institutes of Health: National Heart, Lung, and Blood Institute, 1998), with 22 males and 18 females. The infants enrolled in the study had a mean gestational age at birth of 26.98 weeks (range, 24-30 weeks) and a mean birth weight of 986.75 grams (range, 551-1735 grams). Three infants died before seven days of life, and an additional three infants died before 36 weeks postconceptional age.

**Procedure**

Informed consent for participation in the study was obtained from the parents of eligible infants by the investigator. The parents of eligible infants were approached in their hospital rooms within 12 hours of birth in compliance with the Ohio State University Institutional Review Board regulations. Parents of eligible infants were informed of the study’s methods in detail, which included procedures that were of minimal risk to the infant. The confidentiality of all parents and infants was assured
through the use of coded identification numbers only. Parents consenting for participation in the study were provided with a 27-exposure disposable camera and a $7 gift card to Target stores for film processing after the appropriate signatures were obtained.

Data were obtained from participating infants at two time points: 1) within 24 hours of birth, referred to as day 1 of life, and 2) at 7 days of life. Data collection involved the following: 1) the collection of a 0.5 mL whole blood sample for the analysis of plasma retinol; 2) the collection of a 1 mL urine sample for analysis of IL-1ra concentrations, and 3) the recording of nutritional intake data, including the amount and composition of enteral and parenteral feedings received each day during the first week of life. At 36 weeks postconceptional age, the infants’ oxygen and ventilatory requirements were determined from the medical chart.

The data collection procedures were clustered to minimize infant stress and were timed to correspond with the provision of routine nursing care. All blood draws were obtained concurrently with routinely scheduled laboratory draws to eliminate the need for additional needlesticks and to minimize pain and infection risk related to multiple laboratory blood draws. The collection of all samples was temporarily suspended if the infant experienced any oxygen desaturation, bradycardia, or temperature instability. Whole blood samples were obtained from existing arterial lines, when present, or from a heelstick performed by a registered nurse. Although different blood sampling techniques were used, no differences in plasma retinol concentrations were anticipated, given the high correlations that have been observed between capillary and arterial blood laboratory
values (Johnson et al., 2000). Blood samples were collected into 1 mL lithium heparin coated tubes (RAM Scientific, Needham, MA) that were immediately wrapped in foil to prevent light degradation and placed on ice for transport (Gillis, Jones, & Pencharz, 1983; Greene et al., 1987; Haas, Genzel-Boroviczeny, & Koletzko, 2002). Urine samples were collected from a sterile cotton ball placed into the infant’s diaper during a routine diaper change (Fell, Thakkar, Newman, & Price, 1997). Urine was extracted from the cotton ball with a syringe and collected into sterile, 10 mL plastic urine containers (Fisher Scientific International, Inc., Hampton, NJ) that were placed on ice. The blood and urine samples were immediately transported on ice to The Ohio State University for analysis. A description of the variable measurement is listed below.

**Retinol.** Retinol concentrations were assessed from plasma by the preferred method of high performance liquid chromatography (Olson, 1984) at The Ohio State University Department of Human Nutrition. The collected blood specimens were immediately centrifuged in a cold room at 0°C at 10,000 x g for 5 minutes using an Eppendorf model 5415 D centrifuge (Eppendorf International, Hamburg, Germany). After separation was complete, the plasma portion was removed using a Pasteur pipette and placed into a 0.5 mL freezer-proof plastic container, which was flushed with oxygen-free nitrogen to prevent oxygen degradation of retinol (Olson, 1984). Samples were frozen in the dark at -80°C to promote stability for a 6-month time period (Thomas, Duewer, Kline, & Sharpless, 1998).
For retinol analysis, the frozen plasma samples were thawed at room temperature and separated into two 200-µL aliquots. 200 µL of ethanol containing internal standard, retinyl acetate, and 0.1% BHT was added to release the retinol from retinol binding protein, followed by the addition of 400 µL of hexane. The samples were centrifuged at 5,000 rpm for 5 minutes to separate the hexane layer and protein phases. The hexane layer was then transferred to an 11 mL glass vial using a glass Pasteur pipette and evaporated under a stream of nitrogen. The residue was then re-dissolved in a small volume of methanol and placed into the injector.

The determination of retinol in the plasma was achieved by reverse-phase high performance liquid chromatography using a modified method first described by Catignani and Bieri (1983) for the simultaneous determination of retinol and α-tocopherol. The liquid chromatograph used for this analysis was a Waters® Alliance 2695 XE Separations Module (Waters Corporation, Milford, MA) with a composition accuracy of ± 0.5% and precision of ≤ 0.15% RSD. The chromatography column utilized was a reversed-phase Vydac C18 (5 µm particle size) stainless steel column, 3.9 mm i.d. x 250 mm. The injection volume was 50 µL and the flow rate was 1.5 mL/minute. Absorbance of retinol was monitored at a wavelength of 300 nm, sufficient to capture both retinol and α-tocopherol concentrations, using a mobile phase containing water, methanol, n-butanol and ammonium acetate at a pH of 3.5. Retinyl acetate and α-tocopherol acetate were used as an internal standards and SRM 986C was used as the serum reference control. Retinol concentrations were determined by constructing standard calibration curves of the peak area versus the concentration of the sample solution. A best-fit line was then
calculated for each curve and solved to determine the precise amount of retinol in the sample solution.

**IL-1ra.** To minimize the invasiveness of sample collection, IL-1ra concentrations were measured in the urine of participating infants using the EASIA™ ELISA kit with a sensitivity of 4 pg/mL and detection range of 30-1,700 pg/mL (Biosource International, Camarillo, CA). This kit contained 2 microtiter plates with 96 anti-IL-1ra coated wells and all necessary standards and reagents for conducting the analysis. The collected urine samples were placed on ice and immediately transported to the Ohio State University College of Nursing. Urine samples were frozen at -80°C to promote stability of the specimens over a 6-month time period (Biosource International, Camarillo, CA). Prior to analysis, the samples were thawed at room temperature and diluted with phosphate buffered saline and the standard diluent (Biosource International, Camarillo, CA) in 1:10, 1:25, 1:40, and 1:100 ratios of urine to saline to achieve detection of IL-1ra in concentrations up to 200,000 pg/mL. The assay micro-plate was prepared by adding 100 µL of incubation buffer to the standard, control, and saline/plasma wells followed by the addition of 100 µL of standard diluent to the cell culture supernatant and diluted urine sample wells, which served as internal controls. 100 µL of each urine sample was then placed into the appropriate wells, in duplicate, followed by 50 µL of Biotin conjugate. The wells were incubated at room temperature for 2 hours, followed by aspiration of the remaining liquid. The plate was then washed three times with BioSource Wash Solution, 100 µL of diluted Streptavidin-HRP conjugate was placed in each well, and the plate was
incubated at room temperature for 60 minutes. The plate was then washed four times with BioSource Wash Solution and 100 µL of Chromogenic solution (TMB) was added to each well. After incubating the plate again for 30 minutes at room temperature, 100 µL of Stop Solution was added to each well and read at 450 nm using a Microplate Autoreader Model EL311 with Kinetic Calculator Junior® software (version 1991, BioTek Instruments, Inc., Winooski, VT). IL-1ra concentrations were determined through the construction of a standard curve of the optical density versus the standard concentrations, with higher concentrations indicating more extensive inflammation (Dinarello, 1991).

**Nutritional intake.** Nutritional intake information was obtained through the infant’s medical chart. The exact amount (in mL) and type of enteral and parenteral nutrition received, including formula, human milk, total parenteral nutrition, intravenous fat emulsions, and intravenous fluids were recorded each day from birth through day 7 of life. To ensure reliability and validity of the nutritional intake data, the documentation of intake data in the medical chart was clarified with the nursing staff and nutrition orders prescribed for each infant. Nutritional intake data for each day was hand-entered into NEONova® Nutrition Optimizer software (Version 4.5, 1999, Ross Products Division, Abbott Laboratories, Columbus, OH) and double-checked for accuracy. This software allowed for efficient generation of the total kilocalories per kilogram and amount of vitamin A consumed each day. These values were then averaged to obtain the mean caloric and mean vitamin A intake for each infant during the first week of life.
**Oxygen dependence.** Oxygen dependence at 36 weeks postconceptional age was defined as either: 1) the need for a fraction of inspired oxygen greater than 21 percent; 2) the receipt of oxygen from a nasal cannula; or 3) the receipt of mechanical ventilation or continuous positive airway pressure, regardless of oxygen content. Oxygen dependence was determined from the infants’ medical charts and recorded categorically (i.e., yes or no).

**Data Analysis**

The data obtained from study participants were hand-entered by the investigator into the Statistical Package for the Social Sciences© software program (Version 11.5.0, 2002, SPSS Incorporated, Chicago, IL) and double-checked for accuracy. Graphical descriptive statistics and frequency tables were generated periodically throughout the data entry process to evaluate for the presence of potential outliers or data entry errors. All available data, including that from deceased infants, was included in the analysis. Significance between variables was determined using a significance level of 0.05.

**Description of Results**

The results of this dissertation are presented in a series of three manuscripts, located in chapters 2 through 4. The titles and abstracts of each manuscript are provided below.

**Abstract**

Vitamin A is a crucial antioxidant for premature infants because it protects the lungs from oxidative damage induced by the administration of supplemental oxygen. Although all premature infants are born with decreased vitamin A stores when compared to full-term infants, the vitamin A stores of premature infants at risk for BPD are often significantly deficient. Despite the increases in plasma vitamin A concentrations that have been achieved with vitamin A supplementation, only modest improvements in clinical outcomes and the incidence of BPD have been noted in this population. This article reviews the research on vitamin A supplementation for premature infants, as well as the issues and clinical implications associated with this intervention.
Bronchopulmonary dysplasia (BPD) is a significant cause of neonatal morbidity in extremely premature infants. Although the exact pathogenesis of BPD is unknown, oxidative stress and inflammation are thought to play critical roles in the development of the disorder. Vitamin A is a potent antioxidant and promotes the differentiation of lung tissue in premature infants. Although studies have revealed low plasma concentrations of vitamin A (retinol) in premature infants at risk for BPD, it is unknown how retinol affects the inflammatory process in this population during the first week of life. This study sought to explore relationships among plasma retinol, urinary interleukin-1 receptor antagonist (IL-1ra), and nutritional intake during the first days of life in premature infants at high risk for BPD. Forty infants born at less than or equal to 30 weeks postconceptional age were recruited following birth. At day 1 and day 7 of life, 0.5 mL of whole blood, 1 mL of urine, and nutritional intake data were collected. Plasma retinol and urinary IL-1ra concentrations were analyzed with HPLC and ELISA, respectively.

Plasma retinol concentrations remained stable over the first week despite increasing vitamin A intakes. Most infants had plasma values indicative of vitamin A deficiency. Plasma retinol values on day 1 and day 7 were positively related to IL-1ra concentrations on day 7 of life ($p < 0.05$ for both retinol values), suggesting that infants
with greater inflammation had greater mobilization of retinol into the plasma. IL-1ra concentrations were very high throughout the first week and were indicative of substantial inflammation. For infants who required oxygen at 36 weeks, day 1 IL-1ra concentrations were significantly higher ($p < 0.05$) than those of infants without oxygen needs. Oxygen dependent infants also had a lower mean intake of vitamin A ($p = 0.05$) during the first week of life. These findings confirm that premature infants are vitamin A deficient and experience significant inflammation throughout the first week of life.

Inflammatory markers produced within hours after birth may provide an early measure of BPD in extremely premature infants and increasing vitamin A intake may alleviate some of the early inflammation associated the disorder.
Methodological issues associated with nutrition research in the neonatal intensive care unit. To be submitted to *Neonatal Network: The Journal of Neonatal Nursing*.

**Abstract**

The premature infant is often poorly represented in the research literature, and to date, there are few neonatal nutrition interventions for premature infants that are well validated by research studies. Although more research utilizing this population is needed, conducting research studies in the neonatal intensive care unit poses many problems for investigators as well as their subjects. Although there is considerable evidence that early nutrition may promote the developmental outcome of extremely premature infants, conducting nutrition-related research is particularly challenging due to issues associated with the anthropometric measurement of premature infants, the collection of biological specimens, and general research methodology. In this review, limitations of current neonatal nutrition research are discussed and recommendations for future research are provided.
Despite advances in neonatal care, bronchopulmonary dysplasia (BPD) remains a primary source of morbidity and mortality in extremely premature infants, affecting nearly 85 percent of neonates weighing between 500 and 700 grams at birth (National Institutes of Health: National Heart, Lung, and Blood Institute, 1998). Although BPD was initially described by Northway and associates in 1967, the exact pathophysiology associated with lung injury in affected infants remains unknown. The etiology of BPD is likely multifactorial and influenced by inflammatory, genetic, and nutritional factors. Current research also suggests that oxidative stress from supplemental oxygen administration may play a significant role in the development and course of BPD. When supplemental oxygen is administered to premature infants, the inhaled oxygen ($O_2$) combines with free electrons in the body to form superoxide anion ($O_2^-$) and hydrogen peroxide ($H_2O_2$). These molecules, referred to as reactive oxygen species (ROS), cause structural damage to the pulmonary basement membrane through the peroxidation of lipids and protein within the epithelial cell membrane (Welty, 2000; Welty & Smith,
Biomarkers of lipid and protein peroxidation, such as malondialdehyde, plasma aliphatic aldehydes, and exhaled pentane, are markedly increased during the first days of life in premature infants who later develop BPD (Nycyk, Drury, & Cooke, 1998; Ogihara et al., 1999; Ramsay et al., 2001; Schock et al., 2001; Varsila, Pesonen, & Andersson, 1995).

In healthy full-term infants, ROS are primarily eliminated by circulating plasma and cellular antioxidants, such as vitamin A, which scavenge the ROS and prevent tissue injury (Welty, 2000; Welty & Smith, 2001). However, unlike infants born at term, premature infants are antioxidant-deficient (Phylactos, Leaf, Costeloe, & Crawford, 1985). Studies of fetal animal tissue have shown that antioxidant concentrations increase in the infant up to the end of normal gestation (Frank & Sosenko, 1987a; Frank & Sosenko, 1987b; Sosenko & Frank, 1987). Consequently, because of physiological immaturity, antioxidant stores are low following premature birth, thus predisposing the infant to increased ROS-induced damage and possibly BPD (Drury, Nycyk, Baines, & Cooke, 1998; Moison, Haasnoot, Van Zoeren-Grobben, & Berger, 1998; Vyas et al., 2001).

