I, Barbara Sampson Davidson, hereby submit this as part of the requirements for the degree of:

Master of Science
in Nursing

It is entitled Fucosylated Oligosaccharides in Human
in Relation to Gestational Age and Day
of Lactation

Approved by
[Signatures]
Fucosylated Oligosaccharides in Human Milk in Relation to Gestational Age and Day of Lactation

A thesis submitted to the Division of Research and Advanced Studies of the University of Cincinnati in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN NURSING

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by

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Background
The oligosaccharides found in human milk have structural homology with cell surface glycoconjugates that are used as receptors by enteric pathogens to enter cells and cause infection. Current research findings suggest that breastfed infants may be protected against such pathogens due to the presence of these oligosaccharides, particularly those that are α-1, 2- fucosylated. In the following study, data from a cohort of breastfeeding mother-infant pairs in South Carolina were analyzed for a relationship between the concentrations of total and α-1,2-linked fucosylated oligosaccharides and the variables gestational age of the infant and day of lactation.

Methods
Study participants were 18 lactating mothers whose infants were admitted to a neonatal intensive care unit in South Carolina. The total fucosylated and α-1, 2- fucosylated oligosaccharide concentrations were determined in human milk samples obtained from the study participants. A single 2 mL milk sample was collected from each study participant at one time point between one and five weeks postpartum. The milk samples were analyzed by High Performance Liquid Chromatography (HPLC) with oligosaccharide concentration reported as nanomoles per milliliter (nmol/ml). These data were analyzed using descriptive and inferential statistics for potential relationships between the concentrations of total and percent α-1, 2- fucosylated oligosaccharides and the following variables: infant gestational age (GA), day of lactation (DOL), and ABO blood group type of the mother.

Findings
The mean (SE) total fucosylated oligosaccharides in maternal milk did not differ significantly by gestational age, but there was a trend towards increasing concentration with increasing gestational age. Two of the eighteen (11%) subjects had no α-1, 2- fucosylated oligosaccharide structures. The mean (SE) percentage of α-1, 2-fucosylated oligosaccharides did not differ significantly by gestational age. Milk collected in the first week postpartum differed significantly from milk collected during weeks two to five with respect to the mean (SE) percentage of α-1, 2-fucosylated oligosaccharides.

Conclusion
A larger study with standardized selection of study subjects and milk samples is warranted in order to further characterize this trend.
ACKNOWLEDGEMENTS

In loving memory of my grandmother, Edna M. Langone, whose unconditional love and great pride in her family continue to inspire and sustain me.

This thesis is dedicated to my husband, James Davidson. Thank you for your love, support, and enthusiasm throughout these years. You are the love of my life!

I would like to express my thanks and appreciation to the following family, friends, and faculty for their support and encouragement throughout my graduate education:

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To my sons, Michael and Brian Dunn, my two greatest accomplishments.

To my sisters, Beth Paige and Carolyn Wilkinson, and my brother, Russell Sampson, my confidants and best friends.

To my Aunt Barbara and Uncle Chet, always a source of inspiration to me.

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To my advisor, Dr. Christine Savage, and my committee members, Dr. Ardythe L. Morrow and Dr. Bonnie Brehm, who provided challenges and the guidance to achieve my goals.
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CHAPTER I
INTRODUCTION TO THE STUDY

Evidence that human milk is best for human infants has never been more compelling. Human milk not only contains superior nutritive components as compared to commercial infant formulas (Uauy & Periano, 1999), but also a host of bioactive compounds that promote the growth, development, and function of the infant gastrointestinal epithelium, immune system, and nervous system (Goldman, 2000). Many of these bioactive compounds have immunological functions that protect the infant from invading microbial pathogens (Newburg & Street, 1997). The study of these compounds is critical to our further understanding of the complex relationships that exist between the postnatal development of the human immune system and the immune functions of the mammary gland which result in the enhancement of infant survival (Goldman, Chheda, & Garofalo, 1998).

