University of Alberta

Fat Delivery During Continuous Breast Milk Infusions

by

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Dedication

First and foremost, this is dedicated to my father and mother. Their unending support was invaluable and they were instrumental in allowing this to happen for me. I hope they realize that education is truly the greatest gift parents can give their children. I love you. Thank you.

To my sister and friends in Edmonton (you know who you are). You listened to me, encouraged me, and supported me all of the way – and I mean ALL of the way – through this. I not only thank you, I offer this dedication. You are the best.

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Chapter One

Introduction

Infants who are born prematurely face many challenges. For caregivers, one of the key challenges is providing adequate nutrition (Schanler, Shulman, Lau, Smith, & Heitkemper, 1999; Wilson, Cairns, Halliday et al., 1997). Premature infant nutrition is tailored to an individual infant's needs and clinical status to ensure that the optimum nutrient combination is provided (Bakewell-Sachs, 1999; Pereira, 1995). After the first week of life, the recommended energy intakes for premature infants range from 105-140 kcal/kg per day (Nutrition Committee, Canadian Paediatric Society, 1995, 2000; Gomella, 1999). Breast milk is the recommended milk nutrient of choice (American Academy of Pediatrics, 1985; Atkinson, 2000; Canadian Paediatric Society, 1995, 2000).

Providing adequate nutrition to infants in the Neonatal Intensive Care Unit (NICU) is critical to supporting normal growth and development (Premji, 2002). Good nutrition enhances neurodevelopment in premature infants and improves surgical outcomes, decreases morbidity, and shortens hospital stay (Townsend, Johnson & Hay, 1998). Undernutrition is detrimental to lung growth and development, neurological development, and to respiratory motor function (Wilson, 1995; Ziegler, Thureen, & Carlson, 2002). In the third trimester of pregnancy the fetus is almost fully developed and the normal task at this time is to grow and store energy for birth. Premature infants have less opportunity to grow and store energy than their term counterparts and are at greater risk for nutritional challenges post-natally. Clinically, post-natal nutritional management of premature infants in the NICU endeavours to attain growth similar to that which could have been achieved in-utero (American Academy of Pediatrics, 1985). Unfortunately,

when the premature infant encounters the unnatural circumstances of extra-uterine life sub-optimal growth may result.

Initially, the objective of premature infant nutritional care is to maintain glucose, fluid, and electrolyte homeostasis. Early nutrition is provided with IV total parenteral nutrition (TPN) between 24 -72 hours. When the infant is clinically stable, enteral or gastrointestinal feeds are introduced (Pinchasik, 2001; Pereira, 1995). Parenteral and enteral feeding normally occurs together for a period. The infant is transitioned from TPN to enteral feeding, then oral feeding gradually (Pinchasik, 2001).

Enteral feeding (EF) is started as early as possible using trophic feeds or minimal enteral nutrition. These low volume feedings are initiated within one to eight days of life. Feed volumes range from 10 – 24 mls/kg/day, are not increased, and continue for 5 – 14 days, depending on clinical status (Abad-Sinden & Bollinger, 2002; Ziegler, Thureen, & Carlson, 2002). Feeding volumes are increased as tolerated based on clinical status, gestational age, and individual requirement – an average of 20 ml/kg/d (range 10-35 ml/kg/d) (Rayyis, Ambalavanan, Wright, & Carlo, 1999). No "ideal" rate of advancement exists (Kennedy, Tyson, & Chamnanvanakij, 2003). A premature infant may take four to seven weeks to reach full volume enteral feeding (Schanler, Shulman, Lau, Smith, & Heitkemper, 1999). During this time, an infant may commence and discontinue feedings several times if intolerance develops (Wilson, Carins, Halliday, et al., 1997). Enteral feedings may be administered in one of three ways: bolus infusion by gravity flow, intermittent bolus by pump over 15-120 minutes, or by continuous infusion infused at a constant rate.

When unpasteurized, EBM that sits still at room or refrigerated temperature

undergoes creaming. This occurs because the fat in milk is an emulsion surrounded by a globule which weighs less than the other (aqueous) phases of milk (Jensen, 1989). Milk fat floats to the top of the fluid and other milk components and appears as a distinct layer. The top layer of the EBM is rich in fat globules while the lower one is mostly depleted of globules and is skim milk (Jensen, Lammi-Keefe & Koletzko, 1997; Walstra, 1994). Creaming occurs in storage containers and within all of the components of an EF infusion system, which includes the syringe and tubing. Reported amounts of fat lost vary from from 17 to 60% (Brennan-Behm, Carlson, Meier, Engstrom, 1994; Stocks, Davies, Allen, & Sewell, 1985; Van Aerde, unpublished, 1998). The greatest fat loss occurs during continuous infusions at a low infusion rates (Greer, McCormick, & Loker, 1984).

The recommended energy requirement for stable growing premature infants is approximately 120 kcals/kg/day (range of 105-135 kcal/kg/d) (American Academy of Pediatrics, 1985; Nutrition Committee, Canadian Paediatric Society, 1995, 2000). The following is an estimate of how energy balance is approximated for 120 kcals/kg/d (refer to Table 1 for a summary). The infant would use approximately 83 kcals/kg/d (69% of 120 kcals/kg/d) for energy expenditure which includes physical activity, resting metabolic rate, etc.. Twelve kcals/kg/d (10% of 120 kcals/kg/d) is excreted in the stool and 20 to 30 kcals/kg/d is used for energy storage and growth. This 20-30 kcals/kg is about 16-25% of total energy intake (Groh-Wargo, 2000; Leitch & Denne, 2000) and amounts to an extra 15g/kg/d. One of the greatest challenges in providing nutrition for a premature infant is to supply the energy and nutrients required for their basic metabolic needs and the additional energy required to promote growth (Leitch & Denne, 2000; Wilson, 1995). If energy for storage and growth is inadvertently lost to delivery systems

during EF, growth impedance can occur in the premature infant. The loss of milk fat could deprive the infant of valuable energy, fatty acids, and fat-soluble vitamins.

Table 1.

Energy Requirement of the Premature Infant (kcal/kg/d)

		AAP*	Range
Energy Expenditure	Resting Metabolic Rate	50	45 - 60
	Activity	15	5 - 10
	Cold Stress	10	5 - 10
	Synthesis	8	10 - 25
Energy for Growth and Storage		**25**	**20 - 30**
Energy Excreted		12	10 - 30
Estimated Energy Required		120	95 - 165

* American Academy of Pediatrics.
Adapted from Groh-Wargo (2000).

A Clinical Example. Consider a 1.0 kg LBW infant is receiving 120 kcal/kg/day with 100 kcals/kg from unfortified EBM delivered continuously and the remaining 20 kcals are given parenterally. On average, human milk provides 20 kcals/oz and the fat component is 40 - 60% of the total calories (Nutrition Committee, Canadian Pediatric Society, 1995, 2000). A 25-50% fat loss within a delivery system would equal a loss of 12-25 calories per day. The infant is then receiving milk with only 15 to 18 kcals per oz. Over a 24-hour period an energy loss of 12-25 calories for a 1.0 kg infant could lower the caloric and nutrient intake such that growth might be impaired.

Several variables could affect the delivery of fat to an infant during continuous

EBM feeding. They include the type of infusion pump, the tubing characteristics, the syringe orientation, and hospital feeding protocols. Pumps used for continuous EF have evolved from larger volumetric IV and roller pumps with buretrols and lengthy tubing to smaller syringe pumps with different tubing that is normally shorter in length. Longer infusion systems are associated with fat loss. Even with a syringe pump, elements of the infusion system will vary. These elements include the types and dimensions of the tubing and syringes that are used, the syringe tip orientation, and the placement of the pump itself. Lastly, hospital protocols determine how frequently the tubing and syringes are changed. Discarding infusion tubing without emptying the milk contents that remain inside could result in fat and nutrient loss.

There is a variation in EF methods between and within NICUs. An informal internet survey of clinicians from 35 NICUs in North America was completed in March 2002 and illustrates this variation (refer to a summary in Appendix A). Notable differences include the type of infusion pump used and its orientation, the tubing types used, and the frequency of tubing and syringe replacement.

In the internet survey, clinicians who use continuous feedings in their NICUs were asked about delivery pumps, tubing types, frequency of tubing and syringe changes, and syringe orientation during infusion. Not all respondents answered every question. Ninety-four percent of responding NICU clinicians use syringe pumps. The majority of respondents, 69%, indicated that they orientate the syringe tip up, vertically, for continuous infusions. Two respondents tilted the tip up 45°, two rotated the syringe intermittently, two placed it horizontally, and eight ran the infusion with the tip down. Although it is possible to use many types of tubing, the majority of respondents indicated

that they used microbore IV extension tubing (51%). Forty percent indicated they used the standard or large bore tubing. Only two respondents (6%) indicated that they used an enteral only tubing (Children's Medical Ventures ®), which is actually minibore tubing. Given this variation, it is clear that no standard practice exists.

Purpose & Hypothesis

The purpose of this study was to determine whether there is a difference in the amount of fat delivered through three different tubings currently used in continuous EBM feedings during a low rate, e.g. four ml per hour, simulated infusion. The tubing types chosen for this study reflect current NICU practice. They include microbore tubing (Baxter, #JC9201), minibore tubing (Children's Medical Ventures® #95017-A), and standard bore tubing (Baxter # 2C6223). The infusion conditions were set to reflect clinical practice as closely as possible. The null hypothesis that was tested was that there is no difference in the amount of fat delivered during a four hour, four ml per hour infusion of freshly expressed breast milk through microbore, minibore, and standard bore tubing used in EF systems.

Definition of Terms

Infusion System. For the purpose of this study the infusion system was defined as the equipment required to infuse milk to the infant. This included a 20 ml BD syringe that held the milk, an extension tubing through which milk was passed, and a syringe pump that drove milk to the end of the tubing. It did not include a gastric tube.

Donated EBM. A sample of expressed milk, approximately 100 mls, expressed by mechanical or hand pump, donated by one mother, immediately refrigerated, and used

within 12 hours of pumping.

Run. A four hour, four ml per hour simulated infusion from one donated EBM sample infused through each of three infusion systems that varied by the type of extension tubing used.

Sample. Twenty to 40 mls of breast milk obtained (pre infusion), and 16 mls of breast milk collected over four hours in one container per infusion system (post infusion).

Significance of the Study

Based on the internet survey it was evident that no clear practice standard existed for continuous EF of EBM. There is also a paucity of current literature on the subject of fat loss in continuous EBM infusions. It was important to identify how three extension-tubing types currently used in clinical settings ranked in terms of fat delivery during continuous, low rate infusions of EBM. By providing current and scientific knowledge regarding nutrition loss in EF infusion systems, clinical practices and/or guidelines can be reinforced or changed, as the evidence warrants. If fat loss during continuous infusions is minimized, infants immediately benefit with more calories and better nutrition that aids his or her growth in a precarious time.

Chapter Two

Literature Review

Search Strategy

The following computerized data bases were searched from 1980 to 2003 with a few key studies from earlier years in preparation of this literature review: CINAHL, MEDLINE, Pub Med, EBM Reviews, Cochrane Database of Systematic Reviews, Agricola, Community of Science (COS), and Analytical Abstracts. The literature search evolved from the following key words which were used to find relevant studies. They included the following: "continuous enteral feeding", " continuous infusions", "human milk", "fat loss in continuous enteral feeding", "enteral nutrition", "premature neonate", "premature infant". Secondary searches were performed on identified literature. Scientific and agricultural databases were searched with the following key terms "milk fat analysis", "Roese-Gottlieb or Mojonnier", "solvent extractions", "milk chemistry", "polyvinyl chloride", and "physical properties of milk".

Bolus versus Continuous Feeds

Bolus. In a bolus feeding an indwelling or temporarily placed feeding tube of varied length and composition is used to deliver food to the stomach intermittently, simulating an oral feeding. This type of feeding has many advantages. Schanler, Schulman, and Lau (1999) noted improved feeding tolerance and greater weight gain when infants were bolus fed. Dollberg, Kuint, Mazkereth, and Mimouni (2000) found that infants advanced to full feeding more quickly when on bolus feeds. Bolus feedings stimulate a post-prandial hormonal regulation that promotes gastrointestinal health and

improved motility (Aynsley-Green, Adrian, & Bloom, 1982). It also results in less lipid loss to the delivery system than continuous feeding (Greer, McCormick, & Loker, 1984; Narayanan, Singh, & Harvey, 1984; Stocks, Davies, Allen, & Sewell, 1985). Despite these advantages adverse affects have been reported and may include tachycardia, tachypnea or respiratory distress, bradycardia, apnea, cyanosis and hypoxemia (Blondheim, Abbasi, Fox, & Bhutani, 1993; Grant & Denne, 1991; Toce, Keenan, & Homan, 1987). Brar, Geiss, Brion, and Rios (2001) compared respiratory compliance and functional residual capacity of infants in intermittent versus continuous feeding concluding that infants tolerated bolus feeding well. Despite this evidence (Brar et al., 2001), in clinical practice some infants may not tolerate bolus feedings and may require continuous EF.

Continuous. In continuous EF, indwelling feeding tubes of varied lengths and compositions infuse food into the infant's stomach at a constant rate over 24 hours. From the feeding tube an infusion tubing connects to a syringe or a container and then to a pump which drives the forward flow of food into the infant. The equipment used varies, as described in the introduction.

One advantage of continuous EFs is thought to be the reduction of some or all of the adverse physiologic effects said to be associated with bolus feeding. Compared with bolus feedings continuous EF is thought to be more energy efficient, reduce feeding intolerance, and improve nutrient absorption and growth (Grant & Denne, 1991; Toce, Keenan, & Homan, 1987). A recent systematic review by Premji & Chessell (2003) compared continuous versus bolus or intermittent feeding. Major outcome criteria included feeding tolerance, number of days to full feeds, somatic growth, days to

discharge, and complications of feeding method. Infants who fed continuously took an average of three days longer to reach full volume feedings. They remained on TPN longer, which is more costly than EF, and were at risk for some of the negative effects associated with its use, sepsis for example. There was no evidence of greater somatic growth, feeding intolerance, rate of weight gain, incidence of NEC, or time to discharge: no method could be identified as superior (Premji & Chessell, 2003).

There is also evidence that continuous infusions negatively alter the normal physiologic response to eating. This is due to the absence of cyclical surges in gut hormones thought to encourage gut motility. Ultimately, without these hormone and motility effects, gastrointestinal tissue growth and health may be affected (Aynsley-Green, Adrian, & Bloom, 1982). Post-prandially, the gallbladder secretes bile for digestion. In the absence of routine stomach distension from milk, bile remains in the gallbladder and sludge forms. This may impair the secretion of bile, thus impairing fat digestion and resulting in gastrointestinal upset. This phenomenon has been described in infants who were on continuous feeding (Jawaheer, Shaw, & Pierro, 2001).

Both continuous and bolus EF methods offer specific advantages and disadvantages, both theoretically and to individual infants. For routine use, either is acceptable (Newell, 2000). Continuous EF of EBM will continue to be used in NICUs. Premature infants are at the greatest disadvantage if milk fat is lost in an infusion system.

Breast or Formula Milk

Breast Milk. Breast milk is recommended for infant feeding (American Academy of Pediatrics, 1997; Lucas & Cole, 1990; Meier & Brown, 1996; Newell, 2000; Nutrition Committee, Canadian Paediatric Society, 1995, 2000; Schanler, Shulman, & Lau, 1999;

Townsend, Johnson, & Hay, 1998). Breast milk provides the nutrient composition specifically adapted to the needs of the newborn (Hamosh, 1998). It contains approximately 7% protein, 55% fat and 38% carbohydrate, which provides an optimal caloric distribution for neonates (Bakewell-Sachs, 1999).

Advantages. Breast milk contains anti-infective properties and immunoprotective factors that include secretory IgA, lysozyme, and lactoferrin (American Academy of Pediatrics, 1997; Hamosh, Peterson, Henderson, et al., 1999). Additionally it has protective bacteriostatic properties in the milk fat globule (MFG). MFG membrane glycoproteins act as specific bacterial and viral ligands that prevent pathogens from attaching to the intestinal mucosa (Hamosh, Peterson, Henderson, et al., 1999; Yu, 1999). Other benefits of EBM are the whey predominant protein, low renal solute load, and promotion of maternal-infant attachment (Sapsford, 2000). The whey predominant protein is important to infants, particularly premature infants, because of their metabolic capacity to break down this type of protein. They possess more of the specific enzymes that are required to break it down as opposed to the casein dominant protein found in commercially prepared formulas (Räihä, 1999).