Antioxidant Properties of Vitamin A

The fat-soluble vitamin A exists as retinol in the plasma and is a crucial antioxidant for premature infants. Retinol is thought to function as a chain-breaking antioxidant, thus rendering it an efficient scavenger of lipoperoxyl radicals. In vitro studies have demonstrated suppression of membrane peroxidation and inhibition of ROS
release with retinol supplementation (Palacios, Piergiacomi, & Catala, 1996; Schwarz et al., 1997; Sharma, Lewandoski, & Zimmerman, 1990). Retinol has also been shown to foster pulmonary epithelial differentiation and lung maturation (Perrotta et al., 2003). In studies of vitamin A deficient newborn animals, lung tissue was significantly damaged as a result of low retinol concentrations, evidenced by cilia loss, depressed surfactant synthesis, and replacement of the columnar mucus-secreting epithelium with stratified squamous keratinizing epithelium in the trachea and bronchi (Baybutt, Hu, & Molteni, 2000; Jetten et al., 1987; Stahlman, Gray, Chytil, & Sundell, 1988; Paquette et al., 1996). These alterations are similar to the histopathologic changes that have been noted in premature infants with BPD.

Vitamin A Deficiency in Premature Infants

There is growing evidence to suggest that premature infants are deficient in vitamin A, which may predispose them to the development of BPD. When the plasma retinol values of healthy full-term and premature neonates were compared, median plasma retinol concentrations were significantly higher in the full-term group (Carlson, Peeples, Werkman, & Koo, 1995; Chan, Greenough, Cheeseman, & Gamsu, 1993a). Premature infants also have only half the liver stores of full term controls (Shenai, Chytil, & Stahlman, 1985b). Following premature birth, plasma retinol concentrations continue to decline at 2 postnatal weeks (Tammela, Aitola, & Ikonen, 1999), reaching a nadir at approximately 5 weeks, after which an increase to full-term levels is noted by 4-6 weeks corrected age (Brandt et al., 1978; Mupanemunda et al., 1994). Consequently, premature
infants are likely to experience severe retinol deficiency during the first weeks of life, which may predispose them to lung injury.

For premature infants who later develop BPD, plasma concentrations of retinol following birth are markedly lower than those observed in healthy premature controls (Chan, Greenough, Cheeseman, & Gamsu, 1993b; Inder et al., 1998; Shenai, Chytil, & Stahlman, 1985a). For these infants, plasma retinol concentrations are significantly decreased both during the first week of life and throughout the first 28 postnatal days (Chan et al., 1993b; Inder et al., 1998; Shenai, Chytil, & Stahlman, 1985a). Although plasma retinol concentrations above 200 µg/L (0.70 µmol/L) are necessary to prevent vitamin A deficiency (Pitt, 1981; World Health Organization, 1997), for many premature infants with BPD, plasma retinol concentrations may fall below 100 µg/L (0.35 µmol/L), indicating both severe vitamin A deficiency and depleted liver stores (Pitt, 1981; World Health Organization, 1997). In one study, plasma retinol levels below 100 µg/L were noted in approximately 42% of infants who later developed BPD, compared to only 7% of infants who did not develop BPD (Inder et al., 1998). In a similar study, 85% of the premature infants with severe vitamin A deficiency on day 10 of life later developed BPD (Verma, McCulloch, Worrell, & Vidyasagar, 1996).

Clinical Trials of Vitamin A Supplementation for Premature Infants

Because premature infants with BPD are likely to be retinol deficient, several clinical trials have been conducting using vitamin A supplementation for premature infants at risk for BPD. Although some studies have found no significant differences
between supplemented and control infants with regard to survival and oxygen requirements at 28 days of life (Pearson et al., 1992), others have reported a reduction in supplemental oxygen, mechanical ventilation, and BPD at 36 weeks postconceptional age in infants who received vitamin A supplementation (Shenai, Kennedy, Chytil, & Stahlman, 1987; Tyson et al., 1999). In a meta-analysis of seven randomized, controlled trials of vitamin A administration to premature infants, the pooled data revealed a reduction in death and/or oxygen requirements at 36 weeks postconceptional age in extremely premature infants less than 1000 grams at birth (Darlow & Graham, 2002). Furthermore, in the largest, multi-center clinical trial of vitamin A supplementation to date, it was concluded that for every 14-15 infants supplemented with vitamin A, one additional infant survived without BPD (Tyson et al., 1999). These results suggest that the administration of vitamin A to extremely premature infants may have a beneficial effect on pulmonary outcome by 36 weeks postconceptional age.

Collectively, these studies suggest that the clinical outcomes of premature infants at risk for BPD may be modestly improved through vitamin A supplementation. However, controversy remains as to how premature infants should be supplemented. Pertinent questions have been raised regarding: 1) the “correct” dosage, chemical form, and route of vitamin A administration, and 2) the relationship between retinol deficiency and nutritional intake in premature infants at risk for BPD.
Vitamin A Dosages and Delivery Routes

The research literature remains inconclusive with regard to the correct dosage and route of vitamin A administration. Although randomized controlled trials of oral vitamin A supplementation have been conducted (Wardle, Hughes, Chen, & Shaw, 2001), oral vitamin A supplementation has not successfully increased plasma retinol concentrations in premature infants. It is thought that the absorption of oral vitamin A is not adequate to increase serum levels in this population, potentially due to immature gastrointestinal function (Rush, Shenai, Parker, & Chytil, 1994).

The parenteral administration of vitamin A may also not produce therapeutic increases in plasma retinol concentrations. When vitamin A comes into contact with light and oxygen, the vitamin is inactivated (Gillis, Jones, & Pencharz, 1983; Greene et al., 1987). Although darkly colored, light-protecting parenteral bags and tubing may provide a marginal benefit against photo-degradation of the vitamin, these measures provide little to no protection when phototherapy lights are used (Haas, Genzel-Boroviczeny, & Koletzko, 2002). Furthermore, when vitamin A is added to glucose-amino acid solutions, chemical adherence of the vitamin to standard plastic tubing is common, and as little as 20% of the vitamin dose may actually reach the infant (Gillis, Jones, & Pencharz, 1983; Greene et al., 1987; Haas, Genzel-Boroviczeny, & Koletzko, 2002). Even when polyethylene tubing with a small diameter and higher flow rate is used, retinol delivery may only be increased to about 50% (Haas, Genzel-Boroviczeny, & Koletzko, 2002). However, when vitamin A is added to intravenous lipids instead of glucose-amino acid solutions, recovery is approximately 86% with light protection and 77% without light protection.
protection, due to its fat-soluble nature (Haas, Genzel-Boroviczeny, & Koletzko, 2002). Consequently, when lipid administration of vitamin A was compared to traditional parenteral administration in premature infants with BPD, infants receiving the lipid form attained significantly higher plasma retinol values during the first month of life (Werkman et al., 1994). Although no large-scale clinical trials of vitamin A supplementation have used this delivery method, the addition of vitamin A to lipids may provide more benefit for premature infants with BPD. However, this method is limited to infants receiving parenteral nutrition, and is not practical for infants who are receiving primarily enteral feedings.

Given the problems associated with oral and intravenous vitamin A administration, intramuscular injection has become the preferred route for large-dose vitamin A supplementation. However, the dosage of vitamin A that should be supplied by intramuscular injection is less clear. In a three-phase study of three vitamin A dosages (i.e., 2300 IU, 3500 IU, and 5000 IU) given intramuscularly three times per week for four weeks, the target retinol concentration range of 250-550 \( \mu \text{g/L} \) was attained by 68% of premature infants by four weeks of age while receiving the 5000 IU supplement (Kennedy et al., 1997). However, when the same dosage of vitamin A was administered intramuscularly only during the first week of life, retinol deficiency worsened (Ambalavanan et al., 2003). Because no signs of toxicity have been reported with this dosage, intramuscular injection of at least 5000 IU has been deemed necessary for therapeutic effects (Ambalavanan et al., 2003; Kennedy et al., 1997). Although increases in plasma retinol concentrations and modest reductions in adverse respiratory outcomes
have been noted in premature infants receiving 5000 IU of intramuscular vitamin A three
times per week (Tyson et al., 1999), to date, clinicians have not embraced this practice
due to limited data on the efficacy and benefit of this intervention for premature infants at
risk for BPD.

Vitamin A and Dexamethasone

When determining route and dosage of vitamin A supplementation in premature
infants, another consideration involves the interaction of vitamin A with systemic
corticosteroids such as dexamethasone. In premature infants, systemic dexamethasone
administration has been associated with a rise in plasma retinol levels following
administration, independent of nutritional intake (Georgieff et al., 1989; Inder et al.,
1998). However, this increase is thought to be transient, resulting from initial stimulation
of the liver (Georgieff et al., 1989). Research has shown that despite no differences in
initial baseline retinol concentrations, infants receiving dexamethasone had significantly
increased retinol levels following its administration, which returned to baseline values by
day 15 (Shenai, Mellen, Chytil, & Sundell, 2000). Although the significance of this
finding is unknown, it is thought that dexamethasone enhances the serum concentrations
of transthyretin and retinol-binding protein through increased hepatocyte protein
synthesis. This ultimately leads to a greater release of retinol-binding protein and retinol
from the liver, leading to greater plasma retinol concentrations (Georgieff et al., 1989).
This endogenous release of vitamin A from the liver may ultimately be responsible for its
beneficial pulmonary effects (Shenai, Mellen, Chytil, & Sundell, 2000). If so, then the
findings of earlier studies that did not control for steroid administration must be reevaluated. It is possible that the retinol concentrations of premature infants in earlier studies were falsely elevated from the receipt of systemic corticosteroids. Additional studies that reflect the American Academy of Pediatrics’ (2002) recommendation for limited and judicious dexamethasone administration are warranted to determine if the degree of vitamin A deficiency in premature infants at risk for BPD is greater than what has been previously observed.

Vitamin A Deficiency and Nutritional Intake

Although many studies have revealed decreased retinol concentrations in premature infants with BPD, infants at high risk for BPD also have decreased nutritional intake of energy, protein, and other nutrients during the first week of life when compared to premature controls (Boehm, Bierbach, Moro, & Minoli, 1996; deRegnier, Guilbert, Mills, & Georgieff, 1996). These differences are apparent despite the administration of parenteral nutrition, which is frequently administered in greater amounts to premature infants at risk for BPD due to limited fluid tolerances (Boehm, Bierbach, Moro, & Minoli, 1996). Consequently, premature infants at risk for BPD often have decreased vitamin A intakes when compared to healthy premature controls (Inder et al., 1998; Shenai, Chytil, & Stahlman, 1985a; Verma, McCulloch, Worrell, & Vidyasagar, 1996). Given this difference in vitamin A intake, it is unclear whether the decreased retinol concentrations observed in earlier studies of premature infants were primarily attributable to nutritional intake or to the physiological immaturity of the infant. More research
employing strict controls over nutritional intake in premature infants at risk for BPD is warranted to determine the precise contribution of nutritional vitamin A intake to plasma retinol concentrations. Additional research is also needed to develop age-appropriate ranges of clinically acceptable retinol levels for extremely premature infants.

Clinical Implications

Although research has demonstrated that premature infants at risk for BPD have decreased plasma retinol concentrations and decreased vitamin A intakes when compared to healthy full-term and premature controls, the pathophysiological alterations associated with BPD render the nutritional provision of vitamin A difficult. Limited fluid tolerances, immature gastrointestinal function, and oral aversion due to chronic endotracheal or orogastric tubes are barriers to effective nutrient ingestion, and therefore may decrease vitamin A intake and plasma retinol (Roze, Darmaun, & Gaultier, 1997). Extended parenteral nutrition with insufficient vitamin A concentrations and the delayed initiation of enteral feedings, two common practices associated with the management of BPD, further decrease plasma retinol concentrations. For each day that parenteral nutrition is received, retinol concentrations fall by 5.0 µg/L, whereas no declines in retinol have been noted with enteral feedings (Inder et al., 1998). Furthermore, no universally accepted guidelines for micronutrient administration to premature infants at high risk for BPD have been developed, thus leading to a significant variety of vitamin A supplementation practices among institutions. Although standard vitamin supplementation is recommended for all premature infants at risk for BPD (Cox, 2000),
additional vitamin A for the prevention of lung disease is not currently provided in many hospitals.

Conclusions

The vitamin A concentrations of premature infants at risk for BPD are significantly lower than those of healthy premature infants. Although the exact role that vitamin A plays in the development and pathogenesis of BPD is not well understood, clinical trials of vitamin A administration suggest that supplementation of large-dose vitamin A may help prevent adverse respiratory outcomes and BPD. However, additional studies with increased control of nutritional intake variables and corticosteroids must be conducted before definitive conclusions regarding retinol concentrations and vitamin A administration to premature infants at risk for BPD can be reached. Specific research questions that must be addressed include: 1) the current incidence of vitamin A deficiency in premature neonates with and without BPD; 2) acceptable postconceptional age appropriate ranges of plasma retinol concentrations; and 3) the “correct” dosage and administration route of vitamin A that is efficacious and clinically acceptable. Until these questions are sufficiently answered, vitamin A administration to premature infants at risk for BPD will remain challenging.
CHAPTER 3

VITAMIN A STATUS AND INFLAMMATION IN THE EXTREMELY PREMATURE INFANT DURING THE FIRST WEEK OF LIFE

Despite advances in neonatal care, bronchopulmonary dysplasia (BPD) remains a primary source of morbidity and mortality in extremely premature infants. Although the exact pathophysiology of BPD is yet unknown, oxidative stress and inflammation are thought to play critical roles in the development of the disorder. Both phenomena have been observed within hours following birth in extremely premature infants (Jaarsma et al., 2001; Naik et al., 2001; Nupponen et al., 2002; Schock et al., 2001). Furthermore, significantly higher degrees of oxidative stress and inflammation have been noted in infants who develop BPD versus those who do not, suggesting a causative role of these factors in the development of premature lung disease (Buss, Darlow, & Winterbourne, 2000; Moison et al., 1997; Ogihara et al., 1998; Ogihara et al., 1999; Vyas et al., 2001). Although oxidative stress and inflammation are theoretically separate phenomena, they are closely related. Research has indicated that both may be involved in the development of BPD through the activation of neutrophils following acute lung injury associated with
intubation, mechanical ventilation, and hyperoxia (Buss, Darlow, & Winterbourn, 2000; Munshi, Niu, Siddiq, & Parton, 1997). Following the traumatic insult, the activated neutrophils produce pro-inflammatory cytokines (Groneck et al., 1994; Munshi, Niu, Siddiq, & Parton, 1997) and free radicals (Jankov et al., 2003; Narasaraju et al., 2003), which trigger an inflammatory reaction in premature infants with BPD (Groeneck & Speer, 1995). Interleukin-1β (IL-1β) is the primary cytokine involved in this inflammatory response. High levels of IL-1β have been detected in bronchoalveolar lavage fluid obtained during the first 24 hours of life in premature infants with respiratory distress syndrome (Dammann et al., 2001; Kazzi et al., 2001; Watterberg, Demers, Scott, & Murphy, 1996), and significantly increased concentrations during this time period have been reported in premature infants who later develop BPD (Jonsson et al., 1997; Kotecha et al., 1996; Kwong et al., 1998; Rozycki, 1994). These IL-1β increases are further coupled with inadequate production of the endogenous IL-1β antagonist, interleukin-1 receptor antagonist (IL-1ra), leading to a significant imbalance between pro-inflammatory and anti-inflammatory factors throughout the first week of life (Geiger, Ellemunter, Fink, & Tilg, 1996; Rindfleisch et al., 1996). This ineffective IL-1ra down regulation may be largely responsible for the development of BPD in susceptible infants who experience acute lung injury following birth.