Statement of the Problem

Oligosaccharides are one of the major components in human milk, and have been associated with protection of infants from the development of toxin-induced diarrhea, a major cause of infant morbidity and mortality in the first year of life (Latham, 1999). The fucosylated oligosaccharides are of particular interest since they have been shown to inhibit host cell binding to enteric pathogens (Crane, Azar, Stam, & Newburg, 1994; Newburg, Pickering, McCluer, & Cleary, 1990; Cervantes, Newburg, & Ruiz-Palacios, 1995). The concentrations of both total fucosylated oligosaccharides and specific fucosylated oligosaccharides in human milk vary with a number of factors associated with the mother and infant including gestational age, day of lactation, and Lewis and
ABO histo-blood group types (Glass, Holmgren, Haley, et al., 1985; Thurl, Henker, Siegel, Tovar, & Sawatzki, 1997; Chaturvedi, Warren, Altaye, Morrow, Ruiz-Palacios, Pickering, & Newburg, 2001). Lewis and ABO histo-blood group types in human populations have been associated with the expression of specific glycoconjugates that have been linked with the differential risk of infectious disease (Blackwell, Jonsdottir, Hanson, & Weir, 1986; Newburg, Chaturvedi, Lopez, Devoto, Fayad, & Cleary, 1993). The variation in fucosylated oligosaccharide concentration observed with Lewis and ABO histo-blood group types, and the distribution of these blood group types relative to geographic area is also of interest since this suggests a relationship between the concentration of specific fucosylated oligosaccharides and protection against endemic microbial pathogens (Glass, Holmgren, Haley, Khan, Svennerholm, Stoll, Hossain, Black, Yunus, & Barua, 1985; Glass & Stohl, 1989; Morgan & Watkins, 2000).

Further investigation that examines potential relationships between the concentration of fucosylated oligosaccharides in human milk and gestational age, day of lactation, Lewis and ABO histo-blood group, and the geographic distribution of these blood group types is needed not only to describe mechanisms of action for the fucosylated oligosaccharides against specific pathogens, but also to identify relationships that may exist in populations of mothers and infants with respect to the secretion of specific immunoactive compounds. It has been suggested that the reduction in infant morbidity and mortality associated with the fucosylated oligosaccharides and other immunoactive structures in human milk is of major public health significance (Dewey, 1995).
Purpose of the Study

The purpose of this pilot study was to conduct a secondary analysis to compare the concentrations of both total and α-1, 2-linked fucosylated oligosaccharides to gestational age and day of lactation in milk samples collected from lactating mothers in South Carolina. This comparison in a small population in the United States will provide the foundation for the design of expanded, carefully controlled epidemiological studies to investigate potential relationships between gestational age, day of lactation, and Lewis and ABO histo-blood group types and fucosylated oligosaccharide concentration.

Significance of the Study

Studies of mother-infant nursing pairs for detection of the presence and distribution of fucosylated oligosaccharides in human milk are critically important to increase our understanding of the role that human milk plays in the development of infant immune function and the protection of infant health. These studies may result in the development of methods of intervention effective in the prevention and treatment of infectious disease for both children and adults.

Theoretical Rationale

This pilot study was conducted based on the assumption that human milk is best for human infants due to its unique combination of nutrients and bioactive factors so far unavailable in commercial infant formulas. Identification and analysis of the specific components in human milk that may contribute to the protection of the infant against disease support the efforts of those in the public and private sector who seek to re-establish breastfeeding as the cultural norm in the United States (United States Department of Health and Human Services, 2000; United States Breastfeeding...
Committee, 2001). The social and economic benefits of breastfeeding include reduced health care costs, reduced employee absenteeism for the care of ill infants, reduced direct cost of breastfeeding in comparison to formula purchase, and reduced waste and environmental pollution (Cadwell, 2002).

An epidemiological research model was used as a framework for this pilot study. In this approach, data are analyzed for a linkage or association between a factor and a health outcome. If there is an association, additional research is conducted to determine variation in the amount of disease with the amount of exposure to the factor. Analysis of the data can lead to determination of potential causal mechanisms for health outcomes in populations. When the factor being studied is a molecular or genetic marker suspected of having influence on host susceptibility to disease, the research is described as molecular epidemiology (Friis & Sellers, 1999).