Breast Milk and Premature Infants. For premature infants breast milk can offer many advantages (Schanler, 2001). Breast milk from mothers who give birth prematurely has more energy, protein, sodium, chloride, nitrogen, vitamin D, immune proteins, total lipid, and less lactose than term EBM (Atkinson, 2000; Bitman, Wood, Mehta, Hamosh, & Hamosh, 1983; Pereira, 1995). Unfortunately, over time, EBM does not provide adequate protein, calcium, phosphorus, sodium, iron, copper, zinc, and some vitamins for rapidly growing premature infants (Abad-Sinden & Bollinger, 2002; Anderson, Atkinson,

& Bryan, 1981; Moye, Hall, & Simmons, 1982; Pereira, 1995). Premature infants fed solely on breast milk may experience poor weight gain, decreased linear growth, osteopenia and rickets, and hyponatremia (Hall, 1999).

The premature infant has an impaired ability to digest fat due to decreased pancreatic lipase activity, bile salt synthesis, and reabsorption of bile salts in the distal ileum. When fed EBM, infants have increased gastric emptying, more frequent stooling, and improved fat absorption (Newell, 2000; Premji, Paes, Jacobson, & Chessel, 2002). Armand, Hamosh, Mehta, et al. (1996) found that the gastric hydrolysis of human milk fat was 1.7 to 2.5 times higher than formula fat. More undigested fat is excreted in the stool of formula fed infants (Armand, Hamosh & Mehta, et al., 1996). Breast milk feeding in infants is associated with a decreased incidence of necrotizing enterocolitis (NEC) (Lucas & Cole, 1990). It is suspected that this is due to breast milk constituents not found in synthetic formula (Newell, 2000).

Formula. Synthetic milk formulas emulate breast milk composition as the "gold standard" (Sapsford, 2000). Fat in formula is unlike that of human milk and it does not undergo creaming. Infant formula lacks the immunoprotective properties and growth factors that breast milk contains. In addition, human milk fat is more easily digested than the fat found in formula (Hamosh, 1998). Schanler, Shulman, and Lau (1999) found that breast milk fed infants had a lower incidence of feeding intolerance during feeding advancement, decreased morbidity with sepsis, and less NEC. When possible, breast milk feeding is recommended.

Milk Fat

Energy provision is the most important function of milk fat. The amount of fat in

human milk ranges from 3 – 5 % (Jensen, 1989). Ninety-eight percent of the fat is in the form of triglycerides or TGs. Fat accounts for 40-60% of the energy in milk (Nutrition Committee, Canadian Paediatric Society, 1995, 2000) or an average of 50% of total calories (Butte, Casey, Dewey, et al., 1985; Groh-Wargo, 2000). Fat or lipid alters the composition of cell membranes and provides essential fatty acids (EFAs), which are the precursors to several important prostaglandins, bioactive components, and cholesterol. Premature infants are at risk for EFA deficiency as they are not in utero for the third trimester when EFAs are normally accrued (Leitch, 1998, Pinchasik, 2001). Long chain polyunsaturated fatty acids have been found to enhance neurodevelopment and retinal function (Jensen & Heird, 2002; O'Conner et al., 2001; Uauy & Mena, 2001).

Total fat content is the most variable component of breast milk (Ferris & Jensen, 1984; Jensen, 1989). The fat content of breast milk varies between individuals, within a single feed, and diurnally. In lactating mothers, fat content is higher in the afternoon than in the morning. It may vary in milk expressed using hand and electric breast pumps, the latter having a greater fat yield, and between types of electric breast pumps (Ferris & Jensen, 1984). Milk is released from the breast in two phases, foremilk first and hindmilk afterwards. Foremilk tends to be lower in calories and fat. Hindmilk normally has a fat concentration two to three times higher than foremilk (Kirsten & Bradford, 1999; Valentine, Hurst & Schanler, 1994).

Fat content also varies with a mother's nutritional intake, stage of lactation (Hibberd, Brooke, Carter, Haug, & Harzer, 1982; Rodriguez-Palmero, Koletzko, Kunz, & Jensen, 1999) and gestation at delivery. Variation occurs between breasts, with infections or metabolic disorders, with medications, with mother's menstrual cycle or pregnancy,

the season, the age of the mother, and her parity (Jensen, 1989). Hamosh (1998) found that lactating women in lower socio-economic groups had a decreased fat content in their breast milk.

Measurement of Milk Fat

Human milk is analyzed to determine its biochemical and nutrient composition. Laboratory methods may be used to determine the total lipid content, or to quantify specific lipid classes. Jensen, Bitman, Wood, Hamosh, Clandinin, & Clark (1985) and Jensen (1989) categorize analytical methods of human milk fat as direct and indirect.

Direct methods normally involve many samples of milk, minimal equipment, and determine only total lipid content. Examples of the direct methods include the creamatocrit (CR), enzymatic, colorimetric, and turbidimetric. Indirect procedures are used if information regarding individual lipid classes is required. In an indirect method lipids are separated or extracted from other milk constituents using solvents. In this way, the total lipid content is isolated. It is then weighed (gravimetry) or determined by optical density (spectrography). Lipids of different classes can then be separated and quantified. Examples of indirect methods include: Roese-Gottlieb, Mojonnier, Modified Folch, and dry column. These methods, along with their main advantages and disadvantages, are summarized in Appendix B.

Direct Measures

Creamatocrit. Of all the direct measures available for lipid determination only the CR method is examined in greater detail as it is cited frequently in the literature. Many studies have used the CR method for estimating milk fat loss (Brennan-Behm,

Carlson, Meier, & Engstrom, 1994; Chan, Nohara, Chan, Curtis, & Chan, 2003; Lemons, Miller, Eitzen, Strodtbeck, & Lemons, 1983; Narayanan, Singh, & Harvey, 1984; Spencer & Hull, 1981; Stocks, Davies, Allen, & Sewell, 1985). Small samples of milk are aspirated into a micro-capillary tube and spun in a standard hematocrit centrifuge. During centrifugation, milk fat and fluids form two distinct layers. These layers are measured and fat content is estimated by dividing the length of the fat layer by the total column length (Appendix B). This method of determining fat began when Fleet and Linzell (1964) measured goat milk fat with the CR and a gravimetric method. Lucas, Gibbs, Lyster, & Baum (1978) adapted it to human milk.

There are several advantages of the CR method. Complex thermal and chemical preparations of samples are not required. Minimal equipment (a micro-hematocrit centrifuge) is required, it is inexpensive, and it is suitable for the analysis of multiple samples. The CR is time efficient, it can be done on small samples, and it can be used on fresh or thawed frozen milk samples. It is recommended for the fat determination of multiple samples of milk in field type studies, at a minimal cost, and in the routine analysis of milk (Jensen, Bitman, Wood, et al., 1985; Jensen, 1989). Through regression analysis with reference methods a CR value of milk fat and energy is around ± 10– 11% is obtained (Lemons, Miller, Eitzen, Strodtbeck, & Lemons, 1983 and Lucas, Gibbs, Lyster, & Baum, 1978).

The major disadvantage of this method is that it requires standardization in each different laboratory setting. In each setting the type of milk (human, bovine, or goat for example), the state of the sample (frozen, fresh, and/or pasteurized), and the technician performing the CR must be compared with an established reference method (i.e.: Roese-

Gottlieb, modified Folch) (Jensen, 1989). Standardization must be performed to establish the relationship between the measured proportion of fat (%) in the capillary tube, and the absolute lipid content, determined gravimetrically (Polberger & Lönnerdal, 1993).

Correlations of the CR method through standardization are established with several gravimetric and other reference methods ranging from $r = 0.82$ to 0.99 (Hundreiser, Clark, Jensen, & Ferris, 1984; Lucas, Gibbs, Lyster, & Baum, 1978; Meier, Murtaugh, Vasan, Meier, & Schanler, 1999; Polberger & Lönnerdal, 1993, Prentice, Prentice, & Whitehead, 1981; Wang, Chu, Mellen, & Shenai, 1999). Lucas et al. (1978) stressed the need for each worker to determine their own calibration equation or standardize the method. The relationship between the layers measured in the capillary tube and the percent fat content in milk is not consistent and therefore requires regression analysis for each set of circumstances in which it is used.

Elevated environmental temperatures and those that occur with mechanical centrifugation may influence CR results by disrupting the milk fat globule membrane. Two things could happen: the released fat would occupy less space than the intact globules or the cream layer might dislocate and fat flecks would occur throughout the milk column. Both occurrences lead to erroneous results (Jensen, 1989 & Lucas, 1983). It was not evident that standardization was performed in any of the CR studies on continuous EBM infusions (Brennan-Behm, Carlson, Meier, & Engstrom, 1994; Chan, Nohara, Chan, Curtis and Chan, 2003; Lemons, Miller, Eitzen, Strodtbeck, & Lemons, 1983; Narayanan, Singh, & Harvey, 1984; Spencer & Hull, 1981; Stocks, Davies, Allen, & Sewell, 1985). Another disadvantage is that the reading of the capillary tube milk layer may be subjective, thus less reliable, when measuring with callipers (Polberger &

Lönnerdal, 1993).

Indirect Methods.

Indirect methods use a solvent to extract fat from other milk constituents. In human milk, lipid molecules and TGs gather to form an emulsion, which is surrounded by a film, made of proteins, phospholipids, and FFAs. Triglycerides are protected by this film which is collectively called the milk fat globule membrane (MFGM). The parts of the membrane have differing polarities and are held together by electrical and chemical forces. The MFGM not only protects the TGs, it acts as a messenger, a moderator of chemical processes, and a biochemical communicator (Jensen, 1989). In order to determine the fat content of milk, the protective layer must be broken and the protein film covering it removed (Meloan, 1999).

In order to extract or isolate lipids, all other milk components (proteins, carbohydrates, water, etc.) must be removed. Once isolated, lipids can be weighed. This weight of lipids is determined as a percent of the weight of the milk sample prior to lipid extraction. Chloroform and methanol is the best solvent combination used in lipid research as it most completely extracts total lipids (Nelson, 1991) but many other organic and chemical solvents may be used. The Roese-Gottlieb method uses ammonium hydroxide, diethethyl ether, and hexane (Jensen, Lammi-Keefe, Koletzko, 1997). The Mojonnier method is like the Roese-Gottlieb except for the use of a special Mojonnier flask that hastens the separation of the solvent mixture from the aqueous ethanol layer (Jensen, 1989). The dry column method involves multiple chemicals and steps. Lipids are extracted in a glass column and, when compared to the wet methods, it is a rapid procedure.

Comparisons of Methods to Determine Total Fat Content

Hundreiser, Clark, Jensen & Ferris (1984) compared methods of total lipid content determination in human milk. They compared four common techniques to the Roese-Gottlieb method. Investigators compared the modified Folch (Clark, Ferris, Fey et al., 1982), a dry column method, a spectrophotometric/ turbidimetric method, and the CR method. All were deemed acceptable to determine milk fat content and had high correlation coefficients with the Roese-Gottlieb method ($r \geq 0.993$ - 0.977). The modified Folch, normally the first step in detailed lipid analysis, was the preferred method because of its ability to extract the most lipids.

Lönnerdal, Smith, & Keen (1984) tested the CR, sulpho-phospho-vanillin (colorimetric), and dry column procedures against the Mojonnier (Roese-Gottlieb) and obtained high correlation coefficients ($r \geq 0.977$ - 0.985) with all methods. The greatest variation was with the sulpho-phospho-vanillin method and the investigators suggested it be abandoned. Lastly, Polberger & Lönnerdal (1993) tested thirty samples of frozen human milk for macronutrient content. Their goal was to identify reliable and inexpensive methods for routine lipid analysis. The modified Folch was used as the reference method to test the total lipids assay (phosphovanillin), and CR methods. The total lipids assay had a correlation coefficient of 0.900 with the modified Folch. The CR method (including the liquid fat layer) correlated at $r = 0.870$. Both methods were inexpensive and less time consuming than the modified Folch.

All of the classic methods (Mojonnier/Roese-Gottlieb, Folch) of fat determination are "highly reliable" (Lönnerdal, Smith, & Keen, 1984, p. 292) and achieve the most complete fat extraction. The Roese-Gottlieb/Mojonnier is a recognized standard for

comparison of methods (Jensen, Lammi-Keefe, & Koletzko, 1997). The Folch method has been widely used and is recommended in research settings when studying the nutritional composition of human milk (Jensen, 1989). The Modified Mojonnier method was chosen for this study to ensure that the maximum fat was extracted with a minimal amount of measurement error in order to determine small changes in fat content.

Modified Mojonnier Method. The modified Mojonnier method is an official reference method of the Association of Official Analytical Chemists for testing fat in milk (AOAC International, 2000) and of the Standards Council of Canada (2003). The Mojonnier method is used extensively in the dairy industry. The method is very specific for ether extractable material and will extract virtually all the fat in normal milk (except free fatty acids), and nothing else (Joanna Lynch, Lab Manager, Food Science Department, Cornell University, personal communication, 2004). The limitation of this method is that it does not extract phospholipids as completely as the modified Folch (Jensen, 1989). Phosopholipids comprise about 1.0 % of total lipid content (Lammi-Keefe & Jensen, 1984). In addition, it will not extract all of the FFAs if substantial lipolysis has occurred. One way extensive lipolysis can occur is if milk is stored at –20° C (Jensen, Lammi-Keefe, & Koletzko, 1997). Lipolysis occurs when the MFGM is disrupted and TGs are released. The Mojonnier method has been modified with the addition of a third extraction and the use of NaCl to more completely extract these lipids (AOAC International, 2000; Barbano, Clark, & Dunham, 1988).

Studies of Milk Fat Loss

Several studies on milk fat loss in continuous EBM feedings have been done. Most of those studies were completed in the early 1980's with equipment and protocols

that are not currently used in the clinical setting. In addition, most of these studies used the CR method of lipid analysis without performing the recommended standardization (Jensen, 1989; Lucas, 1983). The effect this has on the results is unclear. These studies are summarized and evaluated in detail in Appendix C. Major findings are briefly described, below.

Brooke and Barley (1978) first observed and studied fat loss during continuous EBM feeding after noticing fatty residue in IV tubing and buretrols. Simulated continuous EBM infusions were conducted and energy content was determined by bomb calorimetry every one to two hours during 8 – 12 hour infusions. Energy (kcal/g) dropped at two hours of infusion, decreasing to 11% below the baseline. The tubing residuals were washed out with distilled water after the infusion was complete and the energy was 24% higher than the lowest value below baseline, illustrating that energy had been lost to the tubing system.

Spencer and Hull (1981) measured the fat content of EBM using a CR method. They also measured fat loss during simulated infusions with the use of an IVAC volumetric pump and a syringe pump placed vertically. Mean fat loss, as measured by CR, was 34% for the IVAC pump and 19% for the vertically placed syringe pump. There are few details of methodology, design, or other results from this study.

Lemons, Miller, Eitzen, Strodtbeck, and Lemons, (1983) compared refrigerated and frozen EBM samples during continuous infusions of EBM. Milk fat and calorie content was estimated using the CR method. The calorie content of fresh EBM fell from 73.9 to 60.4 kcal/100ml during a six-hour infusion. The caloric content of frozen EBM decreased from 67.4 - 57.7 kcal/100 ml during an eight-hour infusion.

Greer, McCormick, and Loker (1984) studied the effect of several variables on fat loss during continuous infusions and compared them to fat loss during bolus feeding. Fat content was measured by the Roese-Gottlieb gravimetric method. Within this research, the study of interest was the comparison of the syringe pump and a larger, roller infusion pump during simulated milk infusions. The syringe pump used a shorter tubing with a smaller filling volume than the roller pump. The effects of the infusion rate, syringe tip position, and degree of homogenization were determined for each pump. The most significant fat loss occurred at a continuous infusion rate of 1 ml/h. Maximum fat delivery was obtained using a syringe pump with the syringe tip in a vertical placement. Loss of fat ranged from 5-39% across methods. A recovery of fat during the eighth hour in the form of a high concentration fat bolus was noted when air was pumped into the tubing. Little or no fat was lost during bolus feeds.

Narayanan, Singh, and Harvey (1984) compared fat concentration determined by CR method at the end of intermittent and continuous pump feedings. Various ways of minimizing fat loss were tested using different syringe pump angles, rates, mixing of milk. These were compared to bolus feeds. The greatest lipid loss occurred with a central nozzle syringe tip in a horizontal position. The greatest lipid delivery occurred when an eccentric nozzle syringe was used and the syringe and pump were tilted upwards at an angle between 25 and 40°.