Retinol, the plasma form of vitamin A, is a potent antioxidant and promotes the differentiation of pulmonary tissue and protects the lungs from oxidative stress (Palacios, Piergiacomi, & Catala, 1996; Perrotta et al., 2003; Schwarz et al., 1997). Retinol also possesses anti-inflammatory properties (Besnard et al., 2001; Swamidas, Basaraba, &
Baybutt, 1999). Significant increases in T-cell proliferative responses and pro-inflammatory cytokine production have been noted in retinol deficient rats when compared to retinol-sufficient controls (Wiedermann et al., 1996). Furthermore, the administration of vitamin A after exposure to various inflammatory mediums has resulted in preservation of lung cell proliferation, decreased inflammatory exudate, and reduced concentrations of mononuclear phagocytes within the lungs (Besnard et al., 2001; Swamidas, Basaraba, & Baybutt, 1999). These findings suggest that vitamin A sufficiency may alleviate much of the cellular damage associated with acute inflammation. Because the clinical manifestations of BPD are largely inflammatory in nature, BPD may be related, in part, to a deficiency of vitamin A. Premature infants have been shown to have significantly decreased plasma retinol concentrations and only half the liver stores of full-term infants (Carlson, Peeples, Werkman, & Koo, 1995; Chan, Greenough, Cheeseman, & Gamsu, 1993a; Greer, 2001). For premature infants who later develop BPD, plasma concentrations of retinol following birth are more greatly depressed and remain low throughout the first 28 days of life (Chan, Greenough, Cheeseman, & Gamsu, 1993b; Inder et al., 1998; Shenai, Chytil, & Stahlman, 1985a).

Collectively, these findings suggest that for premature infants, vitamin A deficiency may be a predisposing factor to the development of BPD through augmentation of the inflammatory cascade. Although numerous studies have examined either the vitamin A or inflammatory status of premature infants at risk for BPD, no study to date has explored the collective relationship between vitamin A and inflammation in this population. Therefore, the purpose of this study was to determine the associations
between: 1) plasma retinol; 2) urinary IL-1ra; 3) nutritional intake, including the mean kilocalories per kilogram (kcal/kg) per day and mean amount of vitamin A consumed per day during the first week of life; and 4) oxygen dependence at 36 weeks postconceptional age in a sample of extremely premature infants at high risk for BPD. These relationships were explored within 24 hours following birth and again on day 7 of life in infants born at less than or equal to 30 weeks gestational age with respiratory distress syndrome. The specific research questions that were examined in this study are as follows:

1. What correlations exist between plasma retinol concentrations, urinary IL-1ra concentrations, mean caloric intake, mean vitamin A intake, and oxygen dependence at 36 weeks postconceptional age?
2. Are there significant differences in plasma retinol concentrations and urinary IL-1ra concentrations between birth and 7 days of life?
3. Are there differences between infants who do and do not require oxygen at 36 weeks postconceptional age with regard to plasma retinol, urinary IL-1ra, mean caloric intake, and mean vitamin A intake?
4. Can oxygen dependence at 36 weeks postconceptional age be predicted from plasma retinol concentrations, urinary IL-1ra concentrations, mean caloric intake, or mean vitamin A intake during the first week of life?
Materials and Methods

Infants were recruited for the study within 24 hours of birth from two Midwestern Level III Neonatal Intensive Care Units that served a 33-county area. Infants were eligible for the study if they met the following criteria: 1) gestational age at birth of less than or equal to 30 weeks, 2) appropriate size for gestational age, 3) evidence of neonatal respiratory distress syndrome within 24 hours of birth, as indicated by a need for exogenous surfactant administration with or without mechanical ventilation, and 4) no evidence of congenital defects, metabolic disorders, sepsis, surgical needs, systemic corticosteroid receipt, or maternal chorioamnionitis. Infants were excluded from the study if positive blood or cerebrospinal fluid cultures were obtained at any time during the first week of life.

This study was approved by the Institutional Review Board of each hospital and The Ohio State University. Informed consent for participation in the study was obtained from the parents of eligible infants following admission to the Neonatal Intensive Care Unit.

Procedure

Data were obtained from participating infants at two time points: 1) within 24 hours of birth, which was defined as day 1 of life, and 2) at 7 days of life. Data collection involved the following: 1) the collection of a 0.5 mL whole blood sample for the analysis of plasma retinol; 2) the collection of a 1 mL urine sample for analysis of IL-1ra concentrations, and 3) the recording of nutritional intake data, including the amount and
composition of enteral and parenteral feedings received each day during the first week of life. At 36 weeks postconceptional age, the infants’ oxygen and ventilatory requirements were determined from the medical chart. All data collection procedures were clustered to minimize infant stress and were timed to correspond with the provision of routine nursing care.

Blood samples were collected into 1 mL lithium heparin coated tubes (RAM Scientific, Needham, MA), wrapped in foil to prevent light degradation (Gillis, Jones, & Pencharz, 1983; Greene et al., 1987; Haas, Genzel-Boroviczeny, & Koletzko, 2002), and placed on ice for transport. Urine samples were collected from a sterile cotton ball placed into the infant’s diaper during a routine diaper change (Fell, Thakkar, Newman, & Price, 1997). Urine was extracted from the cotton ball with a syringe and collected into sterile, 10 mL plastic urine containers (Fisher Scientific International, Inc., Hampton, NJ) that were sealed and temporarily placed on ice.

Retinol. Retinol concentrations were assessed from plasma by high performance liquid chromatography using a Waters® Alliance 2695 XE Separations Module (Waters Corporation, Milford, MA) with a composition accuracy of ± 0.5% and precision of ≤ 0.15% RSD. Blood specimens were centrifuged at 10,000 x g for 5 minutes to achieve separation. Remaining plasma was then removed and placed into a 0.5 mL freezer-proof container that was flushed with oxygen-free nitrogen to prevent oxygen degradation of retinol (Olson, 1984). Samples were frozen in the dark at -80°C to promote stability for a 6-month time period (Thomas, Duewer, Kline, & Sharpless, 1998). Retinol
concentrations were determined within 3 months of collection by a modified method first described by Catignani and Bieri (1983) for the simultaneous determination of retinol and α-tocopherol. The chromatography column utilized was a reversed-phase Vydac C_{18} (5 µm particle size) stainless steel column, 3.9 mm i.d. x 250 mm. The injection volume was 50 µL and the flow rate was 1.5 mL/minute. Absorbance of retinol was monitored at a wavelength of 300 nm using a mobile phase containing water, methanol, n-butanol and ammonium acetate at a pH of 3.5. Retinyl acetate and α-tocopherol acetate were used as internal standards and SRM 986C was used as the serum reference control.

**IL-1ra.** IL-1ra concentrations were determined from the urine of participating infants using the EASIA™ ELISA kit with a sensitivity of 4 pg/mL and detection range of 30 to 1,700 pg/mL (Biosource International, Camarillo, CA). Urine samples were frozen at -80°C to promote stability of the specimens over a 6-month time period (Biosource International, Camarillo, CA). Within 3 months of collection, urine samples were thawed at room temperature and diluted with phosphate buffered saline and the standard diluent (Biosource International, Camarillo, CA) in 1:10, 1:25, 1:40, and 1:100 ratios of urine to saline to achieve detection of IL-1ra in concentrations up to 200,000 pg/mL. The assay micro-plate was prepared by adding 100 µL of incubation buffer to the standard, control, and saline/plasma wells followed by the addition of 100 µL of standard diluent to the cell culture supernatant and diluted urine sample wells, which served as internal controls. Urine samples were added to the micro-plates in duplicate, and the
plates were read at 450 nm using a Microplate Autoreader Model EL311 with Kinetic Calculator Junior® software (version 1991, Bio-Tek Instruments, Inc., Winooski, VT).

**Nutritional Intake.** Nutritional intake information was obtained from the infant’s medical chart. The exact amount (in mL) and type of enteral and parenteral nutrition received, including formula, human milk, total parenteral nutrition (TPN), intravenous fat emulsions, and intravenous fluids were recorded from birth through day 7 of life. The vitamin A and caloric content of these solutions differed for each infant and were calculated according to the prescribed amount of vitamin A and kilocalories per kilogram of body weight. Vitamin A and caloric intakes were then determined based on the total amount (in mL) of solution received. The vitamin A and caloric content of enteral formulas and breast milk received were calculated according to published formula reference guidelines (Ross Pediatrics, 2003).

All nutritional intake data documentation was clarified with the nursing staff and the prescribed nutrition orders to ensure the reliability and validity of the data. The recorded nutrition data were hand-entered into NEONOVA® Nutrition Optimizer software (Version 4.5, 1999, Ross Products Division, Abbott Laboratories, Columbus, OH), which generated the total kilocalories per kilogram and amount of vitamin A consumed each day. These values were then averaged to obtain the mean caloric and vitamin A intake for each infant during the first week of life.
**Oxygen dependence.** Oxygen dependence at 36 weeks postconceptional age was defined as either: 1) the receipt of mechanical ventilation or continuous positive airway pressure with a fraction of inspired oxygen greater than 21 percent; or 2) the receipt of oxygen from a nasal cannula. Oxygen dependence was determined from the infants’ medical charts and recorded categorically (i.e., yes or no). Any infant who was oxygen dependent at 36 weeks was considered to have BPD.

**Data Analysis**

All data were hand-entered into the Statistical Package for the Social Sciences© software program (Version 11.5.0, 2002, SPSS Incorporated, Chicago, IL) and double-checked for accuracy. Graphical descriptive statistics and frequency tables were generated periodically throughout the data entry process to evaluate for the presence of potential outliers or data entry errors. All available data were included in the analysis and any missing data values were excluded from the analysis in a pairwise fashion. Correlation matrices were generated to determine variable relationships and paired-sample t-tests were used to assess for differences in plasma retinol and urinary IL-1ra concentrations between day 1 and day 7 of life. ANOVAs were run to examine differences in the study variables between infants who were and were not oxygen dependent at 36 weeks. The predictive ability of the study variables to distinguish oxygen dependence at 36 weeks was determined with a binary logistic regression. Results were considered to be significant if \( p \leq 0.05. \)

Results
A total of 40 infants were recruited for the study between September 1, 2003 and February 28, 2004. Demographic data from the sample are presented in Tables 3.1 and 3.2. The sample ethnicity, which was representative of the geographical area, included 30 Caucasians, 4 African Americans, and 6 multi-racial infants. Twenty-two infants were male and 18 were female. The infants enrolled in the study had a mean gestational age at birth of 27 weeks and a mean birthweight of 987 grams (Table 3.1).

<table>
<thead>
<tr>
<th></th>
<th>Total Sample (N=40)</th>
<th>Oxygen dependent at 36 weeks (N=17)</th>
<th>Not oxygen dependent at 36 weeks (N=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birthweight</td>
<td>986.8 (342.7)</td>
<td>867.5* (238.1)</td>
<td>1187.8* (337.6)</td>
</tr>
<tr>
<td>Range: 551-1735</td>
<td>Range: 568-1340</td>
<td>Range: 620-1735</td>
<td></td>
</tr>
<tr>
<td>Gestational Age</td>
<td>27.0 (1.8)</td>
<td>26.7** (1.5)</td>
<td>27.9** (1.7)</td>
</tr>
<tr>
<td>Range: 24-30</td>
<td>Range: 24-30</td>
<td>Range: 24-30</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1: Birthweights and gestational ages of enrolled infants. Values represent the mean (± standard deviation).

* Differences between groups are significant at \( p \leq 0.05 \).

** Differences between groups are significant at \( p \leq 0.01 \).

Betamethasone was administered to the mothers of 35 (87.5%) infants prior to delivery. Following birth, all infants received Survanta® (beractant, Ross Laboratories, Columbus, OH) within 24 hours. During the first week of life, 23 (57.5%) infants
received prophylactic indomethacin and 29 (72.5%) received caffeine citrate. By day 7, the overall oxygenation and ventilatory requirements of the sample had lessened in comparison to day 1 (Table 3.2).

Each infant received intravenous glucose solutions after birth. Continuous flow total parenteral nutrition (TPN) solutions with a vitamin A additive (M.V.I. Pediatric®, Mayne Pharma, Inc., Paramus, NJ) of 2,300 IU of retinol per 5 mL of reconstituted solution were administered to all infants by 36 hours of life. By 7 days, small volume enteral gavage feedings had been successfully initiated in 32 infants (Table 3.2).

<table>
<thead>
<tr>
<th>Oxygen/Ventilation Mode</th>
<th>Day 1 (N=40)</th>
<th>Day 7 (N=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High frequency ventilation</td>
<td>5 (12.5)</td>
<td>1 (2.7)</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>27 (67.5)</td>
<td>10 (27.0)</td>
</tr>
<tr>
<td>Continuous positive airway pressure</td>
<td>8 (20.0)</td>
<td>17 (45.9)</td>
</tr>
<tr>
<td>Nasal cannula</td>
<td>0 (0.0)</td>
<td>5 (13.5)</td>
</tr>
<tr>
<td>Room air</td>
<td>0 (0.0)</td>
<td>4 (10.8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Primary Nutrition Source</th>
<th>Day 1 (N=40)</th>
<th>Day 7 (N=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous glucose solution</td>
<td>19 (47.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Exclusive TPN</td>
<td>21 (52.5)</td>
<td>4 (10.8)</td>
</tr>
<tr>
<td>TPN plus enteral gavage feedings</td>
<td>0</td>
<td>32 (86.5)</td>
</tr>
<tr>
<td>Exclusive enteral gavage feedings</td>
<td>0</td>
<td>1 (2.7)</td>
</tr>
</tbody>
</table>

Table 3.2: Oxygenation, ventilatory, and nutritional requirements at day 1 and day 7 of life. Values represent the frequency (percentage).
By day 7, three infants had expired due to complications from extreme prematurity, and blood sampling at 7 days of life was not permitted for one infant due to severe anemia requiring repeated blood transfusions. By 36 weeks postconceptional age, one infant had been transferred to an outlying facility and an additional three infants died due to complications from patent ductus arteriosus ligation (N=1) and necrotizing enterocolitis (N=2). At 36 weeks gestation, 16 infants had no oxygen requirements, while 17 infants were oxygen dependent and considered to have BPD. Infants who required oxygen at 36 weeks were more immature and had significantly smaller birthweights than infants who were not oxygen dependent. These differences are presented in Table 3.1.