In this secondary analysis, data obtained from lactating mothers in South Carolina were examined for potential relationships between total and α-1, 2-linked fucosylated oligosaccharide concentrations in human milk and the day of lactation and gestational age of the infant. Prior research studies have shown an association between the fucosylated oligosaccharide concentration in human milk and the prevention of gastrointestinal infectious diseases in infants. The fucosylated oligosaccharides in human milk inhibit pathogen binding to infant gastrointestinal epithelial cell receptors. This inhibition is accomplished by the homology of the fucosylated oligosaccharide with the cell surface receptor for the pathogen. The soluble oligosaccharide homologues compete with the cell surface receptors blocking pathogen binding (Newburg, 2000). The concentration and type of fucosylated oligosaccharide present in human milk have been shown to vary
with an individual's ABO and Lewis histo-blood group type, secretor status, day of lactation, and gestational age of the infant.

Research Questions

The varied expression of the α-1, 2-linked fucosylated oligosaccharides has been associated with differential protection of infants against pathogen-specific gastrointestinal disease (Morrow, Ruiz-Palacios, Altaye, Jiang, Guerrero, Meinzen-Derr, Farkas, Chaturvedi, Pickering, & Newburg, recently submitted manuscript). This variation has been observed with the ABO and Lewis histo-blood group type of the mother, day of lactation, and gestational age of the infant, leading to the following two research questions for this pilot study:

1. Do the concentrations of total and α-1, 2-linked fucosylated oligosaccharides vary with the day of lactation in this South Carolina population?

2. Do the concentrations of total and α-1, 2-linked fucosylated oligosaccharides vary with the gestational age of the infant in this South Carolina population?

Conceptual Definitions

Oligosaccharides Complex carbohydrates of up to 32 monosaccharide units consisting of glucose, galactose, N-acetylglucosamine, fucose, and sialic acid. Most contain lactose at the reducing terminus, while many contain fucose and/or sialic acid at the nonreducing terminus. More than 130 different types of oligosaccharides have been identified in human milk. The amount and number of different types of oligosaccharides found in human milk are greater than those found in either bovine milk or commercial infant formulas (Newburg, 1996).
Fucosylated Oligosaccharides  Oligosaccharides that contain fucose and are synthesized by fucosyltransferases that are not distributed uniformly throughout the human population. These compounds contain antigenic determinants of the Lewis blood group and/or secretor systems (Erney et al., 2000).

α-1, 2-linked Fucosylated Oligosaccharides  Oligosaccharides synthesized by the action of α-2-fucosyltransferases, the products of the secretor gene. The presence of these structures in human milk has been associated with the protection of infants against gastrointestinal infectious diseases (Erney et al., 2000).

ABH(O) Blood Group and Lewis Blood Group Antigens  A family of genetically and biochemically interrelated mucins comprised of about 85% carbohydrate and 15% amino acids. They were originally detected on red blood cells, but are also widely expressed in other tissues and secretions. (Lloyd, 2001).

Secretor  An individual with soluble ABH antigens, dependent on the ABO blood group of the individual, in salivary secretions. The secretor gene, inherited as a dominant Mendelian trait, controls the secretor characteristic. The secretor gene product is an α-1, 2-fucosyltransferase that transfers fucose to the terminal βGal unit of precursor chains. Secretors are capable of α-1, 2-linked fucosylated oligosaccharide synthesis, while nonsecretors lack this capability (Henry et al., 1995).

Day of Lactation  The postpartum day from which the milk was sampled, used to determine the period of lactation (Colostrum, Transitional Milk or Mature Milk) relative to the milk components of interest in a particular study.

Gestational Age  The number of weeks the infant was in utero. Births at less than 37 weeks gestation are considered preterm. Births at 37 weeks or after are considered...
term. Some human milk components are known to vary in preterm and term milk (Jensen, 1995).
CHAPTER II
REVIEW OF THE LITERATURE

Review

The immunological properties of human milk are no longer associated with the presence of antibodies alone. Other bioactive compounds in human milk including glycoproteins, lactoferrin, oligosaccharides, phagocytic leukocytes, enzymes, bifidus factor, hormones, and nucleotides have been associated with facilitating the development of the infant immune system and enhancing its function, as well as with a decrease in the incidence of acute respiratory infection, otitis media, necrotizing enterocolitis, urinary tract infections, bacterial meningitis, and sudden infant death syndrome (SIDS). Human milk may also reduce the risk of developing a number of chronic diseases like diabetes mellitus, Crohn’s disease, ulcerative colitis, and allergy as well as the risk of developing malignancies such as acute leukemia (Ball & Wright, 1999; Kelleher & Duggan, 1999; Work Group on Breastfeeding, American Academy of Pediatrics, 1997).