Stocks, Davies, Allen, and Sewell (1985) compared fat loss during continuous EBM and bolus feeding. Fat content was analyzed by CR. Four hours of milk was infused at varying rates (4 – 9 ml per hour) and analyzed hourly. A slower rate of infusion resulted in a greater the fat loss. Mean fat loss for bolus feeding was 17% and continuous

infusions were 34% ($p < 0.05$). An inverse relationship between infusion rate and milk fat loss was found for both feeding techniques ($r = 0.865$ and 0.922 respectively)

Lavine and Clark (1989) used a randomized block design to study the effect of short-term refrigeration on fresh EBM and the addition of breast milk fortifier on milk fat delivery in four different "diet" combinations. Syringes were kept horizontal to ensure lipid loss during the simulated three ml/hour EBM infusions. A modified Folch procedure was used to determine the lipid content. The greatest loss of lipid occurred in fresh milk (< 30 minutes from pumping) milk. More lipid was delivered in the refrigerated milk plus fortifier group [50:50], however, the fat delivered was from the fortifier and not from the EBM.

Brennan-Behm, Carlson, Meier, and Engstrom (1994) measured the differences in fat content prior to and following simulated two hour, 2.0 ml per hour EBM infusions, varied only by the type of tubing that was used. The two tubings were standard bore (5.0 ml lumen volume, length unknown) and minibore (0.6 ml lumen volume, length unknown). Thirty samples were thawed and divided into two 15.0 ml aliquots in 20 ml syringes and run at two ml/h in one of the two tubing types. Pre and post infusion CR measures were obtained. The milk that remained in the syringes was unaccounted for and this factor may have affected the post infusion CR, making it erroneously high or low depending on the orientation of the syringe. Despite methodological issues, fat content declined in both infusions but the greatest loss occurred in the large bore tubing.

The effect of IVAC infusion pumps on fat delivery was estimated by CR during EBM infusions. Pump height (18 inches above, level with, or 18 inches below infant) and syringe tip position (vertical, horizontal, and down) were crossed in this 3 x 3 factorial

design. The only significant factor was the vertical, "tip up" syringe position, which increased the amount of fat delivered. Pump height was not a factor in delivery (Coté, personal communication, unpublished data, 1999).

Recently, Chan, Nohara, Chan, Curtis, and Chan (2003) evaluated the effects of adding soy lecithin to EBM during six simulated tube feedings. The CR method was used for lipid determination. Larger Kangaroo pumps were used at infusion rates of 10–50 mls per hour during three to six hour infusions These pumps use larger, longer tubings along with a buretrol or a plastic bag within the infusion system and would not commonly be used for premature infants. Despite these limitations, researchers found that the addition of 1.0g of soy lecithin to 50 mls of milk significantly decreased the amount of fat lost in tubing when compared to no added soy and 0.5g soy. Approximately 50% of milk fat was lost in the other two groups.

In addition to the studies described, above, other examples of attempts at decreasing the amount of fat loss during continuous EBM feeds are available. Van Aerde (1998, unpublished data) saturated the inner lumens of extension tubes with 3 IU per ml heparin prior to infusions of breast milk to determine if lipid adherence was reduced in the inner lumens. Using a modified Folch method he determined that approximately 60% of milk lipid was lost in both control and treatment groups indicating that the heparin did not decrease lipid adhesion or improve fat delivery. Mehta, Hamosh, Bitman, & Wood, (1988) studied the effect of adding medium chain triglycerides (MCTs) to EBM feedings to determine if there was an effect on fat delivery. MCT oils caused a larger cumulative loss of fat when used with bolus EBM feedings. Lining the tubing with MCT oil did not enhance fat delivery during MCT fortified feedings.

Syringes. Central nozzle plastic syringes are the norm in clinical practice. The smallest size syringe that can contain the volume or milk for a 2 – 4 hour infusion is typically used. Using smaller syringes may also help to increase syringe pump accuracy at low infusion rates (Lönnqvist, 2000). Placing the tip of the syringe superior to the plunger vertically and/or at an angle of 25-40° from the horizontal, may help fat to rise and be more evenly and completely distributed (Lemons, Miller, Eitzen, Strodbeck, & Lemons, 1983; Narayanan, Singh, & Harvey, 1984; Schanler, 1995). Narayanan, Singh, & Harvey (1984) found that eccentric nozzle syringes resulted in a decreased fat loss when the syringe tip was superior to the plunger at an angle between 25 and 40°.

Factors that Affect the Fat Content of Milk

Several other factors can influence the lipid content of milk before it is fed to infants. When designing this study these factors were considered and controlled. Factors that influence fat or lipid content considered in the design of this study are summarized in Appendix D. Some factors have been discussed: how mother's milk varies in fat content, how EBM behaves when it is not pasteurized, and that fat loss is well documented among continuous EF delivery systems. In addition, the following factors are briefly explored to illustrate how they affect the fat content of EBM or are relevant to clinical practice.

Fortification of Breast Milk. In the 1980's and 1990's commercially available human milk fortifiers were developed and quickly became the standard of care for LBW infants (Greer, 2001). Human milk fortifiers in powder or liquid form enhance the nutrient and calorie content of EBM with the addition of fat, carbohydrate, protein, calcium, phosphorous, minerals, and vitamins (Sapsford, 2000; Schanler, 2001). Fortifier can increase the fat content of EBM by 1.0g per 100 mls of milk (Mead Johnson

Nutritionals, 2004).

Fortifier may be added to EBM at or near the establishment of full volume feeds, when EF tolerance is established (Atkinson, 2000; Nutrition Committee, Canadian Pediatric Society, 1995, 2000; Yu, 1999). Alternatively, EBM may be fortified earlier, when infants are tolerating 90-100 ml/kg/d of oral feeding (Moody, Schanler, Lau, Shulman, 2000; Pereira, 1995; Schanler, Shulman & Lau, 1999; Yu, 1999). Even though premature infants now commonly have their EBM fortified, if possible, it is important to remember that they may not reach the appropriate EF volumes quickly, tolerate EBM fortification, or switch from bolus to continuous EF. During times of intolerance, fortification may be withheld and the infant is vulnerable to energy and nutrient loss during low rate EBM feeding. While the nutritional goals for many premature infants will require the use of fortified breast milk, unfortified breast milk is commonly used in many circumstances. Given this consideration, EBM used in this study is not fortified.

Pasteurization and Sterilization

Pasteurizing is the heating of EBM to kill most microorganisms to alter bacterial growth. Sterilization kills all microorganisms and involves heating to higher temperatures and for greater lengths of time. Bovine and human milk in milk banks is routinely pasteurized. Pasteurizing does not affect the milk fat, either qualitatively or quantitatively (Fidler, Sauerwald, Koletzko, & Demmelmair, 1998) but it does alter nitrogen retention, fat absorption, and water-soluble vitamin concentration (Jensen, 1989 and Yu, 1999). Antimicrobial factors such as leucocytes and immunoglobulins are destroyed. To preserve the aforementioned factors, routine heating and pasteurizing of milk for neonates is neither advised (Meier & Brown, 1996; Yu, 1999) nor practiced routinely in

NICUs. Sterilization is not recommended because it alters the composition of the milk and decreases the fat content by more than 10% (Fidler, et al.1998; Jensen, 1989).

EBM can be at room temperature (25° C) for four hours, without risking harmful bacterial growth (Evanochko, 1995; Hamosh, Ellis, Pollock, Henderson, & Hamosh, 1996; Lemons, Miller, Eitzen, Strodtbeck, & Lemons, 1983; Meier & Brown, 1996). In the current study a four-hour infusion duration was chosen for this reason. In the current study setting syringes that contain unfortified EBM are left at room temperature for a maximum of four hours. The interval at which extension tubing is changed varies from every 3- 4 hours (with the syringes) to every 24 hours (see Appendix A).

Storage of EBM

After collection, breast milk can be safely stored in a refrigerator (3-4 °C) for up to 24-48 hours during which time bacterial growth and the rate of lipid breakdown is slowed (Lemons, Miller, Eitzen, Strodtbeck, & Lemons, 1983). If milk is to be stored longer than this, it must be frozen.

Freezing and thawing disrupt the protein portion of the membrane binding the MFG resulting in a small but significant decrease in fat content (Silprasert, Dejsari, Keawvichit, & Amatayakul, 1986; Wang, Chu, Mellen, & Shenai, 1999). Cyclical freezing and thawing has been shown to progressively reduce fat content with each cycle of freeze/thaw and is not recommended (Bitman, Wood, Mehta, Hamosh, & Hamosh, 1983; Jensen and Clark, 1984; Sliprasert et al., 1986; Wardell, Hill, D'Souza, 1981). EBM can be frozen for up to six months at -20°C without significantly altering nutritional composition or bacterial colonization (Hamosh, Ellis, Pollock, Henderson, & Hamosh, 1996) but greater lipolysis that alters the FFA composition has been noted when milk is

stored at -20°C rather than at -70° C (Bitman, et al., 1983; Jensen & Clark, 1984). To protect the integrity of the MFGMs in the current study the effects of repeated freezing and thawing were avoided. Only fresh, refrigerated EBM, less than 12 hours old, was used for all 15 experimental runs. To avoid the lipolytic effects of storage at -20°C, milk was stored at -70° C until it was taken to the laboratory for analysis.

Glass containers or plastic containers are recommended for storing EBM as they do not appear to affect milk adversely and minimize the adhesion of live cells (Garza, Johnson, Harrist, & Nichols, 1982; Hopkinson, Garza & Asquith, 1990). Milk for laboratory sampling should be stored in polypropylene or glass containers with Teflon liners to best maintain the integrity of the sample (Jensen, Bitman, Wood, et al., 1985). Baseline or pre infusion samples were frozen in the sterile water bottles to prevent potential fat loss during sample transfer from one container to another. Post-infusion samples were collected in glass containers with Teflon lining.

Warming and Mixing EBM

Using a microwave oven to warm breast milk is not recommended as important immunological factors are destroyed (Lemmons, 2001). Frozen EBM can be slowly warmed to room (25°C) or body temperature (35 - 37°C) in the refrigerator or in a warm water bath (Kubit, 2000). Prolonged heating induces lipolysis in milk. For research purposes, a warmer milk temperature (38°C) allows for better mixing of milk and prevents fat globules from adhering to container surfaces. This provides a more homogeneous sample and enhances the accuracy of analysis (Jensen, Bitman, Wood, et al., 1985). In the experimentation phase of this study, fresh refrigerated milk was slowly warmed to 38°C, shaken by hand, and then aliquots were taken for each run. For lipid

analysis milk samples were thawed in the refrigerator over night, slowly warmed to 38°C, and well mixed prior to testing.

Infusion Devices

Infusion Pumps. Today, common practice is to use a syringe pump in continuous EF infusion systems. Syringe pumps normally require shorter tubing, a variety of syringes can be used with one pump, and they are versatile in their placement and orientation. The accuracy of syringe pumps has come into question, predominantly related to the height variations during low rate infusions of potent drugs and misdelivered fluid volumes (Lőnnqvist, 2000). By using a smaller syringe and more compliant tubing the accuracy of syringe pump infusions are increased (Lőnnqvist, 2000). For continuous EBM delivery the syringe tip should be oriented in an upright position to assist in a more complete delivery of milk fat (Schanler, 1995). Another common EF pump is the Kangaroo Pump, which uses a roller mechanism to infuse milk. This pump is used when larger volumes and faster feeding rates are required and was not considered for this study.

Tubing and Syringes. Lipids have been shown to remain inside of continuous infusion tubings. Lee (1971) tested tubing made of polyethylene, silicon rubber and polyvinyl chloride for lipid loss. Results indicated that large proportions of lipid were lost to the walls of the silicone rubber and polyvinyl chloride tubes with very little lipid being lost to the polyethylene tubing. Most of the tubing used within current EF infusion systems is made of polyvinyl chloride (Macklin, 2001). There is little choice in the clinical setting with respect to tubing composition.

The size of intravenous tubing is differentiated by its length and priming volume. If a diameter measurement is specified, it is the outer diameter. The inner diameter of the

tubing is then determined by the thickness of the plastic tubing (Macklin, 2001). In the clinical setting economy, availability, and safety dictate the type of tubing used. A shorter length of tubing is recommended but in clinical practice, tubing length is variable. (Narayanan, Singh, & Harvey, 1984; Schanler, 1995). For this study, tubing common to clinical practice was chosen, as identified in the internet survey (Appendix A).

Jones (1986) stresses, for safety reasons, that enteral infusion devices should not be compatible with IV infusion devices. Enteral feedings have, unfortunately been connected to intravenous infusions (Ferraro & Huddleston, 1991; J.B.Lippincott Co., 1993; Karl, Ulincny, & Korelitz, 1989; Wood, Creekmore, Green, Huddleston, & Dubik, 1993). The enteral only tubing used in this study has a bright orange stripe along the length of the tubing. The tip is incompatible with IV tubing and is orange. There is also a tag attached to the tubing that says "enteral only".

Conclusion

The literature identifies a number of variables that must be taken into account when feeding premature infants. Good nutrition is important to premature infants but its delivery is precarious. Infants are fed by gavage feeding until they develop the appropriate age related reflexes and strength for oral feeding. Continuous EBM feedings are often given during this time. Fat loss during these feedings can result in energy loss to the degree that growth may be affected. Enteral feeding delivery systems have been studied and fat loss documented. Clear practice guidelines have not emerged. There are several ways that the fat content of EBM can be measured, each with its own purpose, advantages and disadvantages.

Even with its varying fat content and nutritional limitations, EBM is the food of

choice for premature infants. It is important that current delivery methods and equipment are examined for their efficiency in delivering EBM, particularly fat, for the population most vulnerable to the loss of fat. This study will examine which of three extension tubing types currently used within delivery systems in NICUs is the most efficacious in delivering milk fat during a simulated four hour, four ml per hour infusion of freshly expressed breast milk.

Chapter Three

Method

Design

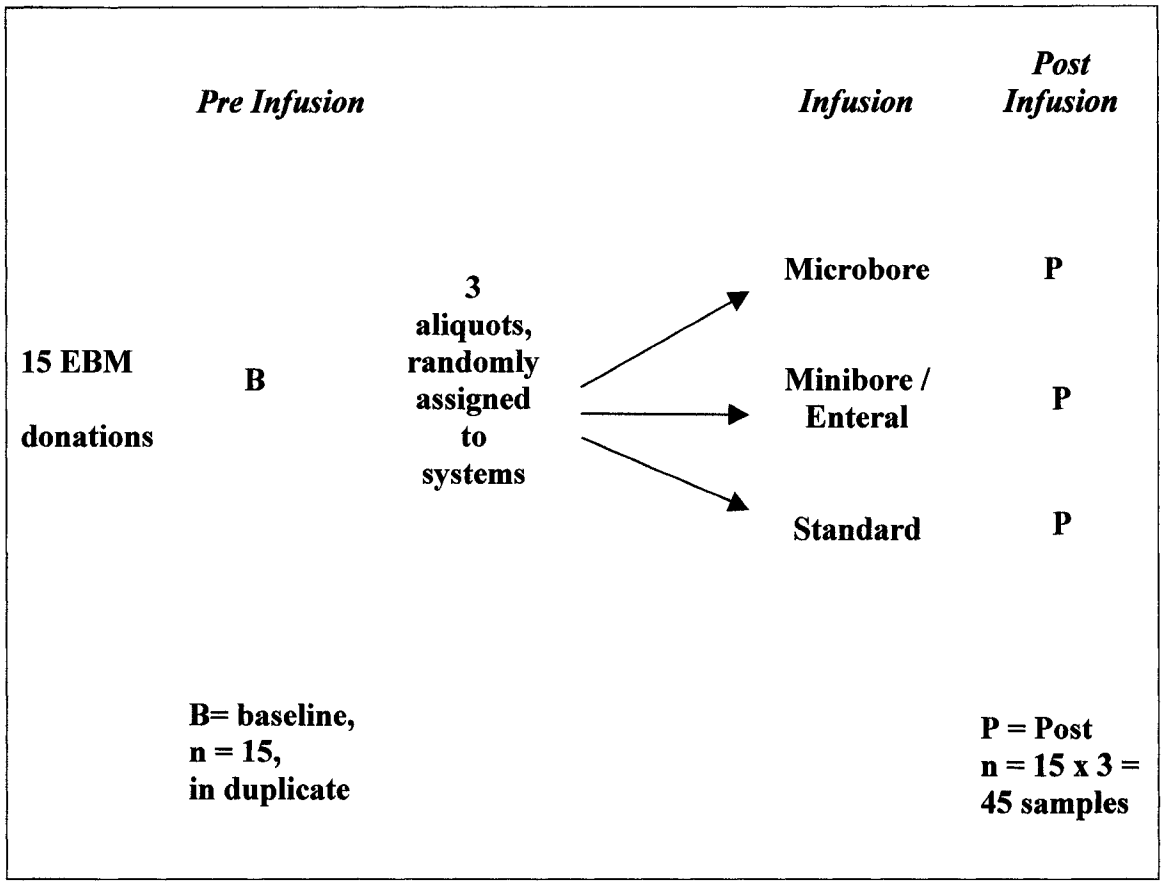
An experimental, three-group design with pre and post measures was used to determine which type of tubing in an infusion system delivered the greatest lipid content. Each system varied by the type of extension tubing used: system M used microbore tubing, system E used minibore or enteral only tubing, and system S used standard bore tubing. The microbore tubing (Baxter, #JC9201) has a length of 152 cm and a priming volume of 0.4 mls. The minibore or enteral only tubing (Children's Medical Ventures® #95017-A) has a length of 152 cm and a priming volume of 2.0 mls. The standard bore tubing (Baxter # 2C6223) has a length of 121 cm and a priming volume of 5.5 mls.