**Correlations Between Study Variables**

Correlations between plasma retinol, urinary IL-1ra, mean caloric and vitamin A intake during the first week of life, and oxygen dependence at 36 weeks postconceptional age are presented in Table 3.3. Plasma retinol on day 1 was highly correlated with plasma retinol on day 7 ($r = 0.926, p < 0.001$) and was also associated with IL-1ra concentrations at 7 days of life ($r = 0.340, p < 0.05$). A similar correlation between plasma retinol on day 7 and IL-1ra on day 7 was also observed ($r = 0.342, p < 0.05$). The IL-1ra concentrations on day 1 were positively correlated with oxygen dependence at 36 weeks postconceptional age ($r = 0.364, p < 0.05$), whereas the mean vitamin A intake
Table 3.3: Correlations between major study variables.

* Correlations are significant at $p < 0.05$. 

<table>
<thead>
<tr>
<th></th>
<th>Plasma Retinol Day 1</th>
<th>Plasma Retinol Day 7</th>
<th>Urinary IL-1ra Day 1</th>
<th>Urinary IL-1ra Day 7</th>
<th>Daily Intake Kcal/kg</th>
<th>Daily Intake Vit. A</th>
<th>Oxygen at 36 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Retinol Day 1</td>
<td>1</td>
<td>0.926**</td>
<td>-0.123</td>
<td>0.340*</td>
<td>-0.042</td>
<td>-0.237</td>
<td>0.055</td>
</tr>
<tr>
<td>Plasma Retinol Day 7</td>
<td>1</td>
<td>-0.064</td>
<td>0.342*</td>
<td>-0.095</td>
<td>-0.188</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>Urinary IL-1ra Day 1</td>
<td>1</td>
<td>0.048</td>
<td>-0.322*</td>
<td>-0.079</td>
<td>0.364*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary IL-1ra Day 7</td>
<td>1</td>
<td>-0.079</td>
<td>-0.016</td>
<td>0.281</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Daily Intake Kcal/kg</td>
<td>1</td>
<td>0.499**</td>
<td></td>
<td>-0.236</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Daily Intake Vitamin A</td>
<td></td>
<td></td>
<td>1</td>
<td>-0.340*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen at 36 Weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
during the first week of life was negatively associated with oxygen dependence at 36 weeks ($r = -0.340, p = 0.05$). The urinary IL-1ra concentrations on day 1 were negatively correlated with the mean caloric intake per kilogram of body weight during the first week of life ($r = -0.322, p = 0.05$).

**Differences in Plasma Retinol and IL-1ra Over the First Postnatal Week**

For the sample as a whole, no differences in plasma retinol were observed between day 1 and day 7 of life (Table 3.4). Of the 40 infants from whom blood samples were obtained on day 1, 30 infants (75%) had retinol concentrations between 0.35 and 0.70 µmol/L, indicating marginal vitamin A deficiency, and 16 (40%) had severely deficient values of less than 0.35 µmol/ L (Pitt, 1981; World Health Organization, 1997). By day 7, plasma retinol concentrations fell below 0.70 µmol/L in 28 (77%) of the 36 infants sampled, and 14 (39%) infants had concentrations below 0.35 µmol/L. These retinol values remained stable over the first week of life despite the increasing provision of dietary vitamin A to all infants during this time period (Figure 3.1).

Urinary IL-1ra was highly elevated both at day 1 and day 7, indicating substantial inflammation throughout the first week of life (Table 3.4). On day 1, only three (7.5%) infants had IL-1ra concentrations less than 10,000 pg/mL, and 16 (40%) had concentrations greater than 100,000 pg/mL. By day 7, the IL-1ra concentrations of eight (22%) infants fell below 10,000 pg/mL, with nine (22.5%) infants remaining above 100,000 pg/mL. Although the mean IL-1ra concentration for the sample as a whole was less at 7 days of life than at day 1, these differences were not statistically significant.
Table 3.4: Plasma retinol and urinary IL-1ra concentrations at day 1 and day 7 of life. Values represent the mean (± standard deviation).

* Values are significantly different between groups at \( p < 0.05 \).
Differences in Study Variables Between Groups

When infants were grouped according to oxygen dependence at 36 weeks gestational age, no significant differences in plasma retinol concentrations on day 1 and day 7 were noted (Table 3.4). However, oxygen dependent infants had a significantly decreased mean intake of vitamin A during the first week of life than infants who were not oxygen dependent at 36 weeks (F = 4.06, \( p = 0.05 \); Table 3.5, Figure 3.1). Ten (58.8%) of the infants requiring oxygen had mean vitamin A intakes of less than 700 IU, compared to only five (31.2%) infants in the non oxygen dependent group. These intakes fell below the minimum daily recommendation of 700 IU of vitamin A per 100 kcal made by the American Society for Nutritional Sciences in 2002 (Klein, 2002). Vitamin A intakes for the sample also fell well below the 1250-2333 IU range per 100 kcal recommended by Shenai (1993) for premature infants with lung disease.

<table>
<thead>
<tr>
<th></th>
<th>Vitamin A (IU)</th>
<th>Mean Calories (Kcal/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sample</td>
<td>717.7 (368.6)</td>
<td>66.5 (10.8)</td>
</tr>
<tr>
<td></td>
<td>Range: 202.5-1601.2</td>
<td>Range: 47.9-88.7</td>
</tr>
<tr>
<td>Requiring oxygen at 36 weeks</td>
<td>613.0 (358.3)*</td>
<td>64.8 (10.3)</td>
</tr>
<tr>
<td></td>
<td>Range: 202.5-1257.8</td>
<td>Range: 47.9-82.9</td>
</tr>
<tr>
<td>Not requiring oxygen at 36 weeks</td>
<td>861.0 (348.0)*</td>
<td>69.8 (10.9)</td>
</tr>
<tr>
<td></td>
<td>Range: 306.3-1601.2</td>
<td>Range: 51.7-88.7</td>
</tr>
</tbody>
</table>

Table 3.5: Mean daily caloric and vitamin A intakes during the first week of life. Values represent the mean (± standard deviation).

* Values are statistically significant between groups at \( p = 0.05 \).
Urinary IL-1ra concentrations on day 1 were significantly greater in infants requiring oxygen at 36 weeks postconceptional age when compared to infants not receiving oxygen at this time ($F = 4.729, p < 0.05$; Table 3.4). Although mean urinary IL-1ra concentrations remained higher on day 7 in the oxygen dependent group, this difference was not significant. In both groups a non-significant decreasing trend in IL-1ra concentrations was noted over the first week of life.

Mean caloric intakes are shown in Figure 3.2 and Table 3.5. No significant differences in caloric intake were noted between groups. However, caloric intakes for
both groups during days 1 to 3 of life fell below the minimum recommendation of 80-100 kcal/kg/day for parenteral nutrition delivery (Cox, 2000; Thureen & Hay, 2000; Figure 3.2).

![Graph showing mean daily caloric intake (kilocalories per kilogram) during the first week of life.]

Figure 3.2: Mean daily caloric intake (kilocalories per kilogram) during the first week of life.

*Predictive Ability of Study Variables at 36 Weeks Postconceptional Age*

Mean weekly caloric intake, mean weekly vitamin A intake, and plasma retinol and IL-1ra concentrations at day 1 and day 7 were not predictive of oxygen dependence at 36 weeks postconceptional age when entered collectively into the logistic regression model.
Discussion

Vitamin A is a critical nutrient for premature infants because it promotes epithelial differentiation and lung maturation (Perrotta et al., 2003). When vitamin A deficiency is present, the lungs are more susceptible to hyperoxic lung injury and inflammation (Besnard et al., 2001; Sharma, Lewandoski, & Zimmerman, 1990; Swamidas, Basaraba, & Baybutt, 1999). Vitamin A deficiency also impairs surfactant secretion from type II pneumocytes (Baybutt, Hu, & Molteni, 2000). These pathological alterations are reduced with vitamin A supplementation (Besnard et al., 2001; Swamidas, Basaraba, & Baybutt, 1999). Premature infants are deficient in vitamin A following birth (Carlson, Peeples, Werkman, & Koo, 1995; Chan, Greenough, Cheeseman, & Gamsu, 1993a), and this deficiency may predispose them to inflammatory damage and BPD.

Plasma Retinol

This study, like others, detected low plasma retinol concentrations in premature infants throughout the first week of life (Chan, Greenough, Cheeseman, & Gamsu, 1993b; Inder et al., 1998; Shenai, Chytil, & Stahlman, 1985a). However, whereas other studies have shown declines in plasma retinol over the first 7 postnatal days (Chabra et al., 1994; Inder et al., 1998; Shenai, Chytil, & Stahlman, 1985a), this study demonstrated remarkable stability in plasma retinol concentrations during the fist postnatal week. This lack of change in plasma retinol was apparent for the sample as a whole as well as for infants who did and did not require oxygen at 36 weeks postconceptional age. Furthermore, unlike other studies that have demonstrated significantly lower plasma
retinol concentrations throughout the first 28 days of life in infants with BPD when compared to healthy premature controls (Chan, Greenough, Cheeseman, & Gamsu, 1993b; Inder et al., 1998; Shenai, Chytil, & Stahlman, 1985a), the present study failed to detect these differences between groups during the first 7 days of life.

Unlike previous studies of vitamin A and premature infants, infants in this study demonstrated extremely low plasma retinol concentrations. Plasma retinol concentrations of $\leq 0.70 \mu\text{mol/L}$ are considered to marginally deficient, and concentrations below 0.35 $\mu\text{mol/L}$ represent severe vitamin A deficiency and depleted liver stores (Pitt, 1981). In the present study, approximately 40% of the total sample had severely deficient retinol concentrations of less than 0.35 $\mu\text{mol/L}$ at day 1 and day 7 of life, and mean retinol values were approximately 0.50 $\mu\text{mol/L}$ during the first postnatal week. In other studies, fewer than 15% of premature infants less than 32 weeks postconceptional age had plasma retinol concentrations below $\leq 0.35 \mu\text{mol/L}$ prior to day 7 of life (Mupanemunda et al., 1994; Verma, McCulloch, Worrell, & Vidyasagar, 1996). Likewise, similar studies have revealed mean plasma retinol concentrations of approximately 0.60 to 0.80 $\mu\text{mol/L}$ in premature infants following birth (Chabra et al., 1994; Ghebremeskel et al., 1999; Inder et al., 1998), compared to ranges of 0.223 to 1.76 in the current study.

Because the accretion of vitamin A and other antioxidants occurs primarily during the third trimester of gestation (American Academy of Pediatrics Committee on Nutrition, 2003; Frank & Sosenko, 1987a; Sosenko & Frank, 1987), the extreme prematurity characteristic of this sample may be largely responsible for these differences. Significant correlations between plasma retinol and gestational age have been reported
elsewhere (Chan, Greenough, Cheeseman, & Gamsu, 1993a; Mupanemunda et al., 1994), although these findings are not consistently supported (Howells, Levin, Brown, & Brooke, 1984; Tammela, Aitola, & Ikonen, 1999). Whereas the mean postconceptional age of earlier studies was 28-30 weeks (Inder et al., 1998; Shenai, Chytil, & Stahlman, 1985a), in the present study, mean gestation approximated 27 weeks. Therefore, it is possible that greater postconceptional age resulted in greater plasma retinol concentrations in previous studies. The higher plasma retinol concentrations observed in earlier studies may also have been a result of widespread use of systemic dexamethasone prior to 2002. Recommendations to discontinue dexamethasone in premature infants were made in 2002 due to adverse neurodevelopmental effects (American Academy of Pediatrics Committee on Fetus and Newborn, 2002). When administered to premature infants, dexamethasone stimulates the liver to release retinol into the plasma (Georgieff et al., 1989; Inder et al., 1998; Shenai, Mellen, Chytil, & Sundell, 2000). This increased secretion of retinol into the plasma may persist for up to two weeks, leading to potential misinterpretation of vitamin A status in premature infants receiving the drug. Therefore, the findings of earlier studies that included gestationally immature infants receiving dexamethasone as part of their routine care may not be representative of the extremely premature infants encountered today.

**Vitamin A Intake**

The increasing vitamin A intake during the first week of life was not associated with an increase in plasma retinol concentrations between day 1 and day 7. Although
similar findings have been reported, the infants in previously studies received considerably less vitamin A as part of the routine nutritional regimen than infants in this sample (Chabra et al., 1994; Mupanemunda et al., 1994). This finding may be related to the infants’ liver stores of vitamin A, which are thought to be depleted when plasma retinol values fall below 0.35 µmol/L (Pitt, 1981; World Health Organization, 1997). Following birth, the liver stores of vitamin A in the premature infant are only about 20% of adult concentrations (Montreewasuwat & Olson, 1979; Olson, Gunning, & Tilton, 1984; Shenai, Chytil, & Stahlman, 1985b). These liver concentrations increase with postconceptional age (Iyengar & Apte, 1972; Montreewasuwat & Olson, 1979). Given the extreme prematurity and the significantly low plasma retinol values observed in this sample on day 1 of life, it may be that early vitamin A intake was used preferentially to restore liver stores (Olson, 1984), and was therefore insufficient to increase plasma retinol concentrations.

In the present study, a significant correlation between mean vitamin A intake during the first postnatal week and oxygen dependence at 36 weeks postconceptional age was observed. Infants requiring oxygen at 36 weeks, when compared to those who did not, had significantly lower mean vitamin A intakes during the first week of life. In other studies, similar differences in vitamin A intake between BPD and non-BPD infants have been noted throughout the first 28 postnatal days (Chabra et al., 1994; Chan, Greenough, Cheeseman, & Gamsu, 1993b; Verma, McCulloch, Worrell, & Vidyasagar, 1996; Werkman et al., 1994). These differences are primarily attributable to the decreased nutritional intake characteristic of premature infants with BPD (Boehm, Bierbach, Moro,
& Minoli, 1996; deRegnier, Guilbert, Mills, & Georgieff, 1996; Tang, Ridout, & Modi, 1997). Because the incidence of BPD is inversely related to postconceptional age, infants who later develop BPD are more gestationally immature and have more critical phases of acute illness that, when coupled with the severity of the infant’s respiratory illness, make it difficult to provide adequate nutrients (Atkinson, 2001; Wilson et al., 1997). Consequently, premature infants who later develop BPD often require sustained TPN delivery and have delayed initiation of enteral feedings (Cox, 2000). This delay in the initiation of enteral feedings has been associated with decreased plasma retinol concentrations in premature infants (Woodruff, Latham, James, & Hewett, 1986).

Because retinyl palmitate provided the sole source of vitamin A in both the enteral and parenteral nutrition preparations used in this study, differences in the vitamin A bioavailability were unlikely. Rather, the enteral feedings provided a greater concentration of vitamin A. This difference was attributable to the large volumes of vitamin A-supplemented TPN that were not completely infused to the infant, due to fluid restrictions associated with critical illness and prematurity.