The oligosaccharides in human milk are of particular interest since they have structural homology with cell surface glycoconjugates that are used as receptors by pathogens to enter cells and cause infection. Based on current research findings, it appears that breastfed infants, due to the presence of these oligosaccharides in human breast milk, are protected against the heat-stable toxin of Escherichia coli and other pathogens including Campylobacter jejuni and caliciviruses known to cause gastrointestinal diseases (Newburg, 1997; Goldman, 2000; Wold & Adlerberth, 2000). However, this protection by human milk against gastrointestinal infectious diseases is not uniform. Some breastfed infants experience bouts of moderate-to-severe diarrhea. It has
been suggested that such bouts are the result, in part, of infant exposure to high doses of enteric pathogens and lack of exclusive breastfeeding (Haider, Islam, Hamadani, Amin, Kabir, Malek, Mahalanabis, & Habte, 1996; Marquis & Habicht, 2000; Morrow, Reves, West, Guerrero, Ruiz-Palacios, & Pickering, 1992). Another possibility is that concentration differences observed in human milk in several bioactive factors, including the oligosaccharides, may also play a role in differential protection. Variation in the oligosaccharide concentration has been reported not only among women, but also within the same woman over the course of lactation (Erney, Malone, Skelding, et al., 2000; Goldman, Garza, Nichols, & Goldblum, 1982; Jensen, 1995).

**Lewis and ABO Blood Group Types**

The oligosaccharide fraction is the third largest solid component of human milk, and contains compounds with α-1,2-linked fucose as well as those with α-1,3 and α-1,4 fucose linkages. The α-1, 2-fucosyloligosaccharides are Lewis antigen homologs, and inhibit host cell binding to enteric pathogens (Crane, Azar, Stam, & Newburg, 1994; Newburg, Pickering, McCluer, & Cleary, 1990; Cervantes, Newburg, & Ruiz-Palacios, 1995). The α-1, 2-fucose linkage is thought to be catalyzed by a fucosyltransferase encoded by the secretor gene (FUT2), while the α-1, 3 and α-1, 4 linkages are thought to be catalyzed by a fucosyltransferase encoded by the Lewis gene (FUT3) (Horton, Sangphi, Phillips, Fiedler, Perez-Escamilla, Lutter, Rivera, & Segall-Correa, 1996; Hamosh, 2001). The term secretor refers to the presence of soluble A, B, and H determinants, dependent on an individual’s ABO blood group type, found in the saliva and other secretions. The Lewis determinants are structurally related to determinants of the ABH blood group system.
Lewis and related antigens are found as free oligosaccharides in human milk and other secretions as well as in association with the cell plasma membrane (Henry, Oriol, & Samuelsson, 1995). Polymorphisms of the secretor and Lewis genes are known to determine expression of the Lewis blood group type, and are thought to control the variation in fucosylated oligosaccharide concentrations in human milk as well as the expression of histo-blood group antigens on infant epithelial cell surfaces (Viverge, Grimmonprez, Cassanas, Bardet, Bonnet, & Solere, 1985; Horton et al., 1996; Hamosh, 2001; Noguera-Obenza & Cleary, 2001). Women who are non-secretors have no α-1, 2-linked fucosylated oligosaccharides in their milk or other secretions. Secretor status varies in human populations with the prevalence of non-secretors in European-derived populations at about 20%. In the mestizo population of Mexico, non-secretors are less commonly seen (Erney et al., 2000; Henry et al., 1995). The differential risk of infectious disease has been associated with differing Lewis and ABO histo-blood group types in human populations.