The study design controlled for a number of variables identified in the review of literature. To review, fresh, refrigerated EBM was used for the simulated infusions to avoid the effects of repeated freezing and thawing (Jensen & Clark, 1984). Milk was refrigerated immediately after collection and transported in a cooler, with ice, to the test setting. EBM was used within 12 hours of expression and no fortifier or supplement was added. A 20 ml BD syringe was used with all infusion systems. All infusions were completed with the syringe tip vertical or pointed up, a position associated with greater fat delivery (Greer, McCormick, & Loker, 1984). The Medfusion 2001 (Medex Inc., Dublin, Ohio) syringe pump was used to infuse EBM at 4.0 ml/hr for all systems. This rate was used for testing purposes as it is commonly used with the premature infant population and is associated with fat loss (Greer, McCormick, & Loker, 1984; Stocks, Davies, Allen, & Sewell, 1985). All syringes were completely emptied each time. Study

samples were frozen at -70°C immediately after the trial infusions until they were taken to the laboratory for analysis. See Figure 1 for a diagram of the study.

Figure 1.

Study Design



Sample

Sample Size. Previous studies of milk fat loss in continuous EBM infusions have shown large differences in the mean milk lipid concentrations before and after simulated infusions. None of the studies on fat loss in continuous EBM infusions identified an effect size (ES). The study by Brennan-Behm, Carlson, Meier, & Engstrom (1994) was

evaluated and I calculated the ES (mean difference / pooled standard deviation) based on their reported findings. The ES for the minibore tubing was 0.69 and the ES for the standard bore tubing was 1.0. According to Cohen's ESs for t-tests (1988, p. 25 - 26), these ESs would be considered large. Assuming a large ES for three groups (ES = 0.5), an alpha of 0.05, and a power of 0.8 the sample size calculated for this study, was 15 per group (Cohen, 1988, pg. 313).

Ethical Clearance and Consideration

Ethical clearance for donation of EBM was obtained from the University of Alberta Health Research Ethics Board, Panel A (Appendix E). Administrative approval was obtained by the Capital Health Authority (Appendix F).

Donated EBM Procurement. Fresh EBM was obtained by voluntary donation. The NICU lactation consultant determined if the mother's milk supply was adequate, independent of the researcher, after which consent was obtained. Mothers who did not have an adequate milk supply or those who had other health challenges that affect milk supply were excluded from donation. EBM samples were used for study purposes only. There were no risks to the mother or her infant and nutrition was not withheld from an infant. Infant satiety and comfort along with mothers' milk supply was monitored during the course of the study. This study was a simulation experiment and no human subjects were used. Anonymity and confidentiality measures were taken. Expressed and donated mother's milk was imprinted with "study only" and without personal information.

A poster was placed in the family lounge of the NICU at the Royal Alexandra Hospital (Appendix G). Contact numbers for the primary investigator were on the poster. More than one mother volunteered but one mother had an ample supply of milk and was

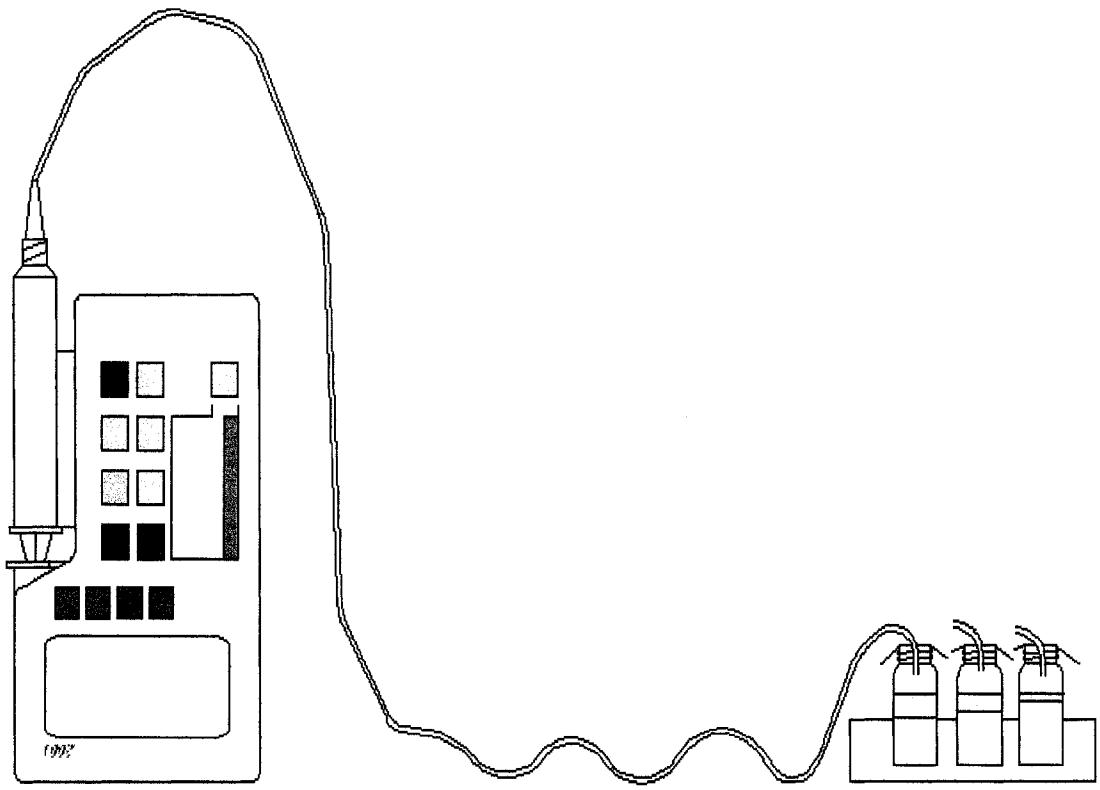
able to donate the milk for the entire study. The researcher contacted interested mothers and the purpose of the study along with the mother's role was explained. An information letter was given to the mother for review before consent was obtained. Consent was obtained on appropriate letterhead and an example is in Appendix H.

Procedure for EBM Donation. Milk was procured between February 1, 2004 and February 28th, 2004. The donor mother was instructed on how much milk to express into the glass collection container, how to store it, and to contact the researcher if her milk supply lessened. Fifteen samples of EBM were collected and each one was used for the study. Milk was expressed with an electric pump most of the time with the occasional use of a hand pump. Milk samples were placed into 90 ml glass sterile water bottles. With overfill approximately 100 mls fit into the bottle. Samples were refrigerated, collected, and transported to the testing site in a hand held cooler with ice.

Setting and Experimental Set Up. The experiment was conducted in a conference room adjacent to the David Schiff NICU at the Stollery Children's Hospital, University of Alberta. The set up was designed to be similar to the infusion set-ups in the NICU. Pumps were placed on the counter, approximately three feet high, with their tips vertical. Tubing was uncoiled and stretched horizontally across the counter; tips were placed into the collection bottles, covered with saran wrap, and secured with tape. See Figure 2 for a diagram of the experimental set up. Each EBM sample was collected, divided, and infused over four hours, at a rate of four mls per hour in a simulated environment using a strict protocol (see procedure, page 36).

Figure 2.

Equipment set up for Simulated Continuous Infusions



Medfusion 2001 (Medex Inc., Dublin Ohio) syringe pumps were used in this study. The Royal Alexandra Hospital NICU, the Stollery NICU, and the Stollery NICU Transport Team donated pumps for research use. This pump is 4.5" wide x 3.0" high x 7.5" long and weighs 2.5 pounds. It infuses at a rate as low as 0.01 ml/h to 378 ml/h depending on the syringe selected. Infusion rates can be adjusted in increments of 0.1 ml per hour. Error is ± 3% (Ardus Medical, 2002).

Procedure for Collection of Samples and Simulated Infusion

The subsequent procedure was followed without exception for all runs. After EBM collection and transport to the testing room, all of the required equipment (syringes, tubing, pumps, tape, collection vials, etc.) was gathered, examined for defect, and organized for use. The pumps were prepared for an infusion of 16 mls over four hours (a rate of four mls per hour), and then placed on standby for 30 minutes. Aliquots were randomized in the following manner. Three slips of paper with the letters ***M***, ***S***, and ***E*** (microbore, standard bore, and enteral only) written on them were shuffled in a way that prevented the researcher from seeing the letters. With the letters hidden from the researcher, the numbers 1, 2, and 3 were written on the backs of the paper. A pen dropped on a random number table was then used to assign a sequence to the three tubing types.

While the equipment was prepared, milk was slowly warmed to 38 ± 2° C (Jensen, 1989) in a warm water bath. During warming, the EBM was inverted in its glass container for examination and to aid mixing. Before allocating milk to the three tubing types, the sample was shaken by hand for 30 seconds. Milk was visually inspected for homogeneity; an absence of fat adhering to the bottle walls or lid before and after warming and mixing. From the large single donated sample the three aliquots were aspirated into 20 ml syringes, determined by the randomization. Care was taken to gently remove bubbles from the syringes and to avoid spilling or wasting any EBM. Volumes of milk were aspirated for each system using the syringe for measurement. System M required 16.4 mls of EBM for one run: 0.4 mls to prime the tubing plus 16 mls for the four hours of infusion at 4.0 mls per hour. System E required 18 mls and system S required 21.5 mls. Each aliquot of milk was systematically connected to the

corresponding tubing then carefully placed in the pump plunger. Milk was infused through the tubing to the tip using the pump's "prime" function. Sequentially, the ends of the tubing were then placed into the top of the glass collection containers, covered with plastic wrap, and secured with tape. The container was placed in a styrofoam holder to avoid inadvertent movement and to prevent spillage. When the infusion was started the time was noted in a log. When all three infusions were in process the remaining milk from the large sample was labelled and immediately placed in the back of a -20° C freezer in the NICU, reserved for EBM storage. At hourly intervals the infusion rates, the system set up, the cumulative amounts on the pump screen, and the amounts remaining in each of the three syringes were checked and recorded. After a completed four hour or 16.0 ml infusion the pumps would stop their pumping and alarm "infusion complete". The time was noted and the pumps were restarted by hitting the "deliver" button, as there was residual milk in the syringes. They continued to infuse at 4.0 mls/hour until the residual that was in the syringes was delivered and the pump rang "occlusion'. The volumes of these residuals ranged from 0.01 – 0.6 mls. On two occasions only 15.96 mls of milk was infused because the syringes were emptied early.

When the syringes were completely empty, the tubing was carefully removed from the glass container and sealed with the appropriately labelled lid. The container was labelled with a corresponding sticker. They were placed in a different styrofoam holder in the hand held cooler, with ice and placed in the freezer with the then frozen baseline sample. After cleaning the workspace and returning all equipment, the samples were immediately transported to the Perinatal Research Centre Laboratory freezer at the Royal Alexandra Hospital. There, all four samples were placed in a plastic zip lock bag labelled

with the run number and date. They were stored at -70°C until they were transferred to the laboratory for analysis. The used syringes and tubing were disposed of in the appropriate Biohazard container in the NICU.

Method Integrity

In order to minimize random error the preceding procedure was carefully constructed and followed with each simulated infusion. Two test runs were completed with frozen breast milk prior to the initiation of the study runs. An observer familiar with the continuous feeding protocol was present for two runs to ensure that the procedure was followed and that the researcher was systematic. Measurements of aliquots and samples were double checked, visually, by the researcher for accuracy.

The Medfusion 2001 syringe pump had several features that enabled consistent treatment of the infusion systems. The priming function provided a consistent rate when each tubing was primed. Each pump has a "volume over time" function that eliminated the use of stop clocks, watches, etc. but a travel alarm clock was used to monitor hourly checks of the milk. Each pump was programmed identically each time. The Medfusion 2001 pumps were supplied to the primary investigator were subject to hospital standards of maintenance. The majority of the study runs (80%) were done with the pumps from the Neonatal Transport Team.

A consistent labelling system was used for the study. Syringe pumps were labelled with the letter that corresponded to their tubing type and this was recorded in a logbook. This logbook contained a copy of the experimental protocol. A table was generated to allow the investigator to record information about each run. For example, the date, start and end time, hourly syringe volume checks, pre and post infusion times,

the hourly total volumes infused for each pump, any notable visual characteristics of the milk, the set-up, and the room temperature.

Lipid Analysis by the Laboratory

Frozen EBM samples were transported to the Centre for Milk Testing in Edmonton, Alberta, on ice in a cooler. The lab is accredited for milk testing, specifically the Mojonnier method, by the Standards Council of Canada (Standards Council of Canada, 2003; Marshall, 1993). Four runs or 16 samples could be analyzed during one week. Sample analysis was complete in March 2004.

Chapter Four

Results

Fifteen EBM samples of 90 – 100 mls each were obtained for the study from one volunteer breastfeeding mother of a term infant. Fifteen simulated infusions were completed yielding 60 samples and 75 results as the baseline samples were analyzed in duplicate. Milk was analyzed gravimetrically for lipid content at the Centre for Milk Testing (Edmonton, AB) using a modified Mojonnier method (Marshall, 1993). Results were reported in percent (weight of fat in kg / weight of milk in kg). All milk samples were included in the results. On occasions where the duplicate results were greater than 0.03% three or four analyses were done. The result nearest to the first determination was selected as the duplicate result. The results are found in Table 2. Data were analyzed using SPSS for Windows (Version 11.5).

Mean Fat Delivery

The mean pre-infusion fat content was 4.489% ± 1.508 (range 2.145– 7.950). The mean post infusion fat content for microbore tubing was 4.492% ± 1.338 (range 2.194 - 6.907). The mean for enteral bore was 3.529 % ± 1.456 (range 1.091 - 6.527) and the mean post infusion standard bore tubing was 1.301 % ± 0.785 (range 0.264 - 2.328) respectively. Figure 3 offers a graphic summary of these results.

Table 2.