Although no differences in plasma retinol concentrations were noted in this study, others have revealed declines in plasma retinol values that were inversely related to the number of days of TPN received (Inder et al., 1998; Peeples, Carlson, Werkman, & Cooke, 1991). In one study, it was determined that by 28 of life, plasma retinol levels had fallen by 5.0 µg/L for each day that TPN was received (Inder et al., 1998). This may be due in part to inactivation of parenteral vitamin A by external light sources and adherence of the vitamin to plastic tubing (Gillis, Jones, & Pencharz, 1983; Greene et al.,
In the present study, vitamin A (retinyl palmitate) was added to each infant’s TPN. Although this was common practice for both hospital institutions, the very slow flow rates of the TPN solutions may have lead to significant vitamin A absorption into the plastic tubing. Studies have shown that this practice may result in the delivery of only 20-50% of the vitamin to the infant (Gillis, Jones, & Pencharz, 1983; Greene et al., 1987; Haas, Genzel-Boroviczeny, & Koletzko, 2002). Consequently, lipid administration of vitamin A has been recommended, due to recovery rates of 77-86% that have been associated with significantly higher plasma retinol values during the first month of life (Werkman et al., 1994).

**Urinary IL-1ra**

IL-1β is the primary pro-inflammatory cytokine associated with inflammation of the lung in extremely premature infants (Dinarello, 1991; Piedboeuf et al., 1998). Following a pulmonary insult, IL-1β is synthesized within the cytosol of alveolar macrophages and monocytes and released from the cell, where it binds to IL-1β receptors in the extracellular space (Dinarello, 1991). This binding leads to chemotaxis of neutrophils and induction of the inflammatory cascade. At the same time that IL-1β is translated, IL-1ra production is signaled from a separate gene within the IL-1 gene family located on the long arm of chromosome 2 (Dinarello, 1991). The IL-1ra that is produced antagonizes the effects of IL-1β through competitive binding of its receptors in the extracellular space (Dinarello, 1996; Dinarello & Wolff, 1993). IL-1ra possesses no agonist activity (Granowitz et al., 1992) and functions purely as an endogenous down-
regulator (antagonist) of the inflammatory cascade (Janson, Hance, & Arend, 1991; Lee et al., 1995; Roberge et al., 1994). Although IL-1ra concentrations provide an indirect assessment of IL-1β status, they are a useful measure of inflammation in premature infants. Approximately 80% of the IL-1ra produced is consistently secreted into the serum (Poutsiaka, Clark, Vannier, & Dinarello, 1991). IL-1ra concentrations are also rapidly secreted into the urine when inflammation is present (Korn et al., 1987; Liao, Grimshaw, & Rosenstreich, 1984). These urinary concentrations are highly correlated with cellular IL-ra secretion (Lynch, Dinarello, & Cannon, 1994).

In this study, the urinary IL-1ra concentrations of extremely premature infants were substantially high throughout the first week of life. In healthy adults, circulating IL-1ra concentrations range from approximately 190 to 1090 pg/mL (Mandrup-Poulsen et al., 1995). These values are increased to a range of 425 to 37,000 pg/mL in adult patients with thermal burns (Mandrup-Poulsen et al., 1995), 6,000 to 7,000 pg/mL in patients receiving low dose *E. coli* endotoxin (Granowitz et al., 1991), and 2,000 to 3,000 pg/mL in patients with meningitis (Ven Deuren et al., 1997). When physiological shock is present, concentrations of IL-1ra of up to 390,000 to 805,000 pg/mL have been reported (Van Deuren et al., 1997). In this sample of premature infants, mean IL-1ra concentrations of 92,625 pg/mL on day 1 and 64,884 pg/mL on day 7 were observed for the sample as a whole, confirming the presence of substantial inflammation. Although these values were high at both time points, only the concentrations obtained on day 1 were correlated with oxygen dependence at 36 weeks postconceptional age. On day 1, oxygen dependent infants had significantly higher mean IL-1ra concentrations than...
infants who did not require oxygen at this time (i.e., 114,093 pg/mL vs. 61,936 pg/mL). Oxygen dependent infants continued to have greater mean IL-1ra concentrations at day 7 of life, although these differences were not statistically significant.

The patterns of inflammation suggested by these IL-1ra concentrations are consistent with previous research. High concentrations of IL-1β and other pro-inflammatory cytokines have been detected in the bronchoalveolar lavage fluid of infants with respiratory distress syndrome during the first day of life (Dammann et al., 2001; Kazzi et al., 2001; Watterberg, Demers, Scott, & Murphy, 1996). As respiratory distress resolves, these inflammatory markers decrease over the first 7-10 days of life in conjunction with decreased neutrophil activation (Contreras et al., 1996; Merritt et al., 1981; Merrit et al., 1983; Nupponen et al., 2002; Todd et al., 1998). This process appears to be interrupted in premature infants who later develop BPD. For these infants, pro-inflammatory cytokine concentrations are significantly greater during the first 24 hours of life than those observed in premature infants with uncomplicated respiratory distress syndrome (Jonsson et al., 1997; Kotecha et al., 1996; Kwong et al., 1998; Rozycki, 1994). This increased cytokine production is apparent throughout the first 7-14 days of life and may persist for up to 28 days (Kotecha et al., 1996; Kwong et al., 1998; Rozycki, 1994; Tullus et al., 1996). Ultimately, the persistence of these cytokines contributes to epithelial fibrosis and decreased pulmonary function in premature infants who develop BPD (Ozdemir, Brown, & Morgan, 1997).

Several studies have revealed decreased cytokine secretion in premature infants when compared to adults, although findings remain inconclusive (Bessler et al., 1993;
Dembinski et al., 2003; Peters, Bertram, Gahr, & Speer, 1993; Weatherstone & Rich, 1989). Recently, Bessler and associates (2002) also noted decreased production of IL-1ra by the mononuclear cells of premature infants. In the present study, the large amounts of IL-1ra observed suggest that extremely premature infants do have substantial cytokine secretory ability. However, this finding must be interpreted with caution. Because IL-1β was not assessed in this study, the ratio of IL-1β to IL-1ra secretion in this sample is unknown. In adult patients with inflammatory diseases, there is frequently a 10 to 100-fold molar ratio of IL-1ra to IL-1β in body fluids (Dinarello, 1996). This increased IL-1ra is thought to be a normal physiological response to illness. However, when significant inflammation is present, physiological increases in IL-1ra may be insufficient to alleviate the effects of IL-1β, leading to a significant increase in the IL-1β to IL-1ra ratio (Dinarello, 1996; Ohlsson et al., 1990). This same phenomenon may occur in premature infants. In a study of premature infants who developed BPD, IL-1ra concentrations remained unchanged during the first week of life, despite increasing concentrations of IL-1β during this time (Rindfleisch et al., 1996). This disparate IL-1β:IL-1ra ratio may therefore be associated with the development of BPD in premature infants (Dinarello, 1996; Rindfleisch et al., 1996).

In the present study, no significant differences in IL-1ra concentrations were noted between day 1 and day 7 of life for either the oxygen dependent or non-oxygen dependent infant groups. Given the high attrition of this sample prior to 36 weeks postconceptional age, this lack of significance may be attributable to the small number of infants within each group. However, this lack of change over time may also reflect
consistent secretion of IL-1ra, perhaps in spite of increased IL-1β in oxygen dependent infants. This notion is congruent with the work of Schultz and associates (2004), who found that overall inhibition of the pro-inflammatory cytokines was markedly reduced in premature infants of less than 30 weeks gestational age at birth when compared to adults.

The finding of high IL-1ra concentrations above 100,000 pg/mL in 40% of the sample on day 1 and 22.5% of the sample on day 7 is of significant clinical interest. Although high concentrations of IL-1ra have been noted in other studies of premature infants during the first week of life (Geiger, Ellemunter, Fink, & Tilg, 1996; Rindfleisch et al., 1996; Tullus et al., 1996), the IL-1ra values obtained in this study are significantly higher than the median concentrations of 3000 to 15,000 pg/mL that have been previously reported (Geiger, Ellemunter, Fink, & Tilg, 1996; Tullus et al., 1996). Because the infants in this study had no evidence of sepsis and were born to mothers without chorioamnionitis, the greater IL-1ra concentrations that were observed may reflect current clinical management practices for neonatal respiratory distress syndrome. It may be that current ventilatory strategies, which involve significantly less barotrauma and volutrauma (Yoder, Siler-Khodr, Winter, & Coalson, 2000), somehow enhance the secretion of IL-1ra from pulmonary mononuclear cells. These differences may also be attributable to the extreme prematurity of this sample in comparison to earlier studies, although no clear association between gestational age and IL-1ra production has yet been identified.
Retinol and IL-1ra

Despite the anti-inflammatory properties of vitamin A, plasma retinol concentrations obtained from this sample were not predictive of oxygen dependence at 36 weeks postconceptional age. Instead, significant positive correlations between plasma retinol on days 1 and 7 and IL-1ra concentrations on day 7 were noted, such that infants with the greatest plasma retinol also had the greatest levels of IL-1ra. In several *in vitro* and *in vivo* studies of vitamin A administration, plasma hyporetinemia has been induced following an inflammatory trigger (Kanda, Yamamoto, & Yoshino, 1990; Rosales & Ross, 1998a; Rosales & Ross, 1998b; Stephensen & Gildengorin, 2000). In these studies, inflammation-induced hyporetinemia was attributed to a decrease in the amount and synthesis of retinol-binding protein (RBP) during acute inflammation (Rosales et al., 1996; Rosales & Ross, 1998b). Under normal physiologic conditions, vitamin A is mobilized from the liver into the plasma in the form of retinol, which is bound to RBP in a 1:1 ratio. However, in the presence of inflammation or protein-calorie malnutrition, liver RBP secretion is blocked, leading to increased accumulation of liver RBP stores and decreased plasma RBP and retinol (Donnen et al., 1996; Morlese et al., 1998; Rosales & Ross, 1998b). Extremely premature infants not only experience significant inflammation and protein-calorie malnutrition during the first week of life, but also have RBP concentrations in the deficient range following birth (Bhatia & Ziegler, 1983; Shenai, Chytil, Jhaveri, & Stahlman, 1981). These factors predispose the premature infant to a state of retinol deficiency. Because RBP concentrations were not assessed in this study,
plasma retinol concentrations alone may not provide an accurate estimation of the vitamin A status of these infants.

The positive correlations that were observed in this study between plasma retinol and urinary IL-1ra during the first postnatal week may also be attributable to enhanced expression of IL-1ra with greater plasma retinol concentrations. Although a precise mechanism for this expression has not been elucidated in the literature, researchers have demonstrated correlations between IL-1β, IL-1ra, and retinoic acid, a retinol metabolite (Hashimoto et al., 1998; Spencer-Green, 1994). In some studies, treatment of cell cultures with retinol and retinoic acid resulted in increased production of IL-1β by the treated cells (Hashimoto et al., 198; Spencer-Green, 1994; Walsh, Seymour, & Powell, 1985). This increased production of IL-1β by retinol and retinoic acid-treated cells suggests that the macrophages responsible for IL-1β release may be stimulated by circulating plasma retinol, which is then converted to retinoic acid. Retinoic acid is thought to exert its effects via retinoic acid receptors, which activate the expression of genes with elements responsive to retinoic acid (Vasios et al., 1989). To date, the exact actions of retinoic acid on IL-1β and IL-1ra production are unknown. However, the positive correlations between plasma retinol and urinary IL-1ra observed in this study may be due to increased transcription of IL-1ra following vitamin A administration.

It is also possible that the correlation observed between increased plasma retinol and increased IL-1ra concentrations on day 7 of life may be due to the inappropriate selection of IL-1ra as a marker of inflammation. It may be that greater plasma retinol concentrations facilitate the immune system with the production of anti-inflammatory
cytokines such as IL-1ra. Perhaps it is this increased ratio of anti- to pro-inflammatory cytokines that is largely responsible for the beneficial effects of retinol in inflamed rats (Swamidas, Basaraba, & Baybutt, 1999). Although the exact mechanism associated with the anti-inflammatory effect of retinol is unknown, the relationship of retinol to IL-1ra warrants further investigation.

**IL-1ra and Caloric Intake**

Despite attempts to initiate early TPN and early enteral gavage feedings in this sample of premature infants, many of the infants in this study had mean caloric intakes well below current recommendations of 80-100 kcal/kg per day during the initial days of life (Cox, 2000; Thureen & Hay, 2000). Although there were no significant differences in week 1 caloric intake between oxygen and non-oxygen dependent infants at 36 weeks postconceptional age, these findings are in agreement with other studies that have noted significantly decreased nutritional intakes of premature infants with respiratory distress syndrome (Hay, 1996; Tang, Ridout, & Modi, 1997; Wahlig et al., 1994; Wilson et al., 1997). In these studies, attempts to increase nutritional intake were unsuccessful, and premature infants with respiratory distress syndrome continued to have inadequate nutrition throughout the first week of life. This nutritional deprivation may have significant pulmonary consequences. In studies of adult rodents, severe calorie restriction led to alveolar destruction and emphysema-like changes within the lungs of starved animals (Karlinski, Goldstein, Ojserkis, & Snider, 1986; Massaro et al., 2003). It was further noted that 35% of alveoli were lost within 72 hours of the onset of calorie
restriction (Massaro et al., 2003). Because premature infants are born with physiologically immature lungs, and because they are further subjected to a variety of pulmonary insults following birth, adequate nutrition is warranted in this population.

In the present study, the finding of increased IL-1ra concentrations in relation to greater mean caloric intake during the first week of life further emphasizes the need for more adequate nutrition during the early neonatal period. In this study, mean caloric intake was used as a global indicator of the adequacy of nutritional intervention, such that infants receiving the greatest calories were also receiving greater amounts of protein, fatty acids, and other nutrients via TPN and enteral feedings. The provision of greater protein and greater antioxidant nutrients may have inhibited the inflammatory response through the scavenging of reactive oxygen species and repletion of vital transport proteins, such as retinol binding protein, which are essential for tissue repair in the lungs.