Day of Lactation

Milk production or lactogenesis occurs in two phases, lactogenesis I and II. These phases are defined both temporally and by the composition of the milk. Lactogenesis I occurs in late pregnancy, and is an indication of preparedness of the mammary glands for milk secretion. Lactogenesis II is defined as the onset of copious milk production and secretion, and occurs in humans on the second or third day postpartum, frequently referred to as the milk “coming in” (Neville, 1995). Three periods of lactation are defined based on the variation in milk composition observed during these phases of milk production and secretion: 1) colostrum, produced late in pregnancy and
the first 36-96 hours postpartum; 2) transitional milk, produced in the first 2 to 3 weeks postpartum as colostrum decreases and milk production increases; and 3) mature milk, colostrum-free, produced after about 2 weeks postpartum. Colostrum is distinguished from transitional and mature milk by its thick, yellow appearance, which is attributed to higher protein content mainly in the form of immunoglobulins. It is also higher in the content of the fat-soluble vitamins A, E, and K, and some minerals such as sodium and zinc. Transitional milk has continuously varying composition, while mature milk is characterized by higher lactose, water-soluble vitamins, and fat content than either transitional milk or colostrum (Riordan & Auerbach, 1998).

Studies on the variation of oligosaccharide concentration with the day of lactation have shown high oligosaccharide content in colostrum and mature milk, with a gradual decrease over the first few months of lactation (Viverge, Grimmonprez, Cassanas, Bardet, & Solere, 1990; Coppa, Gabrielli, Pierani, Catassi, Carlucci, & Giorgi, 1993; Coppa, Pierani, Zampini, Carloni, Carlucci, & Gabrielli, 1999; Nakhla, Fu, Zopf, Brodsky, & Hurt, 1999; Chaturvedi et al., 2001). This decrease in total oligosaccharide concentration was observed in the milk of mothers who had given birth to preterm as well as term infants (Nakhla et al., 1999). In a recent study, both the total and $\alpha$-1, 2-linked fucosylated oligosaccharide concentrations were measured in relationship to the day of lactation. The absolute and relative concentrations of individual oligosaccharides varied substantially over the course of lactation, both between donors and for individual donors throughout lactation. The total fucosylated oligosaccharide concentration decreased over time, and the $\alpha$-1,2-linked fucosylated oligosaccharide to total oligosaccharide
concentration changed from 5:1 to 1:1 during the first year of lactation (Chaturvedi et al., 2001).

**Gestational Age**

Preterm (<37 weeks gestation) infants are more vulnerable to infection than term (≥37 weeks gestation) infants, which suggests that there may be differences in concentrations of fucosylated oligosaccharides in preterm and term human milk. Studies in which preterm milk was compared to term milk have shown concentration differences for a number of nutrients and bioactive factors including secretory IgA, nitrogen, sodium, chloride, and total lipid and fatty acid content (Gross, David, Bauman, & Tomarelli, 1980; Ogra, & Fishaut, 1990; Rueda, Ramirez, Garcia-Salmerón, Maldonado, & Gil, 1998). The relationship of gestational age to the concentration of fucosylated oligosaccharides is less clear. In one study, concentrations of the neutral oligosaccharides, which include the fucosylated oligosaccharides, were measured in preterm milk and were found to be similar to those in term milk (Nakhla et al., 1999). In another study, higher concentrations of the neutral oligosaccharides were reported in the preterm milk as compared to those in term milk (Brand Miller, Miller, McVeagh, & Bull, 1994).

**Summary**

Analysis of milk from specific geographic populations of lactating mothers for ABO, Lewis, and secretor genotypes as well as comparison of total and α-1,2 linked fucosylated oligosaccharide concentrations with gestational age (GA) and day of lactation (DOL) will provide information about the specific roles that the fucosylated oligosaccharides play in the differential protection of infants against microbial infection.
CHAPTER III
METHODOLOGY

Type of Study

A retrospective, descriptive study design was used to examine data related to human milk samples obtained from a cohort of breastfeeding mothers in South Carolina. These mothers had signed informed consent to donate milk samples to a milk bank with the understanding that these samples would be stored and utilized for research.

Subjects

A convenience sample of 18 lactating mothers whose infants had been admitted to the neonatal intensive care unit at the Medical University of South Carolina (MUSC) from 1994-1998 were selected from among a population of mothers who had donated their milk samples to a milk bank for research utilization. The milk samples were collected during weeks 1-5 postpartum and were stored at -80° C. Aliquots (2 ml) were removed from the stored samples, and sent to the University of Massachusetts, Worcester for oligosaccharide analysis by High Performance Liquid Chromatography (HPLC). The concentrations of total and α-1, 2-linked fucosylated oligosaccharides were measured in nanomoles per milliliter (nmol/ml). Only data on the concentrations of fucosylated oligosaccharides, infant gestational age, day of lactation, and ABO blood group of the mother were supplied to our group for this study. Demographic information on race was not available.