Lipid Content by Modified Mojonnier Method

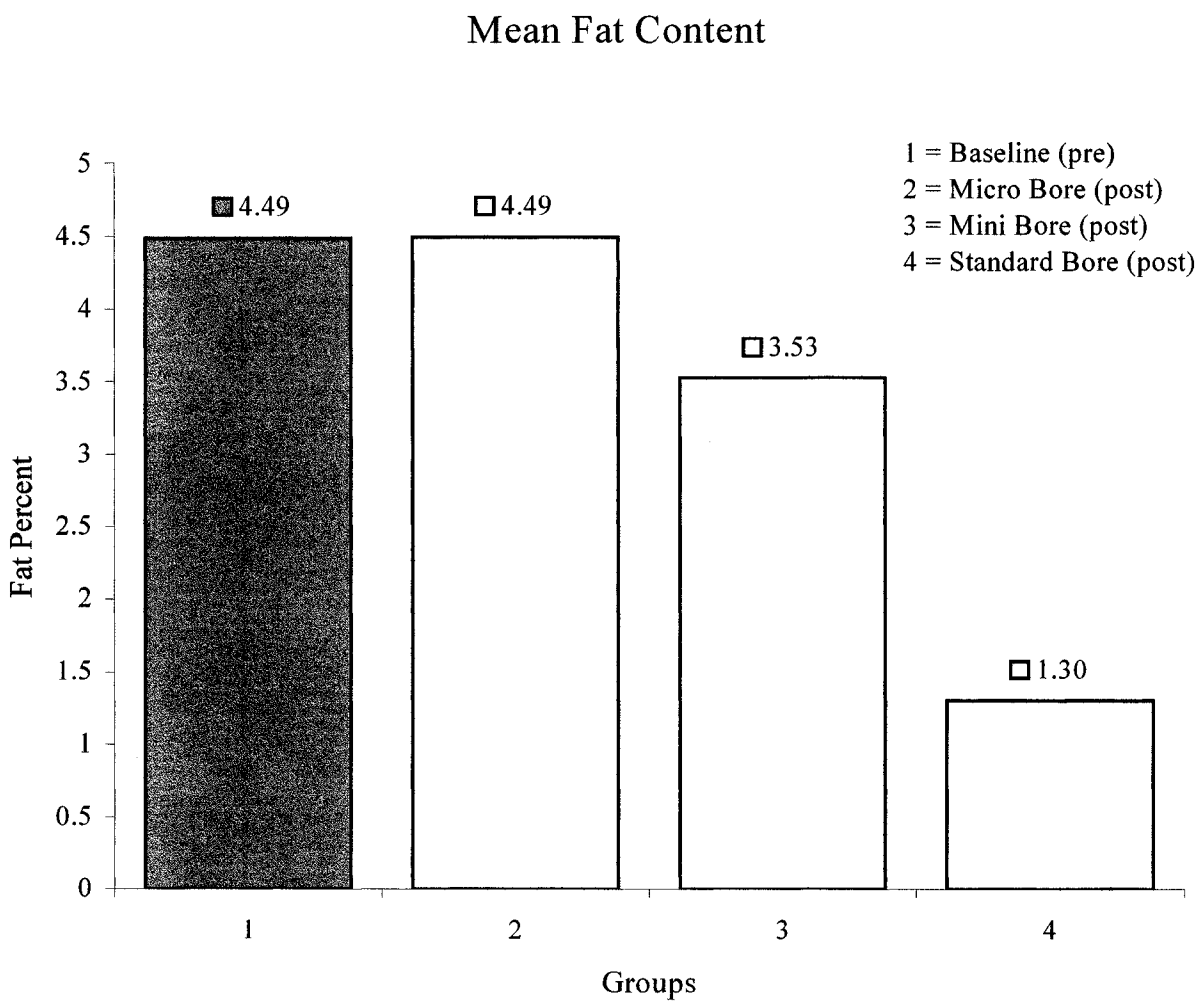
Milk fat content (%)

	Pre simulated infusion (% Fat)			Post simulated infusion (% Fat)			
	Baseline	**Baseline duplicate**	**Differ-ence**	**Baseline mean**	**Micro-bore**	**Mini-bore**	**Standard Bore**
Run							
1	7.903	8.001	0.098	7.952	6.907	6.527	1.882
2	4.679	4.747	0.068	4.713	*4.827	4.152	1.953
3	4.801	4.742	0.059	4.772	*4.844	3.882	2.120
4	5.139	5.185	0.046	5.162	*5.250	4.280	1.733
5	2.798	2.794	0.004	2.796	*2.888	1.779	0.475
6	4.475	4.485	0.010	4.480	*4.625	3.552	0.830
7	4.829	4.827	0.002	4.828	*5.018	4.065	2.328
8	2.269	2.270	0.001	2.270	*2.371	1.534	0.264
9	3.339	3.332	0.007	3.336	*3.452	2.363	0.464
10	4.208	4.294	0.086	4.251	*4.299	3.195	2.234
11	2.160	2.130	0.030	2.145	*2.194	1.091	0.261
12	6.314	6.267	0.047	6.291	*6.307	5.238	1.691
13	5.100	5.039	0.061	5.070	*5.149	4.243	0.914
14	5.302	5.288	0.014	5.295	*5.302	4.361	1.894
15	3.958	3.988	0.030	3.973	3.953	2.669	0.479
Mean			0.038	4.489	4.492	3.529	1.301
Min				2.145	2.194	1.091	0.264
Max				7.950	6.907	6.527	2.328
S.D.				1.508	1.338	1.456	0.785

** Indicates where percentage fat equalled or rose above the baseline content.*

Figure 3.

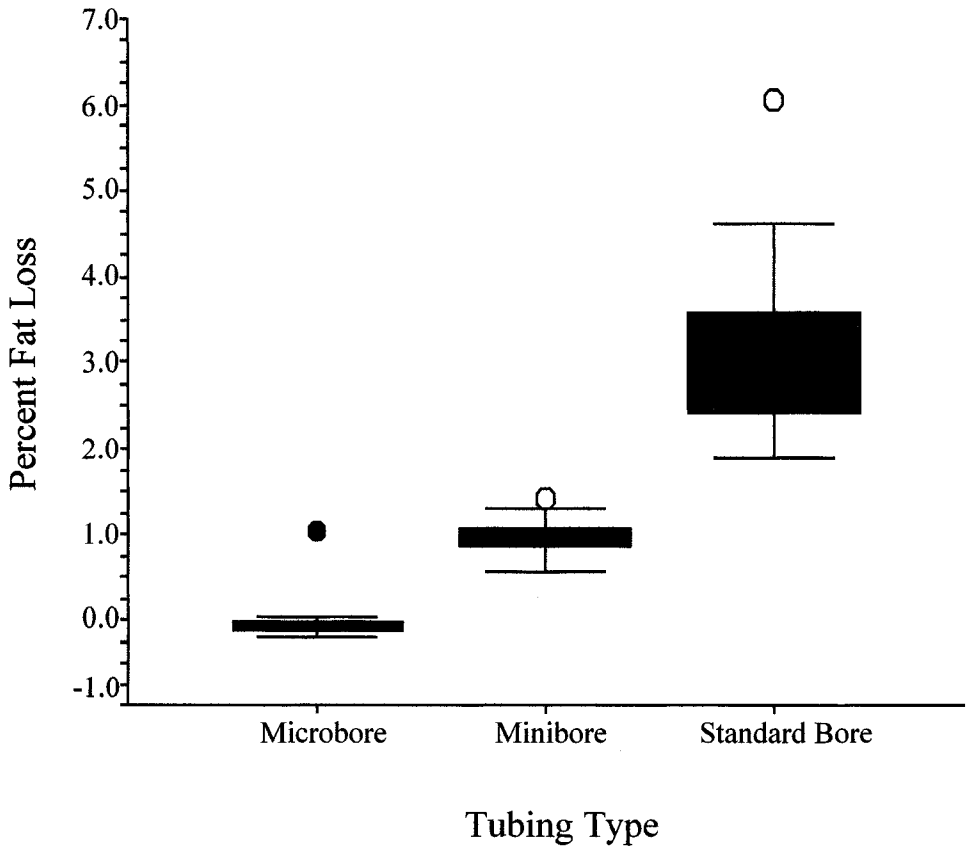
Mean Fat Content of Baseline and Post Infusion Samples



The microbore tubing generally delivered all of the milk fat to infants and, therefore, the caloric density of EBM was maintained. The minibore tubing delivered a mean of 79% of milk fat compared to the pre infusion content, decreasing the caloric density from approximately 20 kcals per ounce to around 17.9 kcals per ounce. The standard bore tubing delivered a mean of 29% milk fat. The fat loss during delivery decreased the caloric density from 20 kcals per ounce to around 12.9 kcals per ounce. See Figure 4.

Figure 4.

Mean Fat Loss During Continuous Infusions



*Circles denote outliers and extreme values

The laboratory reported the lipid content of milk in percent (w/w or kg/kg) to three decimal points. Baseline samples were analyzed in duplicate to establish reliability of the Mojonnier method. The mean of the two results was used as the baseline value. Differences between the duplicate results were also noted. The three post analysis samples were analyzed once only due to the limited volume of milk available. The post infusion values were subtracted from the baseline value for each individual run. The differences constituted the dependent values. Data were entered into SPSS. Categorical codes were assigned to each tubing group. Group 1 was the microbore tubing, group 2 was the minibore or enteral only tubing, and group 3 was the standard bore tubing (Tables 3, 4, and 5).

Table 3.

Fat Content at Baseline and Post Infusion: Group 1

Baseline or Preinfusion (Fat % or kg/kg)				Microbore or Postinfusion (Fat % or kg/kg)		
Run	**Sample 1**	**Sample 2**	**Mean**	**Pre**	**Post**	**Difference**
1	7.903	8.001	7.9520	7.9520	6.907	1.0450
2	4.679	4.747	4.7130	4.7130	4.827	-0.1140
3	4.801	4.742	4.7715	4.7715	4.844	-0.0725
4	5.139	5.185	5.1620	5.1620	5.250	-0.0880
5	2.798	2.794	2.7960	2.7960	2.888	-0.0920
6	4.475	4.485	4.4800	4.4800	4.625	-0.1450
7	4.829	4.827	4.8280	4.8280	5.018	-0.1900
8	2.269	2.270	2.2695	2.2695	2.371	-0.1015
9	3.339	3.332	3.3355	3.3355	3.452	-0.1165
10	4.208	4.294	4.2510	4.2510	4.299	-0.0480
11	2.160	2.13	2.1450	2.1450	2.194	-0.0490
12	6.314	6.267	6.2905	6.2905	6.307	-0.0165
13	5.100	5.039	5.0695	5.0695	5.149	-0.0795
14	5.302	5.288	5.2950	5.2950	5.302	-0.0070
15	3.958	3.988	3.9730	3.9730	3.953	0.0200

Table 4.

Fat Content at Baseline and Post Infusion: Group 2

Baseline or Preinfusion (Fat % or kg/kg)				Minibore or Postinfusion (Fat % or kg/kg)		
Run	**Sample 1**	**Sample 2**	**Mean**	**Pre**	**Post**	**Difference**
1	7.903	8.001	7.9520	7.9520	6.527	1.4250
2	4.679	4.747	4.7130	4.7130	4.152	0.5610
3	4.801	4.742	4.7715	4.7715	3.882	0.8895
4	5.139	5.185	5.1620	5.1620	4.280	0.8820
5	2.798	2.794	2.7960	2.7960	1.779	1.0170
6	4.475	4.485	4.4800	4.4800	3.552	0.9280
7	4.829	4.827	4.8280	4.8280	4.065	0.7630
8	2.269	2.270	2.2695	2.2695	1.534	0.7355
9	3.339	3.332	3.3355	3.3355	2.363	0.9725
10	4.208	4.294	4.2510	4.2510	3.195	1.0560
11	2.160	2.13	2.1450	2.1450	1.091	1.0540
12	6.314	6.267	6.2905	6.2905	5.238	1.0525
13	5.100	5.039	5.0695	5.0695	4.243	0.8265
14	5.302	5.288	5.2950	5.2950	4.361	0.9340
15	3.958	3.988	3.9730	3.9730	2.669	1.3040

Table 5.

Fat Content at Baseline and Post Infusion: Group 3

Baseline or Preinfusion (Fat % or kg/kg)				Standard bore or Postinfusion (Fat % or kg/kg)		
Run	**Sample 1**	**Sample 2**	**Mean**	**Pre**	**Post**	**Difference**
1	7.903	8.001	7.9520	7.9520	1.882	6.0700
2	4.679	4.747	4.7130	4.7130	1.953	2.7600
3	4.801	4.742	4.7715	4.7715	2.120	2.6515
4	5.139	5.185	5.1620	5.1620	1.733	3.4290
5	2.798	2.794	2.7960	2.7960	0.475	2.3210
6	4.475	4.485	4.4800	4.4800	0.830	3.6500
7	4.829	4.827	4.8280	4.8280	2.328	2.5000
8	2.269	2.270	2.2695	2.2695	0.264	2.0055
9	3.339	3.332	3.3355	3.3355	0.464	2.8715
10	4.208	4.294	4.2510	4.2510	2.234	2.0170
11	2.160	2.13	2.1450	2.1450	0.261	1.8840
12	6.314	6.267	6.2905	6.2905	1.691	4.5995
13	5.100	5.039	5.0695	5.0695	0.914	4.1555
14	5.302	5.288	5.2950	5.2950	1.894	3.4010
15	3.958	3.988	3.9730	3.9730	0.479	3.4940

Data Analysis

The Levene test was used to determine whether the data met the homogeneity of variance assumption required for analysis of variance. Due to the homogeneity of variance violation (Levene Test, 13.666, $p < 0.001$), a non-parametric Kruskall-Wallis test was used to analyze change in percent fat among microbore, minibore, and standard bore infusion tubings. Post-hoc pair wise comparisons were done to determine the significance of the rank differences between the three types of tubing.

The non-parametric Kruskall-Wallis test was used to analyze change in percent fat among microbore, minibore, and standard bore infusion tubings. The results of the test indicate that the difference between the groups was statistically significant, χ^2 (3, N = 45) = 37.469, $p < 0.001$.

The mean ranks for the different infusion tubings were: 1) microbore = 8.67; 2) minibore = 22.33; and, 3) standard bore = 38.00. To determine the significance of the rank differences between the three types of tubings, post hoc pair- wise comparisons were completed. To test the significance of differences in % fat content between tubings, a critical difference value of 18.87 was calculated using the following formula:

$$\text{Critical Difference}_{0.05} = = 2.394\sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}$$

Where

N is the total number of subjects in the experiment

n_1 is the number of subjects in one group

n_2 is the number of subjects in the other group

If a difference between a pair of Rank Means is greater than or equal to the

critifcal difference (the right hand side of this equation), then they are significantly different at $p < 0.05$. The differences between the mean ranks were:

1) microbore vs. minibore = 13.66, $p > 0.05$.
2) microbore vs. standard bore = 29.33, $p < 0.05$.
3) minibore vs. standard bore = 15.67, $p > 0.05$.

Only the comparison of the microbore and the standard bore tubing was statistically significant, $p < 0.05$ indicating the difference in fat loss between the two tubing types is significant.

The null hypothesis that there was no difference in the amount of fat delivered by microbore, minibore and standard bore tubing during a four hour, four ml per hour simulated continuous EBM infusion can be rejected.

Chapter Five

Discussion

The purpose of this study was to determine which of three tubing types delivered the most milk fat during a simulate four hour, four ml per hour EBM infusion. There was no significant difference between pre and post measures for microbore tubing. The minibore tubing delivered an average of 79% of the pre infusion fat. The standard bore tubing delivered the least amount of milk fat when compared to the pre infusion sample – only an average of 29%.

Clinical Significance

Assuming EBM has an average of 20 kcals/oz the implications of the fat loss on calories can be determined. Little or no fat was lost within the microbore tubing used in this study. In terms of caloric delivery, all 20 kcals/oz were delivered. The minibore or enteral only tubing delivered an average of 17.9 kcals/oz and the standard bore tubing an average of 12.9 kcals/oz. For the hypothetical 1.0 kg infant described in the introduction (page 4) the 21% fat loss (79% delivery) from the minibore tubing over 24 hours would equal roughly seven kcals. While this was found not to be statistically significant it may warrant clinical consideration. Referring to the energy requirements and expenditure of the premature infant (page 3 - 4) a seven kcal loss would be 6% of the energy requirement for the 24-hour period. Of the kcals required for growth and storage (approximately 25 per kg, per day), seven kcal amounts to 28% of this energy. Energy intake is compromised the infant would, theoretically, receive around 2/3 of the requirement for growth or storage.

For this same 1.0 kg infant a 71% loss (29% delivery) of the fat from EBM is

statistically and clinically significant. Approximately 23 kcals would be lost to the infant over a 24 hour period. Kilocalories used for growth and energy storage are almost completely deficient and the infant would experience sub optimal growth.

Milk Fat in Continuous Infusions

There have been several studies that have examined the issue of fat loss in EBM delivery (Brennan-Behm, Carlson, Meier, & Engstrom, 1994; Brooke & Barley, 1978; Chan, Nohara, Chan, Curtis, & Chan, 2003; Coté, 1999, unpublished; Greer, McCormick, & Loker, 1984; Lavine & Clark, 1989; Lemons, Miller, Strodtbeck, & Lemons, 1983; Narayanan, Singh, & Harvey, 1984; Spencer & Hull, 1978; Stocks, Davies, Allen, & Sewell, 1985; Van Aerde, 1999, unpublished). The results from the current investigation support the aforementioned studies though several important practice and measurement issues limit the applicability of the previous findings into today's practice environment. As indicated, many of these studies were done at a time when clinical practice was markedly different. Previously, volumetric infusion pumps with buretrols, cassettes, and lengthy, large bore tubings were used. Several studies failed to specifically describe the types of tubing used and how the pump, tubing, and collection receptacles were placed in relation to each other. Two studies are similar to the current study in their methodology and design and warrant comparison. Key points of comparison are summarized in Table 6.