Several recent reviews have indicated an important role of nutrition in the modulation of cytokine biology (Alexander, 1998; Grimble, 1998). In particular, the provision of monounsaturated fatty acids and omega-3 polyunsaturated fatty acids has resulted in suppression of IL-1 production through modulation of eicosanoid production and transcription factor activity (Simopoulos, 2002). By contrast, the omega-6 fatty acids induce IL-1 production and exert the opposite effect (Besier & Grimble, 1995). To date, no associations between nutritional intake and IL-1ra production have been determined. Because caloric intake provided a global assessment of the adequacy of early nutrition in this study, it is possible that early aggressive nutrition attempts in this sample supported the anti-inflammatory response of premature infants.
Conclusion

The findings from this study suggest that premature infants are deficient in vitamin A and experience significant inflammation during the first week of life. Inflammatory markers produced within hours after birth may provide an early measure of BPD in extremely premature infants, and increasing vitamin A and nutritional intake may alleviate some of the early inflammation associated with the disorder.
CHAPTER 4

METHODOLOGICAL ISSUES ASSOCIATED WITH NUTRITION RESEARCH IN THE NEONATAL INTENSIVE CARE UNIT

The provision of adequate nutrition to the premature infant is one of the most challenging aspects of neonatal care. Because the majority of nutrient accretion occurs during the third trimester of pregnancy, preterm birth results in sub-optimal nutrient stores in the premature infant, ultimately leading to inadequate growth and development (Ehrenkranz et al., 1999; Hay, Catz, Grave, & Yaffe, 1997; Hay et al., 1999). When prematurity is compounded with acute illnesses such as respiratory distress syndrome, nutritional intake is further compromised, resulting in significant nutrient deficiency (Hay, 1996; Tang, Ridout, & Modi, 1997; Wahlig et al., 1994; Wilson et al., 1997). Despite increasing evidence that early aggressive nutrition enhances the developmental outcome of premature infants (Brandt, Sticker, & Lentze, 2003; Lucas, Morley, & Cole, 1998; McClure & Newell, 2000), there are currently no universally accepted clinical guidelines for aggressive nutritional management of these infants. As a result, nutrition practices vary widely among neonatal institutions, with many premature infants receiving inadequate nutrients for optimal growth and development.
The goal of postnatal nutrition following premature birth is to provide adequate nutrients to achieve weight gains equivalent to those obtained in utero at the same gestational age (American Academy of Pediatrics, 2003). Before this goal can be achieved, research studies that validate or invalidate current nutritional practices must be conducted. Ultimately, this involves large, randomized controlled trials of varying nutrient compositions and intakes for preterm infants within the Neonatal Intensive Care Unit (NICU) setting. These clinical trials, though necessary for the advancement of neonatal care and developmental outcome, pose many problems for both the investigators as well as their subjects. This review highlights these methodological issues and provides recommendations for further neonatal nutrition research.

**Anthropometric Measurement**

Providing adequate nutrition to premature infants is challenging due to the premature infant’s higher growth velocity and metabolic immaturity (Rigo, Morley, & Koletzko, 1999). Despite nutritional intervention, growth deficits are common in premature infants and are especially apparent in infants weighing less than 1000 grams at birth (Berry, Abrahamowicz, & Usher, 1997; Ehrenkranz et al., 1999; Lemons et al., 2001). In the NICU, growth deficiency is frequently determined by plotting birth weight, length, and head circumference measurements on charts of intrauterine growth norms at varying gestational ages (i.e., Battaglia & Lubchenco, 1967; Ehrenkranz et al., 1999; Lubchenco, Hansman, & Boyd, 1966). When these measures are plotted serially on infant growth charts, they provide crude assessments of the overall nutritional status of...
the infant as well as the infant’s tolerance of extra-uterine care (Sparks, 1999). These
growth patterns are also used for daily medical and nutritional management, in that they
provide the basis for medication dosages and parenteral fluid delivery. Yet despite the
clinical usefulness of weight, length, and head circumference measurements in premature
infants, measurement errors are common and may potentially result in misinterpretation
of an infant’s nutritional state. Therefore, it is essential that weight, length, and head
circumference measures are obtained and recorded accurately.

Weight Assessment

In the clinical setting, measurements of daily weight are most often used as a
growth indicator for premature infants. Although changes in weight may indicate
increases and decreases in an infant’s body mass, weight change may also be a reflection
of fluid status and not necessarily an indicator of growth (Ehrenkranz et al., 1999).
Weight changes may also be due to the presence of medical equipment. Both Hermansen
and Hermansen (1999) and Engstrom and associates (1995) found that the presence of
common neonatal medical equipment such as endotracheal tubes, umbilical catheters,
blood pressure cuffs, pulse oximeter probes, and intravenous tubing could add more than
50 grams to the true weight of an infant. For the extremely premature infant of 500
grams, the presence of these devices could potentially account for 10% of the obtained
weight, leading to misinterpretation of the infant’s nutritional status (Hermansen &
Hermansen, 1999). Although in most instances it is not feasible to remove all equipment,
non-critical devices such as blood pressure cuffs should be removed prior to weighing.
Other devices should be weighed separately and subtracted from the total weight as needed (Engstrom et al., 1995; Hermansen & Hermansen, 1999).

Variations in weighing techniques among nurses and health care providers may also lead to inaccurate measurement of an infant’s weight. These techniques may differ as to the time of day the infant is weighed, the number of weighing attempts and the averaging or non-averaging of these readings, and the removal of equipment. For adults, daily weights are best achieved by weighing at the same time every day, generally in the morning after awakening and before eating. However, given the altered sleep-wake cycles and feeding patterns of preterm infants, there is no specific time in which a weight should be obtained. Rather, nurses should weigh the infant at the same time each day prior to any bolus feedings to obtain the most accurate weight possible. Infants should also be weighed on the same scale, because the calibration of different scales may be different, resulting in different measurements (Kavanaugh, Engstrom, Meier, & Lysakowski, 1990; Kavanaugh, Meier, & Engstrom, 1989). In the clinical setting, it is also common practice to weigh an infant multiple times on an electronic or balance scale due to infant movement or crying, which may influence the scale’s readings. When multiple weights are taken, nurses may differ in their approach to weight documentation. Whereas some may take the average of three attempts, others may simply record the weight that best corresponds to that obtained on the previous day. Although these discrepancies between nurses are best resolved by “having the same person weigh the infant on the same scale each day” (Kavanaugh, Meier, & Engstrom, 1989), this practice is not possible in clinical practice. Therefore, to ensure accuracy, protocols for weighing
neonates should be developed and evaluated as a core competency in the nursing staff. Such a protocol would involve the weighing of all infants twice at a certain time each day (e.g., midnight), weighing before bolus feedings are initiated, removing all equipment possible, and subtracting the weights of other non-removable equipment from the infant’s total weight.

Of final consideration when obtaining weights for premature infants is the type of scale used. Kavanaugh and colleagues (1990) found that individual variation and variation between nurses’ recorded weights was greatest when balance scales with a sliding indicator were used. As a result, electronic scales were recommended due to their ability to integrate infant movement through the provision of a single digital readout, which is a “calculated mean of ten automatic measurements taken in rapid succession” (Kavanaugh, Engstrom, Meier, & Lysakowski, 1990). Although in-bed electronic scales are frequently used in clinical practice, their measures may be less reliable than external electronic scales, in part due to the presence of critical medical equipment that cannot be removed (Engstrom et al., 1995). However, for critically ill infants, transfer to an external electronic scale is not possible and in-bed readings must suffice. To ensure accuracy, the weight of in-bed equipment and blankets should be taken into consideration. Equivalent equipment and blankets not being used for patient care should be weighed prior to the infant and subtracted from the total weight obtained. Furthermore, care should be taken as to the handling of equipment above the infant’s chest, which may alter the obtained weight by as much as 100 grams, depending on the height and position of the equipment held (Engstrom et al., 1995).
Length Assessment

Length measurements are essential during the neonatal period because they provide a measure of linear growth, which ultimately reflects nutritional status. In the clinical setting, crown-heel lengths are the most common measure used to assess linear growth. However, when measured alone, crown-heel length may be falsely increased. This is attributable to both skull molding following the birth process positional head deformities associated with postnatal positioning. Consequently, length measures are most appropriately evaluated on a growth chart in conjunction with measures of weight gain and head circumference during the early newborn period, because length measures alone may be a very inaccurate indicator of growth.

Within the NICU, length measures are most commonly obtained using paper measuring tapes. However, the accuracy of these paper tape measures is often poor. For instance, Rosenberg and colleagues (1992) found length discrepancies as great as 2.4 cm between nurses and variations as great as 2.3 cm within an individual nurse’s repeated length assessments. In a similar study, the mean absolute difference of repeated length measurements was as high as 1.18 cm for a single observer and 1.57 cm for different observers (Johnson, Engstrom, & Gelhar, 1997). These differences may be attributable to the easy slippage of paper tape and the potential for stretching with repeated use. Although these differences appear small, they may account for a significant proportion of a premature infant’s obtained length. When these incorrect values are then plotted on a standardized infant growth chart, true growth may remain undetected, or likewise, growth deficiency may be masked.
Although infant lengths are most accurately determined using infant length boards with sliding footboards, these devices are difficult to use in the clinical setting. For extremely premature infants, removing the infant from the incubator and placing him onto a length board is not always feasible, due to the presence of medical equipment and the potential for infant instability due to excessive handling and cold exposure. Consequently, alternative measures of length, such as knee-heel measures, have been explored in newborn infants (Griffin, Pang, Perring, & Cooke, 1999; Skinner et al., 1997). However, such methods have failed to produce consistently reliable results among observers and do not correlate well with the crown-heel measurements of premature infants (Griffin, Pang, Perring, & Cooke, 1999). Furthermore, these measurements rely on expensive, specialized equipment that is not cost-efficient for many NICUs. Therefore, these alternative measures of length are infrequently employed in clinical practice. Given the lack of reasonable alternatives for measuring length in critically ill newborns and premature infants, paper tape measurement of crown-heel length remains the method of choice.

*Head Circumference Assessment*

Accurate head circumference measures are important for neonates and infants in that increased head circumferences are associated with intracranial volume and brain growth. For preterm infants, head circumference measures are especially important due to the strong association that exists between premature birth and neurodevelopmental
disorders. However, similar to length measures, head circumferences in the neonatal period are frequently inaccurate due to skull molding following birth and positional deformities such as plagiocephaly. Consequently, an infant’s head circumference may appear to increase or decrease when no change has occurred.

In the clinical setting, paper tapes are most commonly used to obtain head circumference measurements in newborn infants. In a study comparing cloth and paper tape measures, the measurements obtained by using the paper tape were significantly more reliable than the cloth tape measures, in part due to decreased slippage with movement and inability to stretch with repeated usage (Sutter et al., 1997). Furthermore, paper tapes are more practical for everyday usage in that they are disposable and more cost efficient for the NICU. Yet despite the advantages associated with using paper tapes to obtain head circumference measurements, these measurements are often inaccurate (Ifft et al., 1989; Bhushan & Paneth, 1991). Although a few studies measuring head circumference in full term infants found strong agreement between researchers (German, Mason, & Rosman, 1976; Johnson, Engstrom, & Gelhar, 1997), a study of preterm infants revealed differences as great as 1 cm in the measures obtained by both the same and different nurses, with only 50% of the differences less than or equal to 0.25 cm (Ifft et al., 1989). In a similar study of preterm infants, 5% of the head circumference measurements obtained differed by 2 cm or greater (Bhushan & Paneth, 1991). Such a discrepancy may easily be dismissed as too small for concern, but an obtained head circumference of 1 cm greater than actuality may exceed the expected weekly head growth rates for premature infants, which should fall between the associated in utero rates.
of 0.5 to 1 cm per week (Ifft et al., 1989). As a result, misinterpretation of the infant’s nutritional and medical state is likely to occur, with potential ramifications for future growth and development.

**Growth Chart Usage**

Anthropometric measures such as weight, length, and head circumference are frequently used as outcome variables in neonatal nutrition research studies. When these measures are obtained accurately, their documentation on charts of intrauterine growth norms provide crude assessments of the overall nutritional status of the infant. Although several growth curve references are available for use in the clinical setting (i.e., Battaglia & Lubchenco, 1967; Lubchenco, Hansman, & Boyd, 1966), questions have been raised as to their applicability in current practice (Rigo, De Dcurtis, & Pieltain, 2001; Thomas, Peabody, Turnier, & Clark, 2000). The most commonly used growth charts were developed in the 1960’s with non-diversified infant samples and do not reflect current perinatal medical practices. As a result, the growth charts developed more recently reflect lower average weight, length, and head circumference measurements for extremely preterm infants born at less than 30 weeks gestational age and increased average measurements for infants of greater than 36 weeks gestational age (Ehrenkranz et al., 1999; Thomas, Peabody, Turnier, & Clark, 2000).

Because there are currently no universally accepted growth charts for premature infants, more research is warranted regarding the growth patterns of this population during the neonatal period before a true gold standard for extra-uterine neonatal growth
can be developed. However, regardless of the neonatal growth curve selected for longitudinal nutritional assessment, inaccuracies are likely if gestational age of the infant is unclear. If the mother’s menstrual history is uncertain and other obstetrical estimates of gestational age are unknown, gestational age must be inferred by measures of physical maturity, such as the New Ballard Score (Ballard et al., 1991). In a large, multi-center study of preterm infants weighing 401 to 1500 grams at birth, the New Ballard Score estimates exceeded true gestational age by 1.3 to 3.3 weeks in infants less than 28 weeks’ gestation (Donovan et al., 1999). Although these discrepancies may be reduced by having a single, experienced individual trained in gestational age estimation perform all assessments, often this is not possible in the clinical setting. Therefore, the growth curves of infants of unknown gestational ages prior to delivery should be interpreted with caution. For these infants, serial anthropometric growth assessments may provide valuable information regarding long-term nutritional status, but the initial measures may not reflect true growth percentiles.

The Collection of Biological Specimens

The collection of biological specimens, including blood, urine, and fecal samples, is a common practice in many neonatal nutrition studies because this practice provides quantitative determinations of specific nutrient concentrations, nutrient absorption, or metabolic status. Unlike adults, premature infants are unable to cooperate with instructions and verbalize pain. Therefore, care must be taken when collecting biological
samples to minimize invasiveness and pain. Often, patience is also required because biological samples are rarely collected flawlessly from premature infants.

**Blood Collection**

Blood collection from newborn infants is a common procedure in the NICU. However, unlike adults, premature newborn infants often receive blood transfusions for the treatment of severe anemia during the immediate postnatal period. When blood draws are necessary for nutrition studies, they should ideally be done before any transfusions are initiated. During the transfusion, a small sample of the transfused blood could be collected and evaluated against the infant’s baseline blood draw data. Laboratory blood values from the infant following the transfusion can then be compared to the baseline and transfusion blood laboratory values obtained.

Newborn infants have decreased total circulating blood volumes that make safe blood collection challenging. In an early study of very low birthweight infants during the first 28 days of life, mean blood loss due to diagnostic sampling ranged from 24 to 67 mL per kilogram, with the most critical infants having the greatest blood loss (Obladen, Sachsenweger, & Stahnke, 1988). Likewise, in more recent studies, researchers have found that the mean volume of blood drawn for laboratory testing exceeded required volumes by approximately 19% (Lin et al., 2000), and strong correlations between the amount of overdrawn blood and number of blood transfusions have been reported for extremely premature infants (Madsen et al., 2000). Consequently, neonatal nutrition research involving blood sampling ethically necessitates the withdrawal of small blood
samples to prevent volume depletion and anemia. Often these samples are limited to 0.5
to 1 mL or less, and if clotting or hemolysis occurs, it may not be possible to repeat
sample collection out of concern for the infant’s safety and well-being. Blood collection
may be further limited if the procedure is tolerated poorly by the infant, evidenced by
oxygen desaturation, temperature instability, bradycardia, or apnea. Collectively, these
factors, which are unique to newborn infants, mandate that proper blood collection
techniques be used to achieve meaningful results. Although specific blood collection
protocols may differ among institutions, for research purposes, a single individual should
ideally collect all the blood samples from similar sites in all subjects using the same
technique. If nursing staff or separate researchers must collect blood specimens, these
individuals should be trained with a specific protocol and evaluated on frequent intervals
to ensure reliability. Likewise, laboratory technicians trained in the analysis of very
small blood volumes should also be employed to minimize the amount of blood
necessary for laboratory testing.