Protection of Subjects

Confidentiality of the subjects was maintained through the assignment of a randomly selected subject number for each sample. Clinical data were recorded for each
sample and included the gestational age of the infant, date of birth, date of sample donation, maternal ABO blood type, and maternal age. The subject name linked with sample number was maintained in a separate data file accessible only to authorized staff at MUSC.

Data Analysis

These data were analyzed using descriptive statistics. Frequency distributions were obtained for gestational age (GA), day of lactation (DOL), and ABO blood type. The sample mean and the standard error of the mean (SE) were calculated for both the analysis of gestational age (GA) and concentrations of total and α-1,2-linked fucosylated oligosaccharides and the analysis of day of lactation (DOL) and concentrations of total and α-1,2-linked fucosylated oligosaccharides. The population was divided into three groups for each of the two variables compared with total and α-1,2-linked fucosylated oligosaccharides. The groups for Gestational Age (GA) were ≤ 30 weeks, n = 7; 31-36 weeks, n= 6; and ≥ 37 weeks, n= 5. The groups for Day of Lactation (DOL) were DOL 1-7, n= 4; DOL 8-14, n= 10; and DOL ≥ 15, n= 4. Comparison of the population means for these variable groupings were analyzed by ANOVA. The stage of lactation variable was further analyzed by dividing the population into two groups according to week of lactation to correspond with the stages of milk maturation: Week 1, n=4 and Weeks 2-5, n=14. These data were analyzed by t Test for comparison of the sample means. Because of the small sample size and the disparity in size of the above two groups, nonparametric analysis of these data was by the Wilcoxon Two Sample Test. Secretor status was determined by the presence of α-1, 2-linked fucosylated oligosaccharides in the milk sample.
CHAPTER IV
DISCUSSION OF THE FINDINGS

Introduction

The purpose of this retrospective, pilot study was to examine data obtained from milk samples donated by a cohort of breastfeeding mothers in a United States population for potential relationships between gestational age (GA) of the infant and day of lactation (DOL) and the concentrations of both total and percent \( \alpha-1,2 \)-linked fucosylated oligosaccharides. Relationships of these variables with the neutral fucosylated oligosaccharides, which includes \( \alpha-1,2 \)-linked fucosylated oligosaccharides, in multiple populations outside the United States have been associated with the differential protection of infants against gastrointestinal disease, a major cause of infant morbidity and mortality around the world.

Discussion

Frequency distributions for the 18 study subjects were obtained for the variables of gestational age (GA), day of lactation (DOL), and ABO blood group type of the mother. The types of oligosaccharides and their concentrations in human milk are genetically determined and related to ABH (O), Lewis, and secretor status of the mother. Some human milk oligosaccharides are found in the milks of all mothers, however, others are dependent on the mother's ABH, Lewis, and secretor status, e.g., oligosaccharides with A specificity have been found in the milk of mothers with blood group A (Sabharwal, Sjoblad, & Lundblad, 1991; McVeagh & Miller, 1997). In addition, it is known that those mothers identified as nonsecretors have no \( \alpha-1,2 \)-linked fucosylated oligosaccharides in their milk. The \( \alpha-1,2 \)-linked fucosylated oligosaccharides have been
associated with protection of infants against gastrointestinal infectious disease (Morrow et al., recently submitted manuscript).

These data were analyzed for frequency of ABO blood type and determination of secretor status by the presence of $\alpha$-1, 2-linked fucosylated oligosaccharides in the milk sample. Two of the 18 (11%) subjects were identified as nonsecretors since they had no $\alpha$-1, 2-linked fucosylated oligosaccharides in their milk. The ABO blood type for one of the study subjects was unknown.