Table 6.

Design Comparison for Knuth, Greer et al. and Brennan-Behn et al.

	Knuth (2004)	Greer, McCormick, & Loker (1984)	Brennan-Behm, Carlson, Meier, & Engstrom (1994)
Question Asked	Which tubing during a four ml per hour, four hour continuous infusion delivers the most milk fat?	Are there fat losses in EBM during continuous delivery with syringe pumps and roller pumps and compared to bolus feeding?	Does the type of infusion tubing used with syringe pumps affect lipid loss?
Sample	Fresh EBM, less than 12 hours from time of pumping, frozen at -70°C.	Frozen EBM, -20°C for up to 6 months.	Frozen EBM in a standard deep freezer (-20°C).
Sample Size	N=15 in each group	N=4 for each combination of variables possible (21).	N=30 per group.
Design	True experimental.	Multi-study, multi-factorial with repeated measures. Not randomized.	Experimental, blinding.
Fat Measure	Modified Mojonnier	Roese-Gottlieb	Creamatocrit
Limitations		-Complex, several studies, reported at once.	-Creamatocrit, not standardized. - 15 mls milk into syringe, 4 mls infused, 11 mls unaccounted. -Depending on the syringe position and the rate and amount of creaming, post infusion results would be erroneous.
Strengths	-Methodology: milk fat measurement. -Milk volume: priming plus only amount for infusion. -Method of lipid analysis. -Design controlled for variables. -Adequately powered.	-Methodology: milk fat measurement. -Milk volume: only amount for infusion. -Method of lipid analysis. -Design controlled for variables - - clear description of the many combinations.	-Inter and intra rater reliability of CR method.
Question answered?	Yes.	Partially.	No.

Greer, McCormick, and Loker (1984) studied fat content in EBM (shaken or blended) during continuous infusions at various rates (1, 4, and, 7 ml/hour) using a syringe (horizontal or vertical positioning) or roller pump, and taking hourly measurements. They compared continuous versus bolus feeding. Each combination of variables, a total of 21, was performed and analyzed four times. The current study did not test as many variables as Greer, et al. but the method of lipid analysis was similar. Reliability of the method was incorporated into the study design with duplicate testing of the baseline samples. The present study builds on the findings of Greer et al. by testing fat loss in continuous feedings by syringe pump infusions, in a tip up position at four mls per hour by testing the tubing type. The current study found that the microbore tubing delivers the most milk fat during simulated, continuous EBM infusions.

The study by Brennan-Behm, Carlson, Meier, & Engstrom (1994) most closely resembles the current study as investigators tested the fat content of EBM pre and post simulated EBM infusions using different types of tubing. Several differences, however, are noted between their study and the current study (refer to Table 4). These included the method of lipid analysis, the power of the study, and the design with respect to the volume of milk in the syringes. Despite the methodological concerns there was a clear pattern of lipid loss pre and post infusion – and between the two tubing types. The standard bore tubing lost significantly more fat than the minibore tubing, $t(29) = 2.36$, $p = .025$, losing a mean of 2.77 kcals/oz. The minibore tubing lost a mean of 2.32 kcal/oz.

The current study more accurately answers the question "which tubing delivers the most milk fat during a simulated continuous infusion of EBM?" than the Brennan-Behm et al., study because it more closely approximated clinical practice, the method of

lipid analysis was stronger than the CR, and more extraneous variables were controlled.

Two key questions are raised by the current study. Why did the minibore tubing deliver most or all of the milk fat? Moreover, what happens in the standard and minibore tubing to prevent significant amounts of fat from being delivered? The exact manner in which milk fat is lost in continuous EBM infusions was not examined in this study but possible explanations can be proposed so that they may be tested in future studies.

Mechanism of Fat Loss in Continuous EBM Infusions

Despite the use of syringe pumps that allowed syringe tips to be placed vertically, a significant amount of fat was lost from the standard and minibore tubings. Conclusive evidence of "how and why" fat is lost in infusion tubing was not found in the literature. Several researchers describe milk fat "sticking" or "adhering" to the inner lumens of the tubing (Brennan-Behm, Carlson, Meier, & Engstrom, 1993; Chan, Nohara, Chan, Curtis, & Chan, 2003; Van Aerde, 1999, unpublished). Some of the possible mechanisms worthy of exploring are the following: the structure of the human milk fat globule, the components and surface area of tubing, the position of the tubing and pump, some aspects of fluid mechanics within a tube, and the instability of EBM.

Properties of the Human Milk Fat Globule. Human milk is a complex system of many fluids that can be considered as different compartments: aqueous phases, colloids, emulsions (fat globules), fat globule membranes and other cells (Jensen, 1989). Fat globules vary in size from 1 μm to 5 μm in diameter (Ferris & Jensen, 1984; Jensen, 1989) and are dispersed as an oil-water emulsion stabilized by the milk fat globule membrane (MFGM). Human MFGMs have glycoprotein filaments on them that may attach to the wall of small intestinal cells and enhance digestion (Bucheim, Welsch,

Huston, & Patton (1988) in Jensen, 1989). Perhaps these filaments or other components of the MFGM somehow attach or stick to the inner lumen of infusion tubing, aiding clustering and the process of creaming.

All of the tubings used in the study are made of polyvinyl chloride or PVC. Polyvinyl chloride is commonly used to manufacture syringes and tubing (Loft, Kabs, Witt, Sartoris, Mandl, Niessen, & Waag, 2000). PVC tubing is known to leach toxic plasticizers (Loft et al., 2000). Perhaps the inner lumen of the PVC tubing is irregular or leaches a substance (plasticizer) to attract MFGMs, causing it to adhere to the surface. Future studies in biophysics could test these theories.

Surface Area of Tubing. If we assume that fat globules somehow "stick" to the inner lumen of the tubing, one might hypothesize that the surface area (SA) available for milk fat globules would be the key factor in fat loss. The diameter and SA of tubing's inner lumen is not normally explicit on the packaging information. If a diameter is given, it is the outside diameter of the tubing. The internal diameter varies according to the thickness of the tubing (Macklin, 2001). Frequently, the length and priming volume differentiates one IV tubing from another. As the names imply, micro is smaller then mini, which is smaller than standard or large bore tubing. By knowing the volume and length of the tubing (see page 31) the formula $V = L\pi r^2$ can be used to determine the radius of the tubing. Then, using the formula $SA = 2\pi rL$ the SA of each tubing can be calculated for each tubing. Microbore tubing has an approximate SA of 27.6 cm^2. The SA of minibore tubing is approximately 57.3 cm^2 and the SA of the standard bore tubing is approximately 91 cm^2. If one assumes that adherence is the primary mechanism of fat loss, as the SA increases, the amount of fat loss also increases.

Tubing Position. The positioning and associated angle of infusion tubing may play a role in how fat accumulates and is subsequently lost during an infusion. The tubing in this study was extended from inverted syringes that were placed in Medfusion 2001 pumps, across a countertop, and in a horizontal fashion (see Figure 2). Based on their description, the tubing in the Brennan-Behm, Carlson, Meier & Engstrom (1993) study seemed to have been extended in a downward fashion as the syringe and pump were 18 inches above the collection container. In two other studies the tubing was extended into incubators and the milk was collected within the incubator (Lemons, Miller, Eitzen, Strodtbeck, and Lemons, 1983; Greer, McCormick & Loker, 1984) but the relative position of the pump was not described. Lucas (1993 – in Premjii, Paes, Jacoson, & Chessell, 2003) observed that 33% of human milk energy can be lost over 40 hours if the syringe is placed above the infant. Lemons, Miller Strodtbeck, & Lemons (1983) stated that the infusion pump was placed on top of the isolette. No other literature could be located regarding the level of the pump relative to the infant. Locally, pump height relative to the location of the infant (below, level with, or above) was not a factor in the delivery of milk fat during continuous infusions (Judi Coté, 1999, personal communication). In this study the horizontal placement of the tubing with minimal height variation may have aided in the delivery of fat particularly in the microbore tubing by enabling straighter laminar flow.

The Instability of Human Milk. Walstra (1994) describes four types of milk instability: creaming, aggregation, coalescence, and partial coalescence. Creaming occurs when milk fat globules, which are lighter or less dense than the aqueous phase of milk, rise against gravitational forces when milk is stored or left to sit (Jensen, 1989; Walstra,

1994). This phenomenon should be called creaming and not fat "separation". Fat separation occurs when the TGs are released from the MFG and oil is visible on the surface of the milk (John Komarnicki, Center for Milk Testing, personal communication, 2004).

Several factors affect the process and rate of creaming. Homogenization decreases the rate of creaming as fat globules are disrupted and form smaller globules. They remain suspended in the fluid portion of milk (without rising to the cream layer) for a longer period of time (Goff and Hill, 1993). The fat content of milk affects the rate of creaming in an inverse relationship: an increased fat content results in decreased creaming.

When milk fat globules cluster, the rate of creaming increases. In cold, raw milk creaming takes place faster when an immunoglobulin in milk forms a complex with lipoproteins. This complex, known as cryoglobulin, precipitates onto the fat globules and causes flocculation (globules come into contact, adhere to each other, and form larger clusters). This is known as cold agglutination. Cold agglutination increases creaming because flocculated fat globules rise to the surface much more quickly than individual fat globules. As fat globules cluster, the speed of rising increases and sweeps up the smaller globules with them. The cream layer forms very rapidly, within 20 to 30 min., in cold milk. Floccules can be disrupted by gentle stirring and spontaneously and completely disperse when milk is warmed to 37°C or higher (Goff, 2004; Walstra, 1994).

When milk fat globules aggregate, they stay closer together than if they would just bump against one another when dispersed in fluid. As with clusters aggregates, flocculates, or larger coalesced globules rise faster than smaller fat globules and pick up smaller ones as they rise, increasing the speed of rise, and the rate of creaming. When fat

globules coalesce, they flow together to form a larger globule. Partial coalescence occurs when true coalescence fails due to crystals forming and then irregular shapes results. Coalescence and partial coalescence are not well described in human milk. During the literature review I was unable to find detailed information regarding the physio-chemistry of human milk. There was a great deal more available on bovine milk. In 1989, Jensen noted that there was a large amount of research on bovine milk physics and chemistry, but not human milk.

During each of the simulated infusions in this study visible fat accumulation was noted each time in the upper aspects of both the standard and minibore tubing. The standard bore tubing had visible milk accumulation – within 15 minutes of infusion commencement. Milk fat lined the superior portions of the tubing. It also gathered more heavily in the most superior and curved portions of the tubing (see Figure 5, page 60). On steeper rises and falls of the tubing, as it randomly lay uncoiled on a horizontal surface, unmoved for four hours, there was only a small visible accumulation of fat. Fat accumulation was not visible in the microbore tubing. It is unknown whether this was due to an inability to visualize any accumulation in to the very small inner lumen or that little or no fat accumulated in the tubing. A question for future studies is whether the fat accumulates in tubing is merely aggregated or whether coalescence occurs. Aggregated milk globules can be broken apart while milk fat that has coalesced cannot be broken apart again (Goff, 2004). It is unclear the effect coalesced MFGs have on the absorption and digestion of fat in infants.

Fluid Mechanics. Greer, McCormick and Loker (1984) and Stocks, Davies, Allen, & Sewell (1985) describe how fat loss is inversely proportional to infusion rate. A

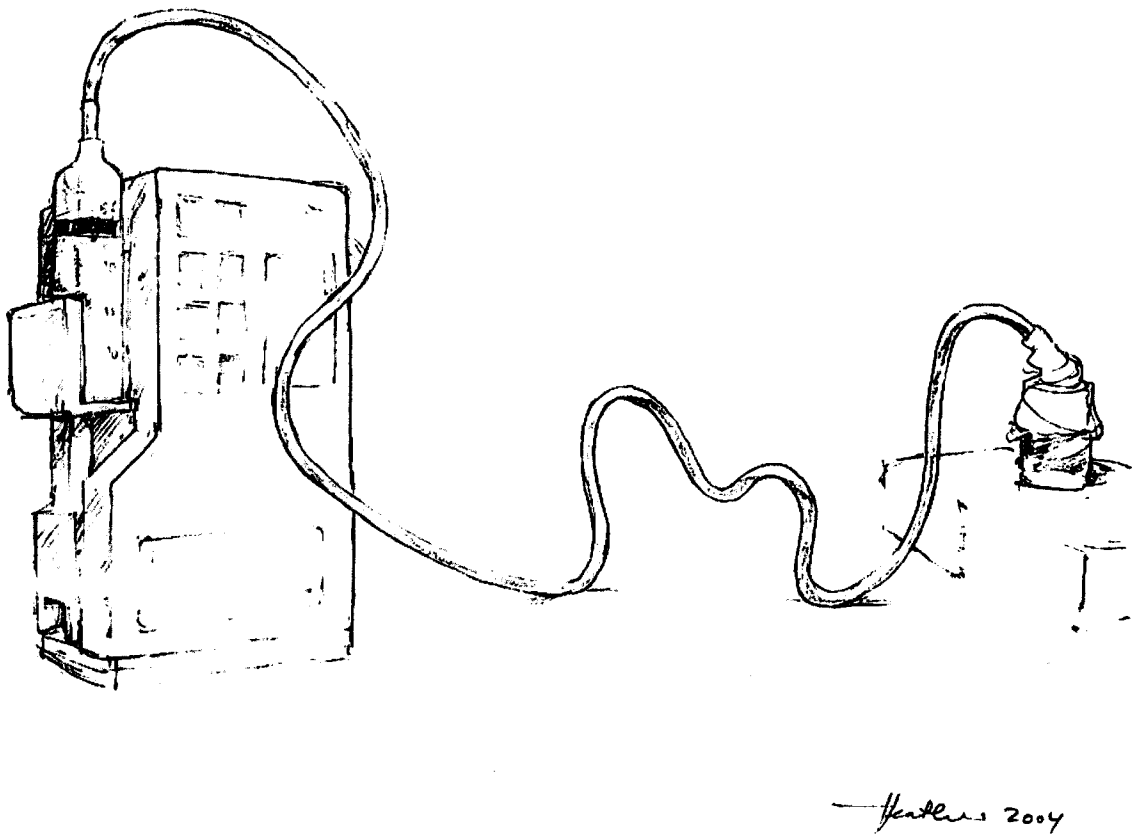
slower rate of flow within an infusion system would provide a smaller velocity gradient, thus, allowing more creaming. Laminar flow is the movement of high viscosity products through a pipe in concentric layers where the fastest particle may move at twice the speed of the average particle. Within an infusion system, milk and cream may flow in this manner. According to the principles of laminar flow, the cream layer would aggregate on the upper surface of the inner lumen and flow more slowly than the aqueous phases. Laminar flow is also influenced by viscosity, which is how resistant the fluid is to flow. Human milk will always vary in its components and its viscosity. Fluid resistance to flow within tubing is a function of the administration set length and diameter, as well as fluid viscosity (Macklin, 2001).

If a large velocity gradient exists, creaming is undone and aggregates are disrupted, as in with stirring or fast pumping (Walstra, 1994). As the volume or space available within the tubing lumen decreases, flow velocity of milk increases, and the amount of creaming (and consequential fat loss) decreases. In the large bore tubing there is a relatively large amount of space and during a continuous infusion, milk is flowing at a slower velocity. This may be why creaming is occurring at a faster rate than in the other two tubings and why so much fat is lost within the standard bore tubing. Future studies might investigate this principle or focus on substances that prevent or decrease creaming.

In conclusion, it is likely that a collection of factors influence fat loss within an infusion system. When the natural tendency milk has to cream combines with a low flow rate (velocity) within a larger area (volume) then creaming will occur. When it does fat globules will rise above other milk components and aggregate. In this way, milk fat globules do not simply "stick" to the tubing – they are "allowed" to remain inside.

Figure 5.

Lipid Accumulation in Standard Bore Tubing



Strengths of the Study

Power. Sample size calculations indicated that a power of 0.80 was achieved with a sample size of 15 in each group. This was adequate to show a large ES (0.5) with an alpha level set at 0.05. An adequate sample size and power decreased the risk of a Type II error, failing to reject a false null hypothesis.