When laboratory blood draws are necessary for neonatal nutrition research, great
care should be taken to minimize the stress of the blood collection procedure. Although
some studies have demonstrated less infant pain with venipunctures as opposed to
heelsticks (Larsson, Tannfeldt, Lagercrantz, & Olsson, 1998; Shah & Ohlsson, 2001), for
the clinically unstable infant, venipunctures should be avoided because they may
traumatize veins that may be needed for intravenous delivery of fluids or medications.
However, if indwelling intravenous or arterial catheters are present, they should be used
for blood draws in lieu of heelsticks whenever possible to minimize infant pain.
Frequently, it is not possible to obtain all blood samples from the same site in every infant. Often there is no way to control for this event because subjecting all infants to heelsticks or venipunctures when indwelling lines may be present would be unethical. This poses some concerns for neonatal researchers. For instance, laboratory values obtained from capillary samples often differ from those obtained from arterial lines (Andrew & Mueller, 1992; McLain, Evans, & Dear, 1988; Meites, 1988; Johnson et al., 2000). This is due in part to the squeezing of the extremity that is associated with capillary draws, which may result in false elevations of laboratory values such as potassium, lactate, and ionized calcium (Meites, 1988; Johnson et al., 2000). Likewise, collecting blood from arterial or intravenous lines may result in false elevations of glucose, electrolytes, or macro- or micro-nutrients if those lines are also used for parenteral nutrition and fluid delivery (Cowett & D'Amico, 1992; Johnson et al., 2000).

When indwelling lines are accessed for blood draws, they should be first flushed with appropriate amounts of saline. Secondly, a small “waste” sample of blood should be drawn into a syringe, and then the blood sample should be obtained. The collection of a “waste” sample prior to the blood draw is necessary to eliminate the presence of intravenous fluids or nutrition in the obtained sample, which could alter the laboratory results. After the collection of the blood sample, the “waste” blood is replaced to minimize blood volume depletion associated with laboratory blood draws.

For newborn infants, heelsticks are often needed to obtain laboratory values due to the limited duration of indwelling arterial and intravenous catheters. However, few heelstick protocols are based on clinically relevant research. The use of automated
incision devices is generally considered to be the most reliable and efficient method of capillary blood collection in newborn infants, in that they reduce the incidence of hemolysis and shorten the time required to collect the blood sample (Meehan, 1998; Paes et al., 1993). However, care must be taken to ensure that the depth of the automated incision device does not exceed 2.4 millimeters, because at greater depths, calcaneum puncture and subsequent infection may occur (Jain & Rutter, 1999). Furthermore, although many nurses and phlebotomists prefer that heel warmers be used prior to heelsticks, there is little support for this practice in the research literature. When blood was collected from newborn infants with and without heel warmers, no differences in the rate of blood flow, the degree of heel squeezing, blood collection time, or the number of repeated blood draws was found following warming of the extremity (Barker, Willetts, Cappendijk, & Rutter, 1996; Janes et al., 2002; Johnson et al., 2000). These findings warrant reevaluation of current heelstick methods employed in the NICU. When heelsticks are needed for neonatal nutrition research, heelstick protocols reflective of these findings should be piloted and employed in research studies to ease sample collection, minimize researcher cost, and decrease infant pain.

Urine and Stool Collection

The collection of urine and stool is often indicated for many neonatal nutrition research studies. When these specimens are collected, special considerations must be taken into account to prevent harm to the infant. Urine sampling involving suprapubic needle insertion or urethral catheterization is only indicated in emergent situations in
which a sterile urine sample is required. Research involving neonates should avoid the use of these methods due to the likelihood of tissue traumatization and potential for infection associated with these techniques. When a non-sterile urine sample is sufficient, urine is often collected through the application of an external bag that is affixed to the infant’s perineum with an adhesive. However, unlike adults, premature infants have a very weak bond between the dermal and epidermal layers of the skin (McManus-Kuller, 1984), and the application of adhesive bags may result in significant skin excoriation. Furthermore, leakage from adhesive bags may allow for direct contact between urine and the skin, which may lead to irritation. When a urine specimen is needed, it may be safely collected through the aspiration of urine from the infant’s diaper using a syringe. This process is facilitated through the use of diapers without absorbent gel fillings. An alternative approach when absorbent gel diapers are used would involve the application of a sterile cotton ball to the infant’s diaper. After the infant voids normally, the urine can be aspirated from the cotton ball with a syringe. Both methods for the collection of urine from a diaper are easily initiated and do not alter laboratory values such as specific gravity, blood, ketone, protein, and glucose levels and poses no risk to the infant (Reams & Deane, 1988; Strohbach & Katrina, 1982).

Depending on the nature of the research, the collection of stool may be simple or quite challenging. Often it is sufficient to remove a stool sample from the infant’s diaper. However, in the case of metabolic balance studies, it may be necessary to collect all stool from the infant over a designated time period. Although elaborate beds for the collection of stool in these instances have been developed (Cooke et al., 1988), they are not
frequently used in the clinical setting. Rather, stool is most commonly collected through
the application of an external bag to the perianal area with adhesive. Similar to external
urine bags, this practice may also result in skin excoriation. This trauma may be reduced
by applying the bag to an ostomy adhesive wafer to prevent skin breakdown (Ehrenkranz,
Gettner, & Nelli, 1989).

Ethical and Other Methodological Considerations

The recruitment of newborn infants for research studies involves many ethical,
scientific, and practical dilemmas that are not apparent when recruiting adult subjects.
Ethically, when research utilizing infant samples is necessary, it should address issues
that are of relevance to the clinicians, the research subjects, and society as a whole (Rigo,
Morley, & Koletzko, 1999). Given that newborn infants are a vulnerable population,
studies should not seek answers that are already known, but should instead provide novel
information, to eliminate excessive subject burden. Furthermore, it is generally accepted
that for research involving children, the benefit to risk ratio should be higher in children
than in adult subjects (National Institutes of Health: Office of Extramural Research,
1998; Underwood, 1999). This notion asserts that researcher manipulations should be
minimally invasive and the least psychologically and physically intrusive (Underwood,
1999). Consequently, a research study is deemed to be of minimal risk if “the physical or
psychological risks are no greater than those the infant would encounter in daily life or
during routine tests” (Holditch-Davis & Conway, 1992).
Although increasing the benefit to risk ratio is likely to increase study enrollment and consent by caregivers, it is not possible to conduct all research studies of preterm neonates with noninvasive measures that do not cause pain or disrupt the normal physiological functioning of the infant (e.g., an external temperature probe or external respiratory monitor). Rather, invasive measures such as blood draws that have the potential to cause pain and harm the subject are frequently needed to assess the adequacy of research interventions. Furthermore, because certain illnesses are limited primarily to premature newborn infants, the use of noninvasive measures for all studies would result in very limited findings, given that no other populations are available for assessment. Although these studies of greater than minimal risk may be necessary for the advancement of knowledge and future ameliorization of disease in preterm infants, they are subject to increasingly stringent guidelines and are often rejected by institutional review boards (Koren, 1990). However, because no clear definition of “greater than minimal risk” has been established, the assessment of risk in research protocols is highly variable among scientists and review boards (Janofsky & Starfield, 1981). As a result, many study protocols involving preterm infants may be rejected due to perceived risks to subject enrollment.

*Informed Consent and Psychological Stress*

Informed consent presents another challenge for neonatal nutrition researchers. Whereas adults may choose whether or not to participate in a research study based on personal beliefs, informed consent for infant participants must be obtained from a
competent adult legal guardian (Holditch-Davis & Conway, 1992; National Institutes of Health: Office of Extramural Research, 1998). Obtaining consent from the parents of a newborn in the NICU may be challenging for researchers (Hoppu, 1999; van Stuijvenberg et al., 1998). Because many caregivers with preterm infants do not expect to deliver prematurely, they must not only cope with the birth of a new child, but must also adjust to new roles as parents of a fragile infant (Casteel, 1990; Hughes et al., 1994; Pinelli, 2000). Furthermore, parents of preterm infants must also undergo the complete grieving process, which includes grieving for the healthy, “normal” child that was expected (Affleck & Tennen, 1991), overcoming feelings of guilt (Whetsell & Larabee, 1988), addressing feelings of helplessness and uncertainty related to the child’s prognosis (Casteel, 1990), and adjusting to NICU policies and procedures (Bass, 1991; Kenner & Lott, 1990).

As a result of these normal parental psychological adjustments to having a preterm infant in the NICU, informed consent may be difficult to obtain. Parents who are frustrated with the amount of procedures already done on their infant, as well as those parents who are poorly attached, may not be willing to consent for research participation, especially if the imbalance between potential participation and inconvenience is perceived as large. Furthermore, differences in culture and socioeconomic status may further inhibit consent. If the study’s description, purpose and utility are poorly understood, or if participation is perceived as a time constraint or hindrance, caregiver consent is unlikely. Likewise, in cultures where hospital-based medical care is not
revered, informed consent for NICU research may be impossible, due to incongruent philosophical notions of holism and care delivery.

Sampling Issues

Any research study involving newborn infants must also consider sampling theory. Although the assumption of random sampling is inherent in most inferential statistical methods, random sampling of premature infants is often not possible. Rather, samples of preterm infants are typically selected from one NICU only and are limited to those infants fitting specified inclusion criteria for whom informed consent was obtained (Thomas & Conway, 1992). This often results in a very small sample of infants that is further limited by mortality or a loss of subjects to outlying facilities, which is common in the high-risk population of extremely premature infants. Because small sample sizes are common in neonatal nutrition research studies, their findings must be interpreted with caution. With small sample sizes, generalization to the larger population of preterm infants is not appropriate (Beal & Betz, 1993). Therefore, large, multi-center clinical trials are necessary to obtain adequate numbers of subjects.

Another sampling problem involves the selection of a comparison group. Newborn infants are an extremely heterogeneous group, with varying prenatal histories, postconceptional ages, genders, and ethnicities. As a result, often grouping of infants for research purposes are not mutually exclusive or exhaustive (Dyke & Conway, 1992). Furthermore, for comparison studies examining differences between two preterm infant groups, such as those with and without a specified clinical diagnosis, randomization to an
experimental and control group is not possible, in that diagnosed illness is a naturally occurring event (Aylward, 2002). Because preterm controls are typically healthy infants without medical problems, enrollment of these infants may be difficult, due to few perceived benefits of participation (Hoppu, 1999; van Stuijvenberg et al., 1998). Therefore, significant discrepancies in sample numbers, environmental situations, or infant characteristics between groups may be apparent, resulting in a selection threat which may be impossible to control (Thomas & Conway, 1992). Furthermore, if marked physical differences between control and experimental groups are present, blinding is not possible and may bias results (Lester & Miller-Loncar, 2000). Although no neonatal research study is successful in controlling all extraneous variables, proper research design that addresses these issues may enhance the validity and clinical usefulness of the study. However, all research findings related to neonatal nutrition should be evaluated and interpreted within the individual context of each study, given the wide variability in practices that exist among NICUs.

Environmental Issues

For many neonatal nutrition studies, the NICU environment itself may pose difficulties for researchers. For multi-center studies, differences in NICU policies and procedures may confound results. Even when the medical and nursing staff are provided with new protocols for studies examining various nutrient delivery or administration methods, it may not be accurately followed if it differs greatly from the NICU’s norm. Furthermore, given the extremely critical nature of many infants within the neonatal care
unit, unanticipated nursing or medical interventions may be warranted during the study period, which have the potential to influence research results. These interventions may include: 1) medications or parenteral fluids that directly or indirectly interfere with normal nutrient absorption and utilization, 2) the withholding of feedings or total parenteral nutrition when an infant becomes medically unstable, or 3) inadvertent overstimulation of the infant, leading to poor integration of sucking, swallowing, and breathing, which are necessary for enteral feedings. Because it is unethical to deny medical intervention to a participating research study infant, researchers must address such aberrations to the research protocol when interpreting results and consider the delivery of such interventions as a threat to the external validity of the study.

Conclusions

Neonatal research, and neonatal nutrition research in particular, is important for the advancement of neonatal care and improvement of developmental outcomes. The physiological immaturity and medical instability characteristic of premature infants in the NICU render research design and implementation challenging. It is hoped that the considerations addressed in this review assist neonatal researchers in the attainment of reliable and valid research results.
APPENDIX A

THE OHIO STATE UNIVERSITY CONSENT TO INVESTIGATIONAL TREATMENT OR PROCEDURE

THE OHIO STATE UNIVERSITY                         Protocol No.  2003H0140

CONSENT TO INVESTIGATIONAL TREATMENT OR PROCEDURE

I, _________________________, hereby authorize or direct Anne M. Mentro or
associates or assistants of his/her choosing, to perform the following treatment or
procedure (describe in general terms),

My infant has been asked to participate in this study because he or she was born
prematurely and has experienced difficulty breathing during the first 48 hours of life. My
infant is one of 50 infants who will be participating in this study. The purpose of this study
is to better understand dietary and infection factors that may play a role in the development
of lung disease in preterm infants. Previous research shows that infants with lung disease
may have decreased dietary intake and increased infection during the first days of life. At
day 2 and day 7 of life, nutritional intake data, my infant's medical history, and information
about the pregnancy and birth will be collected from my infant's medical chart. My infant
will also donate 4-5 drops of blood (0.7 mL) and 20 drops of urine (3 mL) at day one and
day 7 of life for this study. My infant will not receive a needlestick by the researcher. The
blood will be collected from my infant's intravenous line ("I.V.") or arterial line if present,
which is a small catheter that is placed into the blood vessel for the administration of
medications and fluids. If my infant does not have an intravenous or arterial line, then the
blood will be collected at the same time as a required hospital laboratory blood draw. In
this event, a heelstick will be performed by a registered nurse for the blood draw and the
blood will be collected at this time only. My infant will not receive additional needlesticks
for participating in the study.

upon _________________________.

(myself or name of subject)
The experimental (research) portion of the treatment or procedure is:

If I agree to participate, researchers will perform the following actions when my infant is 2 days old and again when he or she is 7 days old:

1) Investigators will look at my infant's medical record to retrieve information regarding the amount of formula and/or intravenous feedings my infant is consuming and their nutrient composition. If my infant is given breast milk, a 5 mL sample (about 35 drops) of that breast milk will be collected from the nursery and analyzed for nutrient composition. The nutritional information obtained from the medical chart will help determine the total amount of calories, vitamin A, and selenium that my infant has consumed.

2) Investigators will also obtain information about my infant's birth and medical care from his or her medical chart. The data collected from the medical chart will include the history of my infant's labor and delivery, my infant's gestational age, ethnicity, birth weight, medications received, APGAR scores, duration of mechanical ventilation, liters of oxygen received per day, and daily weights.