Table 1

<table>
<thead>
<tr>
<th>Gestational Age (GA)</th>
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For Gestational Age (GA), the subjects were divided into 3 groups: GA ≤ 30 weeks, n=7; GA 31-36 weeks, n=6; and GA ≥ 37 weeks, n=5. The frequencies were 39%, 35%, and 18% respectively.
For Day of Lactation (DOL), the subjects were divided into 3 groups: DOL 1-7, n=4; DOL 8-14, n=10; and DOL ≥ 15, n=4. The frequencies were 24%, 56%, and 24% respectively.

<table>
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<th>Day of Lactation (DOL)</th>
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<td>37</td>
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For ABO blood type of the mother, the frequencies were 17% A, 17% B, 50% O, and 11% AB. The ABO blood type for one study subject was unknown.

<table>
<thead>
<tr>
<th>ABO Blood Type</th>
<th>Frequency</th>
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<td>O</td>
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Figure 1. Mean Total Fucosylated Oligosaccharide Concentration by Gestational Age

The oligosaccharide concentration in the colostrum and mature milk of mothers of term infants is high and gradually decreases over the first few months of lactation (Coppa et al., 1993). Since these oligosaccharides have been associated with the protection of infants against infectious disease, the high oligosaccharide content in human milk at birth suggests that human milk is uniquely designed to protect infants when they are most susceptible to infection. These observations, along with the evidence that a number of other factors such as secretory IgA, nitrogen, sodium, and chloride differ in preterm and term milk, have led investigators to postulate that the oligosaccharide concentration in preterm milk may be higher than it is in term milk, thereby offering greater protection to preterm infants.
In this pilot study of a United States population, the mean (SE) total fucosylated oligosaccharide concentration did not differ significantly by gestational age of the infant, however, there was a trend towards increasing concentration with increasing gestational age: GA ≤ 30 weeks 499 (184); GA 31-36 weeks 663(362); and GA ≥ 37 weeks, 1012 (361). (Figure 1). In addition, the mean (SE) percentage of α-1, 2-linked fucosylated oligosaccharides did not differ significantly by gestational age of the infant: GA ≤ 30 weeks, 31 (13); GA 31-36 weeks, 54 (12); and GA ≥ 37 weeks 24 (11) (Figure 2).

Figure 2. Mean Percentage α-1,2–Linked Fucosylated Oligosaccharides by Gestational Age
In this study, milk collected in the first week postpartum differed significantly from milk collected during weeks 2-5 postpartum (p < 0.001) with respect to the mean percentage of $\alpha$-1,2-linked fucosylated oligosaccharides. The mean (SE) percentages for these groups were 7% (3) and 45% (8) respectively. (Figure 3).

![Figure 3. Mean Percentage $\alpha$-1,2-Linked Fucosylated Oligosaccharides by Day of Lactation](image)

Conclusion

No significant difference was found in this pilot study in the total fucosylated oligosaccharide concentration in milk with respect to the gestational age (GA) of the infant. However, there was a trend towards increasing total fucosylated oligosaccharide concentration with increasing gestational age. These findings appear to support an earlier
study in which the authors concluded that there was no significant difference in total fucosylated oligosaccharide concentration in preterm and term milk (Nakhla et al., 1999). The trend towards increasing fucosylated oligosaccharide concentration with increasing gestational age was seen only over a relatively short course of lactation (through the fifth week postpartum). This trend suggests that the total fucosylated oligosaccharide concentration in human milk may be at its highest when the infant immune system is most immature. One would expect to see a decrease in the total fucosylated oligosaccharide concentration as the infant immune system is reaching maturity; however, in this pilot study, milks obtained from a longer course of lactation were not examined.

A significant difference was observed in the mean (SE) percentage of α-1,2-linked fucosylated oligosaccharide to total fucosylated oligosaccharide concentration from milk samples obtained during week 1 of lactation as compared to milk samples obtained during weeks 2-5 of lactation (p < 0.0001). This finding lends even more support to the theory that human milk contains greater concentrations of fucosylated oligosaccharides when the infant immune system is least able to effectively combat infection, especially infection of the gastrointestinal tract against where the α-1,2 linked fucosylated oligosaccharides have been shown to be effective (Morrow et al., recently submitted manuscript).