Optimal Measurement Method. The Mojonnier method offered a reliable and precise measure of fat content (Lynch, Barbano, Healy, & Fleming, 1994) that provided conclusive evidence to answer the question: Which of three tubing types used in continuous infusion of EBM delivers the most milk fat? The Mojonnier method has long since been used as a reference method and an official method for determining fat in milk for the AOAC and the Standards Council of Canada. Quality standards of measurement for this method are established and monitored by testing duplicate samples of milk and calculating reliability. Recommended agreement is within 0.03% (AOAC International 2000; Marshall, 1993). Up to 0.06% is acceptable depending on the quality of the sample. (Joanna Lynch, Lab Manager, Food Science Department, Cornell University, personal communication, 2004). Most of the duplicate results of this study fall within this range. This range is far more precise than that of the CR method used in other published studies. Unfortunately, some of the milk samples became grainy, despite efforts to maintain the integrity of the sample according to recommendations in the literature. The coefficient of variation (CV) calculated for the Mojonnier method is approximately 0.51. Generally, a CV of less than 1.0 is acceptable. For this study, the CV was calculated to be 0.76 (Joanna Lynch, Lab Manager, Food Science Department, Cornell University, personal communication, 2004).

Control of Extraneous Variables. This true experimental design controlled for many variables. Each aliquot of milk was randomly assigned to a tubing type during each experimental run but the researcher was open to this. Each tubing (group) served as its own control. The researcher consistently followed a specific research protocol. Procedural reliability was enhanced as someone familiar with continuous EBM infusion studies and protocols evaluated the researcher during two study infusions. The researcher was the only person to perform the study for all 15 runs, closely following the protocol.

Limitations of the Study

Volume Measurement Precision. It was evident that no matter how precise the researcher attempted to be placing exact milk volumes in the 20 ml BD syringes, it was extremely difficult to complete a simulated infusion without residual milk in the syringe. All of the syringes and tubings contained residuals. The Medfusion 2001 syringe pumps are accurate to +/- 3% or 0.48 mls of a 16 ml infusion (Arudus Medical, 2001). The 20 BD syringes are accurate to +/- 5% (BD Medical Surgical Systems, 2004), which could amount to 0.8 mls in for a 16 ml volume. This could account for the consistent syringe residual. The minibore tubing consistently had the greatest residual in the syringe despite strenuous efforts to ensure only 16 mls of milk was in the syringe after the tubing prime, when the infusion began. Conversely, on two occasions the infusions stopped because the syringe was empty. Only 15.96 mls was infused.

Ambient Temperature. Temperature is known to affect the rate of creaming, as described earlier. Some of the previous studies on continuous EBM infusions have dripped the EBM through infusion tubing, feeding tubes, and into incubators at a controlled temperature (Greer, McCormick, & Loker, 1984; Lemons, Miller, Eitzen,

Strodtbeck, & Lemons, 1983). The room temperature where the current study took place ranged from 22.6 – 23.5 ° C during all study runs with a median temperature of 22.8° C. Using an incubator would have more closely approximated clinical practice.

Potential Measurement Error in Lipid Determination. The strength, precision, and accuracy of the Mojonnier method of analysis for fat in milk, and as a reference method, is well documented (Barbano, Clark & Dunham (1988); Jensen, 1989; Polberger & Lönnerdal, 1993). Some technicians achieved better reliability than others. The Centre for Milk Testing is an experienced, licensed, and accredited laboratory (Standards Council of Canada, 2003). While the technologists all had substantial experience with the Mojonnier method – from 10 to 20 years – theoretically, reliability might have improved if one staff member had analyzed all of the samples.

During laboratory analysis fat globules may have coalesced or were altered from freezing and thawing despite precautions to protect the sample. Separation was reported from the laboratory on two occasions. When this occurred they worked harder to obtain a homogenized sample before removing the test aliquot.

The greatest potential for error existed with baseline milk sampling and aliquot preparation. The milk used in the study was fresh and without visible creaming or separation in all samples obtained prior to experimentation. Only very small oily flecks were occasionally visible on the surface of the milk and remained with warming. Results would have been erroneously low or high and more variable if milk had been aspirated from a non-homogenized sample. Care was also taken not to spill or waste milk in the syringes or the baseline sample container when aliquots were taken. As possible, air bubbles were removed from the syringes.

Nursing Practice Implications

The findings of this study are specific to the three tubing sets tested. However, important issues with respect to current nursing practices and continuous EBM infusions can be addressed. Based on these findings the standard bore tubing should no longer be used for low rate, continuous EBM infusions for the premature infant population vulnerable to nutrient and fat misdelivery. If possible a smaller diameter, microbore, tubing should be used. The minibore tubing should be reserved for infusions when a higher infusion rate is used. A good marker of this would be when the infant reaches full volume EF.

It might be possible to modify the dimensions of the enteral only tubing to enhance fat delivery and provide safe EF. New EF tubing has been introduced at the study site since this study commenced. The new tubing is an enteral only tubing with a length of approximately 140 cm and a fill volume of 0.9 mls. A trial could be done on this tubing to see how fat delivery compares to the tubings used in the current study. Children's Medical Ventures (CMV®) manufactures a feeding tube, used in this study, whose tip is incompatible with IV tubing. This tubing has a continuous orange stripe, a tag stating "enteral only", and a large orange connection tip – making it easier to discern enteral from parenteral.

In this study fat creaming was clearly observed in the larger bore tubings. This reinforces the recommendations from other studies that if larger bore tubings are being used nurses should be aware that syringes require complete emptying. If current clinical practice involves changing the syringe and tubing every four hours nurses need to be aware of the potential nutrient loss when syringes and tubings are thrown away.

While this study raises the issue of residual fat being left in the tubing nurses should also be aware of potential excess fluid delivery if tubing contents are purged into the infant at each changing. Simply flushing the tubing into the infant to deliver the fat is not the answer. For example, the standard bore tubing has a volume of 5.5 mls. Emptying this tube into the infant every four hours, without accounting for the fluids, could cause an excess fluid delivery of 33 mls in 24 hours. This could cause an overloading of fluid and could compromise the fluid status of a critically ill neonate. Only the amount of milk required for four hours of EF should be aspirated into the syringe, primed through the tubing, infused until it is completely emptied, and then flushed with an amount of water or air equal to the priming volume. Air or water boluses into the syringe are an area of future study.

Caution must be taken not to simply equate a shorter length of tubing with increased fat delivery. Recommendations to use shorter tubing for continuous infusions are found in the literature (Schanler, 1995; Narayanan, Singh, & Harvey, 1984). Based on this study, clinicians should not assume that a shorter tubing length in and of itself increases the amount of milk fat delivered – both length and diameter must be considered. This is evidenced in this study by the differences in milk content of the milk delivered by micro, mini, and standard bore tubing. In this case the standard bore tubing was 20% shorter than the mini and microbore tubings but was associated with significantly more fat loss.

Lastly, there are potentially salient variables that were not tested in this study. Not all NICUs can safely and/or completely invert their syringe pumps and deliver EBM with the syringe tip vertical due to the space and type of equipment available for use.

Additional studies using the microbore tubing with various syringe tip orientations and pump heights should be done to determine if one position is superior. Some NICUs are now using slow bolus feedings given over one to two hours. As an alternative to continuous or bolus feedings, the degree of fat loss, if any, in these infusion systems could be determined.

Implications for the Infant

Fat loss in EF of EBM is a concern not only because nutrition and calories are lost but also because of what caregivers must do to make up for those losses. When infants fail to gain weight adequately attempts are made to optimize the caloric intake so that an infant demonstrates growth. There are common practices used to address failing growth in infants in the NICU. They include optimizing glucose infusions, increasing the total fluid intake (TFI), and increasing the caloric density in parenteral and/or enteral nutrition. Unfortunately, they are not entirely without risk.

Optimizing calorie delivery can be achieved by increasing the amount or concentration of glucose in intravenous infusions, including total parenteral nutrition (TPN). This intervention must be carefully executed and monitored as preterm infants generally do not have the ability to metabolize glucose as efficiently as term infants (Gomella, 1999; Pereira, 1995). They may be stressed and have and elevated cortisol production, which results in higher glucose production, and/or they are unable to suppress their own glucose production when exogenous glucose is introduced (Pinchasik, 2001; Ziegler, Thureen & Carlson, 2002). Consequently, hyperglycemia could develop. Hyperglycemia increases the osmolarity of the blood causing problems with increased urine output and dehydration (Pinchasik, 2001). Hyperosmolarity may increase the risk of

intraventricular hemorrhage (IVH) (Gomella, 1999). If hyperglycemia is persistent, the amount of glucose delivered could be reduced or further intervention taken with an insulin infusion to ameliorate hyperglyemia (Gomella, 1999; Pereira, 1995; Pinchasik, 2001; Ziegler, Thureen & Carlson, 2002).

To increase calories, the TFI prescribed per kg, per day of an infant's body weight can be raised based on the current volume intake and the infant's clinical status. Fluid restrictions are sometimes in place if an infant is in a postoperative state, has a patent ductus arteriosus (PDA), or is not voiding adequately. Weight gain may not be true growth; it may simply be fluid retention. Fluid overloading is associated with increased incidence of PDA, bronchopulmonary dysplasia (BPD), IVH, or necrotizing enterocolitis (NEC) (Gomella, 1999). Premature infants may not be physically able to handle an increased TFI, as their kidneys are not yet functioning optimally (Gomella, 1999). When feeding enterally, an infant may not physically tolerate the increase in food volume and signs of intolerance, e.g. gastric residuals, abdominal distension, vomiting, may occur. Fluid balance must be carefully monitored to avoid adverse effects.

Whether an infant is feeding enterally or receiving TPN the caloric density in existing nutrition can be increased to provide the most calories possible. They must be provided in appropriate ratios of carbohydrates, fats, and proteins (Pinchasik, 2001; Ziegler, Thureen, Carlson, 2002), though no clear standards exist. There are limits to how much nutrition can be concentrated in TPN due to the osmolarity of the solution and whether or not the infant has a central line, which allows for the delivery of fluid with greater osmolarity. Skin damage occurs if TPN fluids leak into the surrounding tissues with peripheral infusions (Abad-Sinden & Bollinger, 2002) and greater osmolarity

augments damage to the tissues. Long term TPN comes with its own set of risks and complications, e.g. metabolic derangement, sepsis, thromboses, and liver disease (Heine & Beines, 2002), and the risk of infection or sepsis is always present with centrally placed intravenous access (Pinchasik, 2001; Wolf, 2001).

Lastly, if the infant is feeding enterally the caloric density of their food can be increased or concentrated within the TFI that they are prescribed, but again, not without risk. Enteral feedings may have liquid or powdered formula concentrate added to them. Concentrating infant food increases the osmolarity with each additive (Abad-Sinden & Bollinger, 2002). High osmolarity could exacerbate the adverse effects of giving too much feed volume (described above) as it delays gastric emptying (Williams, 1997). Recently, in NICUs across the world the bacterial contamination of milk powders used to supplement infant food has resulted in sepsis, NEC, and death (Bar-Oz, Preminger, Peleg, Block, & Arad, 2001; Centers for Disease control and Prevention (CDC), 2002; Chan, 2003; Nazarowec-White & Farber, 1997;Van Acker, De Smet, Muyldermans, et al., 2001). Infants must be carefully monitored for signs of feeding intolerance and sepsis.

Conclusion

Continuous, low rate, EBM infusions will remain to be a standard of care in NICUs. Even with recommendations from the literature in the 1980s it is clear that no practice standard exists. Premature and low birth weight infants continue to lose precious nutrients and calories when milk fat is lost during continuous EBM infusions. Preventing fat loss will benefit infants by providing them the most complete nutrition possible from their own mother's milk. It will also minimize the amount of intervention required by practitioners, minimizing the risks associated with them.

This study examined one variable in continuous, low rate feedings of EBM – the tubing type. Fat loss differed by tubing type. Results of different fat loss by tubing type are congruent with previous studies during this type of feeding. Results indicate that standard or large bore tubing should not be used for continuous, low rate infusions of EBM. Of the tubing types tested in this research the microbore tubing offers the most complete fat delivery of fat during continuous, low rate EF of EBM.

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Appendix A: Summary of Internet Survey

Summary of Internet Survey on Continuous Feeding Equipment and Practices

	n	% of responses
Pump Type		
Volumetric	2	6
Syringe	32	91
Both	1	3
Syringe		
Angle		
Up	22	63
Horizontal	1	3
Down	8	23
Variations:	3	9
Change Frequency		
Q3h	3	9
Q4h	25	80
Q6h	2	6
Q8h	1	3
Tubing		
Type	14	40
Standard/Large bore		
Microbore	17	49
Enteral only extension set	2	6
Change Frequency		
Q3h	2	6
Q4h	13	37
Q6h	2	6
Q8h	3	9
Q12h	3	9
Q24h	10	29

Appendix B: Methods of Determining Total Fat Content

Direct			
Method	**Description**	**Advantages**	**Disadvantages**
Creamatocrit	-Small volumes of milk aspirated into capillary tubes, centrifuged. Fat layer measured with callipers and ruler then compared to total layer of milk plus fat. -Regression equation used to calculate calorie content	-Small volumes of milk (50 uL). -Used when many samples must be analyzed and volume of milk sample is small. -Fresh or frozen milk can be used. -Inexpensive, minimal equipment required. - Easy to perform.	-Must be calibrated by reference method with the laboratory, technician, and type of milk being used. - Heat influences results. - Subjectivity may occur with caliper reading, which may or may not include FA layer. -May not function if cream column dislocates (occurs in repeatedly frozen samples). - Accuracy ± 10%.
Enzymatic	-Enzyme production and measurement of glycerol from acylglycerols (ie: Triacylglycerols).	-Small volumes of milk (250 uL).	-Only for use with acylglycerols (TGs, DGs and MGs). -Phospholipids not included. -Requires colorimeter or spectrophotometer.
Colorimetric	-Sulfophospho-vanillin: at an acid pH TGs and a chemical called vanillin produce chromogen, which is a measured, color reaction.	-Small volumes (50 – 100 uL). - Commercial kit available. -Requires minimal equipment.	-Diet may influence content of certain FFA. -Requires colorimeter or spectrophotometer.
Turbidimetric /Spectrophoto -metric	-Protein is made soluble and then the optical density is determined.	-Small volumes (50 uL).	-Must be calibrated by solvent extraction.
Babcock & Gerber	-Sulphuric acid used to destroy non- lipid contents of milk, releasing fat. -Volume of fat is read as a percent on long neck of special bottles.		-Does not recover phospholipids as well as the Roese-Gottlieb method. -Large amounts of milk required.

Appendix B: Methods of Determining Total Fat Content (cont'd)

Indirect			
Method	**Description**	**Advantages**	**Disadvantages**
Roese-Gottlieb	-Milk is treated with ammonium hydroxide, extracted with ethanol, diethyl ether, and hexane.	-Can be done with as little as 1 ml of milk. - Accurate, precise and proven standard method.	-Requires 1 – 10 ml milk volumes. -If lipolysis has occurred all of the FFA may not be extracted. -Phospholipids not completely extracted.
Mojonnier	-Modification of Roese-Gottlieb with a special flask that the speeds separation of phases of the method. - Modified Mojon-nier has three extractions.	- Accurate, precise and proven standard method.	- Requires 10 mls of milk to perform but can be done with less. - Modified with 3rd extraction. - See above (Roese-Gottlieb).
Modified Folch	-Chloroform Methanol, 2:1 v/v. -Complex solvent extraction with several phases.	-Can use 0.1 – 10 mls of milk in analysis.	-Expensive and time consuming. -Chemicals are known to be carcinogenic and toxic. -Common for emulsions to form that are difficult to break.
Dry Column	-Sodium sulfite and Celite mixed with milk. -Extraction with dichloromethane; methanol (9:1) in a glass column.	-Rapid, no problem with emulsions, extract is dry, quick. Method simple. Can be used under simple laboratory conditions.	-Column packing and elution steps are time-consuming.

Hundrieser, Clark, Ferris, Jensen (1984); Jensen (1989); Jensen & Clark (1984); Lucas, Gibbs, Lyster & Baum, (1978); Lucas (1983); Lönnerdal, Smith & Keen; Polberger & Lönnerdal, (1993).