3) Approximately 4-5 drops of blood (0.7mL) will be collected from my infant. I understand that my infant will not receive an additional needlestick for participation in this study. Blood will be obtained from an intravenous or arterial line if present. If an intravenous or arterial line is not present, then my infant will receive a heelstick by a registered nurse for routine medical laboratory draws and the blood will be collected at this time only. The blood that is collected from my infant will be analyzed for the amount of vitamin A, selenium, and glutathione peroxidase that is present.

4) Approximately 20 drops of urine (3 mL) will be collected from my infant. This urine will be obtained by placing a small cotton ball in my infant's diaper, which will be squeezed to remove the urine after my infant has wet his or her diaper normally. The urine that is collected from my infant will be analyzed for a specific marker of inflammation called interleukin-1 receptor antagonist (IL-1ra).

This is done as part of an investigation entitled:

Antioxidant, Inflammatory, and Nutritional Correlates in Preterm Infants at Risk for Bronchopulmonary Dysplasia

1. Purpose of the procedure or treatment:

The purpose of this study is to better understand dietary and infection factors that may play a role in the development of lung disease in preterm infants. Previous research shows that preterm infants with lung disease (also called bronchopulmonary dysplasia) may have decreased antioxidants in their blood, increased inflammation or infection in their bodies, and decreased dietary intake. No research has been done to determine the relationships
between the antioxidants, inflammation, and nutrition in these infants. It is hoped that the information obtained from this study will also provide a more complete understanding of lung disease in premature infants.

2. Possible appropriate alternative procedure or treatment (not to participate in the study is always an option):

I may refuse to allow my infant to participate in this study. This will not affect my infant's medical treatment or my access to health care resources in any way. I can find information regarding risk factors for bronchopulmonary dysplasia in preterm infants from other sources, including my health care providers, nurses, and printed publications.

3. Discomforts and risks reasonably to be expected:

Because the blood collected for this study is obtained at the same time that blood is collected as a routine part of my infant's medical care, the risks to my infant for participating in this study are minimal. However, if the blood collected from my infant is obtained from a heelstick by a registered nurse in conjunction with a hospital blood draw, then my infant may possibly experience slight discomfort, pain, or bruising related to the squeezing of his or her foot. There is also a slight risk of infection following any heelstick or intravenous or arterial line draw. My infant may also experience oxygen desaturation, respiratory distress, and temperature changes during the blood collection. If this occurs then the sample collection will immediately stop and medical care will be given to my infant. All information collected in this study will be kept confidential.

4. Possible benefits for subjects/society:

There are no direct benefits to my infant. The results obtained from this study will add to our understanding of factors associated with lung disease in preterm infants. It is planned that the information obtained from this study will be useful in the development of future interventions to decrease the severity, duration, and incidence of lung disease in premature infants. If desired, I will be provided with a detailed analysis of my infant's nutrient intake in the hospital nursery.

5. Anticipated duration of subject's participation (including number of visits):

Participation in the study will last for 7 days. The blood sample, urine sample, and nutrition data will be collected on 2 occasions in the hospital nursery: when my infant is 2 days old and again when he or she is 7 days old. I will be given a disposable camera with 12 pictures and a gift certificate for free film developing for my infant's participation in this study, even if my infant does not complete the entire study.
I hereby acknowledge that Anne M. Mentro has provided information about the procedure described above, about my rights as a subject, and he/she answered all questions to my satisfaction. I understand that I may contact him/her at Phone No. 614-481-3059 should I have additional questions. He/She has explained the risks described above and I understand them; he/she has also offered to explain all possible risks or complications.

I understand that, where appropriate, the U.S. Food and Drug Administration may inspect records pertaining to this study. I understand further that records obtained during my participation in this study that may contain my name or other personal identifiers may be made available to the sponsor of this study. Beyond this, I understand that my participation will remain confidential.

I understand that I am free to withdraw my consent and participation in this project at any time after notifying the project director without prejudicing future care. No guarantee has been given to me concerning this treatment or procedure.

I understand in signing this form that, beyond giving consent, I am not waiving any legal rights that I might otherwise have, and I am not releasing the investigator, the sponsor, the institution, or its agents from any legal liability for damages that they might otherwise have.

In the event of injury resulting from participation in this study, I also understand that immediate medical treatment is available at University Hospitals of The Ohio State University and that the costs of such treatment will be at my expense; financial compensation beyond that required by law is not available. Questions about this should be directed to the Office of Responsible Research Practices at (614) 688-4792.

I have read and fully understand the consent form. I sign it freely and voluntarily. A copy has been given to me.

Date: __________ Time _______ Signed __________________________

AM

PM

Witness (es) ____________________________ (Person Authorized to Consent for Subject if Required)

if required
I certify that I have personally completed all blanks in this form and explained them to the subject or his/her representative before requesting the subject or his/her representative to sign it.

Date: ______________________  Signed

(Signature of Project Director or his/her Authorized Representative)

HS-028A (Rev. 7/93)
TITLE OF THE STUDY: Antioxidant, Inflammatory, and Nutritional Correlates in Preterm Infants at Risk for Bronchopulmonary Dysplasia

VOLUNTEER NAME:

RESEARCH STUDY DIRECTOR: Deborah K. Steward, PhD, RN
Anne M. Mentro, MS, RN, CPNP

RESEARCH STUDY SPONSOR: The Ohio State University

1) INTRODUCTION

NOTE: The words “you” and “your” are used in this consent form. These words refer to the study volunteer whether a child or an adult.

We invite you to be in this research study. Before you decide to be in this study, you need to understand the following:

1. We must explain the study to you;
2. You must have a chance to ask questions. Ask about anything you do not understand before you agree to be in this study;
3. You must sign this consent form if you want to be in this study. This means you are agreeing to take part in this study. If you are under 18, your parent(s) or legal
4. guardian must sign this consent form. If you are between 9 and 18, you must agree to be in the study by giving assent;
5. You will be given a copy of this consent form;
6. Being in this study is up to you;
7. You might not benefit from being in this study, but we may learn something that could help others; and
8. You are free to quit this study at any time. If you decide to quit, it will not in any way affect your regular medical care.

2) WHY ARE WE DOING THIS RESEARCH STUDY?

This is a study to find out if the diet of preterm infants is related to inflammation. By performing this study, we hope to increase our understanding of factors that may play a role in the development of lung disease in preterm infants. Your infant has been asked to participate in this study because he or she was born prematurely and has experienced difficulty breathing during the first days of life. Your infant is one of 50 infants who will be participating in this study.

3) WHERE WILL THE STUDY BE DONE AND HOW MANY VOLUNTEERS WILL BE IN THE STUDY?

This study is being done in the Neonatal Intensive Care Unit at Riverside Hospital in Columbus, Ohio. About 50 infants will take part in this study across the United States. We hope to have at least 25 volunteers here at Riverside Hospital.

4) WHAT WILL HAPPEN DURING THE STUDY AND HOW LONG WILL IT LAST?

If you agree to participate, the researcher will perform the following actions when your infant is 2 days old and again when he or she is 7 days old:

1) The researcher will look at your infant’s medical chart to obtain the amount and type of diet that my infant is receiving. If you are breastfeeding your infant, a 5 mL sample of your breast milk will be collected from the nursery stores and analyzed for the amount of selenium and vitamin A that it contains.

2) About 4-5 drops of blood (0.7mL) will be collected from your infant by an experienced registered nurse during a required hospital blood draw. No additional needlesticks will be received by your infant for participating in this study. The blood that is collected will be analyzed for the amount of vitamin A and selenium that is present.
3) About 20 drops of urine (3 mL) will be collected from your infant. This urine will be obtained by placing a small cotton ball in your infant’s diaper, which will be squeezed to remove the urine after your infant has wet his or her diaper normally. This urine will be used to determine the amount of inflammation that is present in the body.

5) WHAT BAD THINGS CAN HAPPEN TO ME IF I AM IN THIS STUDY?

Most likely, no bad things will happen to your infant for participating in this study. However, if the blood is collected by a heelstick, then your infant may possibly experience slight discomfort, pain, or bruising related to the squeezing of his or her foot. Because the blood will be collected only during a required hospital blood draw, pain will be minimized because no additional needlesticks will be received. Although there is also a slight risk of infection following any blood draw, good handwashing will help minimize this risk.

6) WHAT GOOD THINGS CAN HAPPEN TO ME IF I AM IN THIS STUDY?

Your infant will not benefit from being in this study, but we might learn something that could help others.

7) WHAT HAPPENS IF I AM HURT WHILE IN THIS STUDY?

If your infant is hurt because of this study, Children's Hospital will provide medical care to you. You may have to pay for this care. If you have questions or are worried about your legal rights, call Children’s Hospital Legal Services at (614) 722-3940. This does not mean that you give up any of your rights under state or federal laws to ask for this care to be paid by someone else. If you have questions or are worried about your rights as a research volunteer, please call (614) 722-2874, Children's Hospital, Office of Research Services.

8) SPECIAL INFORMATION FOR FEMALES:

Not applicable to this study.

9) WHAT WILL HAPPEN IF NEW INFORMATION IS FOUND OUT ABOUT THE TREATMENT?

If we find out any new information during this study that might affect your infant’s health or that might change your mind about your infant’s participation in this study, the researcher will call you.
10) OTHER IMPORTANT INFORMATION:

The Study Director is not being paid for the time and knowledge needed to do this study.

Being in more than one research study at the same time may hurt your infant. You should tell the Study Director if your infant is already in another research study. The Study Director will decide if it is OK to be in this one at the same time.

11) IF I DECIDE NOT TO BE IN THIS STUDY, WHAT OTHER TREATMENTS CAN BE GIVEN?

This study does not involve a treatment. Your infant’s care will not be affected by participating in this study.

12) WHAT WILL HAPPEN IF I DECIDE TO DROP OUT OF THE STUDY?

Your infant’s participation in this study is your choice. You can say no to being in the study or stop at any time. If you decide to stop your infant from taking part in this study, call the Study Director at (614) 481-3059.

Any of the people in charge of this study may stop your infant from taking part at any time if they decide that it is in your infant’s best interest. If your infant has other medical problems, the Study Director or the sponsor will decide if your infant may be in this research study.

13) WILL THERE BE ANY ADDITIONAL COSTS TO ME?

There are no additional costs to you for participating in the study. For allowing your infant to participate in this study, you will receive a small disposable camera and a gift certificate for film developing.

14) HOW WILL MY STUDY INFORMATION BE KEPT PRIVATE?

Information collected for this study will be kept confidential to the extent allowed by law. Information used and/or disclosed (shared with someone outside of Children’s Hospital) may include information that can identify you or your infant. This is called “protected health information” or PHI. By agreeing to be in this study, you are giving permission or authorizing the Study Director and study staff to collect, use, and disclose your PHI for this research study. Information collected is the property of the Ohio State University and
Children’s Hospital. In the event of any publication regarding this study, your identity will not be revealed.

- **People or Companies authorized to use, disclose, and receive PHI collected or created by this research study:**
  - The Ohio State University Institutional Review Board
  - Children’s Hospital Institutional review Board
  - Dr. Deborah K. Steward and Anne M. Mentro

Because of the need to give information to these people, absolute confidentiality cannot be guaranteed. Information given to these people may no longer be protected by federal privacy rules.

- **PHI that may be used or disclosed:** Your infant’s age (date of birth).

- **Reason(s) why the use or disclosure is being made:** This disclosure is necessary for the analysis of the study results. It is also necessary to conduct the research and to ensure that the research meets legal, institutional, safety, or accreditation requirements.

- If your infant has a bad outcome or adverse event from being in this study, the Study Director and staff or other health care providers may need to look at your infant’s entire medical records.

- The PHI collected or created under this research study will be used/disclosed as needed until the end of the study. The records of this study will be kept for an indefinite period of time.

- You may decide not to authorize the use and disclosure of your infant’s PHI; however, your infant may not be able to be in this study. If you agree for your infant to be in this study and later decide to withdraw, you may also withdraw your authorization to use your infant’s PHI. This request must be made in writing to the Study Director. If you withdraw your authorization, no new PHI may be collected and the PHI already collected may not be used unless it has already been used or is needed to complete the study analysis and reports.

For this study, the researcher will keep a database of all subjects who participate in a research study. This database is used to keep track of the research studies conducted and who participated in each study. This database is also used to contact people about future studies. Only Dr. Steward and her staff have access to this database.
PARTICIPANT'S STATEMENT

I have read this consent form and have been given a chance to ask questions about this research study. These questions have been answered to my satisfaction. If I have more questions about my participation in this study or a research-related injury, I may contact the Study Director Anne M. Mentro at 614-481-3059.

I consent to participate/I consent to have my child participate in this study. I will be given a copy of this consent form with all the signatures for my own records.

I have been given a copy of the Children's Hospital Notice of Privacy Practices. I understand that my right to my patient information that is created or collected by Children's Hospital in the course of this research can be temporarily suspended for as long as the research is in progress. I also understand that my right to access will be reinstated upon completion of this research.
CONSENT SIGNATURES

A. Adult Participant's Consent

I have read the explanation about this study and have been given the opportunity to discuss it and ask questions. I hereby consent to take part in this study.

Signature of Adult Participant & Date Signed

B. Parent’s Permission for Minor Participant

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.

Signature of Parent(s)/Guardian & Date Signed

(Guardians must provide proof of legal guardianship)

C. Child’s Assent (If age 9-17 or sign Assent Form)

The information about this study has been read and explained to me and I understand it. I agree to participate in this study.

Child’s Signature & Date Signed

D. Person Obtaining Consent

I certify that I have explained the research, its purposes, and the procedures to the patient or his/her legal representation before requesting their signature.

Signature of Person Obtaining Consent & Date Signed

Signature of Investigator & Date Signed

Signature of Witness (if applicable, see SOP IRB-010) & Date Signed

IRB-3
Consent Version Date 9/22/03

Consent will be obtained by Study Doctor, Study Nurse or Study Coordinator

Parent Initials
LIST OF REFERENCES


Temprosa, M., Wright, L.L., Ehrenkranz, R.A., Fanaroff, A.A., Stark, A., Carlo, W.,


Lin, J.C., Strauss, R.G., Kulhavy, J.C., Johnson, K.J., Zimmerman, M.B., Cress, G.A.,


Mandrup-Poulsen, T., Wogensen, L.D., Jensen, M., Svensson, P., Nilsson, P., Emdal, T.,


Piguet, P.F., Vesin, C., Grau, G.E., & Thompson, R.C. (1993). Interleukin-1 receptor antagonist (IL-1ra) prevents or cures pulmonary fibrosis elicited in mice by bleomycin or silica. *Cytokine, 5,* 57-61.


Wilson, D.C., Cairns, P.C., Halliday, H.L., Reid, M., McClure, G., & Dodge, J.A. (1997). Randomised controlled trial of an aggressive nutritional regimen in sick...
very low birthweight infants. *Archives of Disease in Childhood Fetal and Neonatal Edition, 77,* F4-F11.