Scope and Limitations

The descriptive design of this study allowed for the collection and categorization of the data. It was not within the scope of this pilot study to make predictions based on the results. The purpose of the study was to examine the data for potential relationships
between the selected variables and the concentrations of both total and α-1,2-linked fucosylated oligosaccharides as justification for the development of carefully designed, epidemiological studies on a larger scale.

The limitations of this study are inherent in the study design. The sample was a nonrandom, convenience sample (n = 18) of milk donors from a neonatal intensive care unit in South Carolina. This limits the generalizability of the findings to this sample. Because of the small sample size, there is a high probability of Type II error that reduces the ability to detect differences in oligosaccharide concentration and the selected variables.
CHAPTER V
SUMMARY OF THE STUDY AND RECOMMENDATIONS

Summary

Studies of human milk suggest that oligosaccharides may be responsible for the reduced risk of infection in breastfed infants. Fucosylated oligosaccharides are known to act as receptors for enteric pathogens preventing both attachment of these pathogens to infant gastrointestinal epithelial cells and subsequent infection. The reduction in infant morbidity and mortality in the first year of life as a result of breastfeeding has great public health significance. The social and economic benefits of breastfeeding cannot be underestimated, and include reduced health care costs, reduced employee absenteeism for the care of ill infants, reduced direct costs of breastfeeding in comparison to commercial formula purchase, and reduced waste and environmental pollution (Dewey, 1995; Cadwell, 2002).

The purpose of this retrospective, pilot study was to examine data obtained from the milk of a cohort of lactating mothers in a United States population for potential relationships between the selected variables of gestational age (GA) of the infant, the day of lactation (DOL), and the ABO and Lewis histo-blood group type of the mother and the concentrations of both total and α-1, 2-linked fucosylated oligosaccharides. In previous studies comparing the concentrations of the fucosylated oligosaccharides and the day of lactation, the levels of total fucosylated oligosaccharides were initially high and then declined over the course of lactation. In studies in which the concentrations of the fucosylated oligosaccharides and the gestational age (GA) of the infant were compared,
conflicting results have been obtained. Some investigators have reported a higher concentration of these oligosaccharides in preterm milk than in term milk, while others have reported no differences. These studies have been limited in terms of sample size as well as methodology.

The ABO and Lewis histo-blood group type of the mother have been associated with the appearance and types of oligosaccharides in human milk. These genetic characteristics of the mother differ geographically, and suggest differential protection of infants against infectious disease. Further examination of these observations could result in the development of prophylactic interventions for the protection of infants and adults against endemic agents of disease (Newburg, 2000).

There were no significant differences observed in the comparison of gestational age (GA) of the infant with oligosaccharide concentration in this analysis, however, a trend towards increasing total oligosaccharide concentration with increasing gestational age was observed. No significant differences were observed when comparing the concentrations of total fucosylated oligosaccharides with the day of lactation (DOL), however, milk collected during the first week postpartum did differ significantly from milk collected during weeks 2-5 postpartum with respect to the percentage of α-1,2-linked fucosylated oligosaccharides (p<0.001). Two of the 18 study subjects were nonsecretors as indicted by the lack of α-1,2-linked fucosylated oligosaccharides in their milk.

Recommendations

Predictions and conclusions about the relationship between the concentrations of total and α-1,2-linked fucosylated oligosaccharides and the selected variables of

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gestational age (GA) of the infant, day of lactation (DOL), and ABO and secretor status of the mother are beyond the scope of this study because of its very nature. The study design was descriptive and retrospective allowing only an analysis to determine trends observed in this particular sample.

Further research is warranted, however, based on these results. A longitudinal study in which a milk donor population of sufficient statistical power is followed over the course of lactation with standardized milk collection is recommended. The milk sample collection can be standardized with respect to time of day, method of milk expression (pump or manual), and volume of the milk sample (contents of one whole breast) to minimize any differences that would be due to these factors. In addition, the donated milk samples should include milk from mothers who have delivered preterm as well as term infants. For a more extended and informative analysis of the ABO, Lewis histo-blood group type, and secretor status of the mother, saliva samples can be collected along with milk samples to determine the appearance and type of ABH antigens present. Specific types of oligosaccharides in milk have been associated with the presence of antigens of this blood group system according to the blood type of the mother (McVeagh et al., 1997; Newburg, 2000).
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