Author/Date	Methods	Method of Analysis	Findings	Recommendations	Notes
Brooke & Barley (1978)	-Simulated EBM infusions, N=9. -Milk volume approximately 15 – 25ml. -Flow rates: 10 – 25 ml/h. -Milk fat tested at 1 – 2 h intervals for 8 – 12 h. -2 m tubing (Metriset). -Room temperature 30 - 31° C.	-Bomb calorimetry in triplicate testing of tubing washings and milk from infusions.	-Variations of energy in kcal/g up to 24%, - - A rise in energy at the end of the 8 – 12h of infusion. -Dramatic energy loss at two to four hours of infusion.	- Agitate burette hourly to ensure mixing.	-Practice differs vastly in today's clinical practice. - Tested for kcals and not fat loss.
Spencer & Hull (1981)	- Tested breast milk for fat content and also tested continuous infusion of EBM using a volumetric (IVAC) and syringe pump, N = 6 - Syringe tip vertically placed. -Fresh EBM,	-Creamatocrit.	-Mean fat loss of 24% for the IVAC volumetric pump and 19% for the syringe pump.		- Very little detail given in this study. - Small sample size.
Lemons, Miller Strodtbeck, & Lemons (1983)	-Tested bacterial growth in EBM and fat loss in refrigerated (4° C) and frozen EBM samples, n=9. -Syringe pumps placed on top of incubator. -Extension tubing (?length), stop cock, and feeding tube into an incubator (31 – 37° C) for collection. -Infusion rate 3 ml/h, for 8 hours. -Unspecified milk volume into a 60 ml syringe.	-Creamatocrit, in duplicate.	- Significant fat losses (p<0.001) at 8 hours for both frozen and refrigerated EBM. - Frozen EBM had a greater caloric loss than fresh EBM. -↓ in calorie content from 73.9 to 60.4 kcal/100ml (fresh). -↓ in calorie content from 67.4 to 57.7 kcal/100 ml (frozen).	- Use a syringe loaded pump with minimal extension tubing. -Change all feeding equipment Q4H for safety from harmful bacterial levels. - Use refrigerated EBM by 48 hours.	-Change in practice to shorter duration that equipment was used.

Author/Date	Methods	Method of Analysis	Findings	Recommendations	Notes
Greer, McCormick, & Loker (1984)	- Factorial, pre/post design with repeated measures: --mixing milk: hand shaken vs. blending. --rates 1, 4, and 7 ml/h. --continuous vs. bolus. --syringe tips horizontal vs vertical. --pumps in continuous: roller vs. syringe. -n=4 for each combination of variables, total of 21. -Bolus feeding: 8 hour infusion volume (1, 4, or 7 ml/h for 8 hour) given hourly via gavage tube for 8 hours and cumulative sample analyzed. - Terminal air bolus used to recover all the milk. -Infusion tubing plus feeding tube into incubator at 31° C. -tubing: --syringe pump: 84 cm, 3.3 ml volume. --roller pump: 212 cm, 12 ml volume.	-Gottlieb gravimetric method, reliability established in their laboratory.	-Greatest fat lost at rate of 1 ml/h (mean of 32%), syringe tip placement not significant (61-73% recovered). - Syringe tip vertical at 4 and 7 ml/h = ↑ fat delivery (89-99% and 77-94% recovered respectively). - Blending milk made little difference with fat content with syringe pumps. -Fat recovered with air bolus. - Large terminal fat bolus delivered during last hour of infusion. - Intermittent bolus feeding methodology had very little difference in pre/post fat content –92 – 100% of fat recovered. -Roller pump performed poorly with 30 – 31% losses. -Range of fat loss 5 – 39% across methods.	-Intermittent feedings are preferred. - Keeping syringe tip vertical and use syringe pump if continuous feedings are given.	-Complicated design. - No comment of gravimetric method used with <10 mls of milk. -Small sample size.

Appendix C: Studies of Fat Loss in Continuous Infusions (cont'd)

Author/Date	Methods	Method of Analysis	Findings	Recommendations	Notes
Narayanan, Singh, & Harvey (1984)	-Pre/post comparisons of milk fat at the end of intermittent and continuous pump feeding. -Variety of positions of syringes (with the aid of a ramp), syringe types, and routine agitation. --central vs. eccentric nozzle syringes, N=110. --syringe angles from 17 - 45°. --half hourly and hourly mixing of milk in syringe. - Feeding stopped when 2.0 mls left in syringe.	-Creamatocrit – no discussion of method-ology.	- Terminal fat boluses in continuous feeding. -1/2 hourly mixing may have helped to retain fat. -Greatest lipid loss occurred with central nozzle syringe tip in horizontal position during continuous EF.	-Empty syringe during each use. -Use shorter infusion tubing (continuous). -Use eccentric nozzle syringe with nozzle tilted up. -Start early bolus feeds.	-Busy design, somewhat scattered. -No details of infusion systems, infusion set ups, room temperature, etc.
Stocks, Davies, Allen, & Sewell (1985)	- Fat loss in continuous and intermittent bolus feeding. Also studied protein (IgA) loss. -Continuous infusions of varying rates – 4 – 9 ml/h. -Infusion duration 3 – 4 hours. -Used pasteurized banked milk. -Bolus infusions -- 5 minutes, reused gavage tubes for entire study after a water rinse. -Used standard 4 fr. feeding tube, 60 ml syringe, and 150cm manometer line. -Syringe tip vertical.	-Creamatocrit, stated it was accurate in the region of 10% or 95% confidence limits.	- Mean fat loss of up to 17% occurred for bolus feeding -Mean fat loss of up to 34% for continuous infusions. - Difference in fat losses between two methods significant: (p<0.05). -Significant inverse correlation between fat loss and flow rates for both techniques (r^2=0.865 and 0.922).	-Use intermittent bolus infusions when possible. -Efficacy of continuous method questioned.	-Several methodologica l issues: Crematocrit, re-using feeding tubes, -? Volume of milk used? -Pasteurized milk should not have affected the fat content.

Appendix C: Studies of Fat Loss in Continuous Infusions (cont'd)

Author/Date	Methods	Method of Analysis	Findings	Recommendations	Notes
Lavine & Clark (1989)	-Randomized block design with repeated measures. -Effect of short-term refrigeration and EBM fortification on fat content. -Total of four 'diets': --Fresh EBM. --Fresh EBM and liquid fortifier [50:50]. --3 day refrigerated EBM. --3 day refrigerated EBM plus liquid fortifier [50:50]. -Syringe pumps with tip horizontal to encourage fat loss. -Rate of infusion 3 ml/h. -Glass syringes. -Room temperature 28°C.	-Modified Folch- well described, each sample analyzed in duplicate for reliability.	-Greatest lipid loss occurred in fresh EBM (only 9% of the fat in the fresh milk delivered after 8 hours of pumping). -Fortifier and refrigeration storage improved delivery of lipid through tubing ($p<0.05$). - Source of increased delivery of lipid in milk with fortifier added was from the fortifier and not from the human milk fat. -There was visible milk fat separation in tubing and residual in syringes. -Milk did not separate in samples stored at 4° C.	-Use refrigerated (up to 48h only) rather than frozen milk for continuous EBM feeding.	-Strong design and methodology. -Accurate and precise method of testing. -Clear conclusions. -Rationale for glass syringe and effect not discussed.

Appendix C: Studies of Fat Loss in Continuous Infusions (cont'd)

Author/Date	Methods	Method of Analysis	Findings	Recommendations	Notes
Brennan-Behm, Carlson, Meier, & Engstrom (1994)	-Pre/Post design testing two tubing types for fat/calorie delivery. -Tubing types; --standard bore (5.0 ml volume). --minibore (0.6 ml volume). -N=30. - 15 m aliquots of milk aspirated into 20 ml syringes. -Infusion rate 2.0 ml/ h for 2h. -Pumps positioned above the collection containers.	-Creamatocrit- extensive discussion on the method and reliability, particularly during testing. -Performed in triplicate.	-Mean percent of decrease in lipid concentration found: --standard bore = 1.59% (SD 1.10), mean calorie loss of 2.77 kcal/oz. --minibore = 1.34% (SD 1.02), caloric loss of 2.32 kcal/oz. -loss from the standard bore tubing was significantly different than the minibore ($p < .025$).	-Recommend minibore rather than standard bore tubing. -Because there were significant losses from each tubing intermittent gavage infusions might be more feasible.	- Creamatocrit method, not standardized. - Large volume of milk left over from syringe unaccounted for and might have affected post infusion results obtained.
Van Aerde (1998) (unpublished)	-Pre/post EBM infusion fat testing with tubing treatment (heparin 30 IU / ml) and control. - Terumo Medfusion 2001 pump. -1.8 m of extension tubing, fill volume 7.0 ml. - 30 ml syringe. -Infusion time 1 hour, at 10.0 ml/h. -Frozen milk used for study; thawed, aliquot taken, refrozen, then analyzed.	-Modified Folch, each sample analyzed in duplicate.	-Fat loss approximately 60% in treatment and control groups.	-Heparin should not be considered for continuous EBM infusions.	-Rate of infusion fast but lipid lost still occurred.

Author/Date	Methods	Method of Analysis	Findings	Recommendations	Notes
Coté (1999) (unpublished)	-Effect of pump height and syringe angle on fat delivery with repeated measures each hour of a four-hour infusion. --Pump 18" above or below infant, or level with infant. --Syringe tip vertical, horizontal or pointed down. -N=135, 15 per cell. -IMED volumetric infusion pumps with lengthy tubings. -Frozen EBM samples. -Rate of infusion 3 ml/h.	-Creamatocrit -analyzed in triplicate with reliability established.	-The only significant variable that increased fat delivery was placing the syringe tip up, in a vertical position. -Pump height relative to the infant had no significant effect on fat delivery. -Fat lost almost immediately to infusion system.		- Validity of creamatocrit method questioned. -Use of IMED pumps now obsolete.
Chan, Nohara, Chan, Curtis and Chan (2003)	-Effect of lecithin on fat delivery during continuous EBM infusions. -Three groups tested: control (straight EBM), 0.5 or 1.0 gram of soy lecithin added to 50 ml of EBM. -Kangaroo (roller) pump and appropriate tubing with two different feeding bags. -donated EBM frozen at -20°C and then thawed. -rate of infusions: 10, 20 and 50 mls/hour, range of 3 – 6 hours.	-Creamatocrit – analyzed in duplicate.	-Significant loss of fat in control and 0.5g soy/50ml EBM ($p<0.001$). -Average fat loss for control samples was 58% ± 13%. - Mean fat loss for 0.5g soy/50mls EBM 55% ± 2%. -Mean fat loss for 1.0 g soy/50 mls EBM was 2% ± 2%. -Rate:no effect on fat loss. -greatest fat loss during first four hours of infusion (70% ± 6%).		Methodological issues: design not clear, creamatocrit measurement. -Not necessarily applicable to premature infants but finding of increased fat delivery may warrant further study within this population.

Appendix D: Factors that Affect Milk Fat

Factor	Variations/options	Fat content ↑ or ↓or ∅	Reference
Maternal Influences	-Dietary, time of day, socio-economic, pump type	**↑ or ↓**	Ferris & Jensen (1984); Jensen et al. (1985); Jensen (1989).
	-Premature Delivery	**Slight ↑**	Wood, Hamosh, Hamosh, & Mehta (1983); Anderson, Atkinson & Bryan (1981); Clark et al. (1982).
	-Term Delivery	↓	
EBM Fortification	-Unfortified	↓	Lavine & Clark (1989); Mehta, Hamosh, Bitman & Wood. (1988); Sapsford (2000); Yu (1999).
	-Fortified	↑	
EBM Modifications & Storage	-Pasteurized	↑	Evanochko (1995); Fidler, Sauerwald, Koletzko, & Demmelmair (1998); Jensen (1989); Yu (1999); Garza, Johnson, Harrist, & Nichols (1982).
	-Unpasteurized	↓	
	-Fresh – Refrigerated 4°C	↑	
	-Prolonged storage at 4°C	↓	
	-Frozen and thawed	↓	
	-Storage (glass or plastic)	∅	
Infusion type	-Continuous	↓	Greer, McCormick, & Loker (1984); Narayanan, Singh, & Harvey (1984); Stocks, Davies, Allen, & Sewell (1985).
	-Bolus	↑	
	-Intermittent bolus	**Slight ↓ ?**	
Rate of Infusion	-Slower (<7.0 mls/hour)	↓	Greer, McCormick, & Loker (1984); Stocks, Davies, Allen, & Sewell (1985); Chan, Nohara, Chan, Curtis, & Chan (2003).
	-Faster	**↑ or ∅**	
Composition of Feeding	-Foremilk	↓	Jensen (1989); Schanler,(2001).
	-Hindmilk	↑	
	-Fortified	↑	

Appendix D: Factors that Affect Milk Fat (cont'd)

Factor	Variations/options	Fat content ↑ or ↓or Ø	Reference
Thawing Process and Temperature	-Microwave and High heat	↓	Jensen (1989); Mehta, Hamosh, Bitman & Wood. (1988).
	-Warm Water	↑	
	-Room Temperature	↑	
	-Refrigerator Temperature	↑	
Mixing	-Blending	↓	Jensen (1989)
	-Shaking	↑ or Ø	
Pump Type	-Syringe pump	↑	Wessel (2000); Schanler (1995); Spencer & Hull (1981).
	-IV pump	↓	
Extension Set Tubing Type	-Large diameter and volume	↓	Brennan-Behm, Carlson, Meier, & Engstrom (1994).
	-Small diameter and volume	↑	
Tubing Length	-Longer	↓	Schanler (1995).
	-Shorter	↑	
Pump Level		Ø	Coté, unpublished, (1999).
Syringe Angle	-Tip up	↑	Coté, unpublished, (1999); Lavine & Clark, (1989);Narayanan, Singh, & Harvey (1984); Greer, McCormick, & Loker (1984).
	-Tip horizontal	↓	
	-Tip 30 - 45°	↑	
	-Tip down	↓	
Syringe Nozzle	-Centre Nozzle	↓	Narayanan, Singh, & Harvey (1984).
	-Off-centre nozzle (Eccentric)	↑	
Syringe Type	-Plastic versus Glass	Ø	Lavine & Clark, (1989).
EBM additives	-Heparin	↓	Chan, Nohara, Chan, Curtis, & Chan (2003); Mehta, Hamosh, Bitman & Wood. (1988); Van Aerde, unpublished, (1999).
	-Lecithin	↑	
	-MCT oil	Ø	

Appendix E: Ethical Approval

Health Research Ethics Board

2J2.27 Walter Mackenzie Centre
University of Alberta Edmonton Alberta T6G 2R7
p: 780 492 0724
p: 780 492 6459
f: 780 492 7303
ethics@med.ualberta.ca

ETHICS APPROVAL FORM

Date: **July 2003**

Name(s) of Principal Investigator(s): **Ms. Lynne Ray**

Department: **Faculty of Nursing**

Title: **Fat delivery during continuous breast milk infusions**

Protocol:

The Health Research Ethics Board (Biomedical Panel) has reviewed the protocol involved in this project which has been found to be acceptable within the limitations of human experimentation.

Specific Comments:

The Research Ethics Board assessed all matters required by section 50(1)(a) of the Health Information Act. Subject consent for access to identifiable health information is required for the research described in the ethics application, and appropriate procedures for such consent have been approved by the REB Panel. The REB has also reviewed and approved the patient information material and consent form.

Signed - Chairman of Health Research Ethics Board (Biomedical)

for _____
D. W. Morrish, M.D.
Chairman, Health Research Ethics Board
Biomedical Panel

This approval is valid for one year

Appendix F: Capital Health Authority Approval

Regional Research Administration
Clinical Trials Centre
1800 College Plaza
8215 - 112 Street
Edmonton, AB T6G 2C8
Phone (780) 407-1372

NOTICE OF ADMINISTRATIVE APPROVAL FOR PROPOSED RESEARCH

Site: RAH

Project Title: Fat Delivery During Continuous Breast Milk Infusions

Project Number: R-1817

Investigator Name: Ray, Lynne Dr.

Department / Faculty: Faculty of Nursing

Division:

Supporting Documents

Ethics Appoval Date: 24-Jul-03 **Ethics File #:** 4866

Study Protocol

Sponsor: Perinatal Research Centre

CRO:

Type of Funds: Award

Overhead rate: 0%

Legacy Account: U of A account **Oracle Account:**

Contract Finalized Date:

Project Approved: 18-Aug-03 **Comment:**

Kathy Brodeur-Robb
Regional Research Administration
Copies to: Finance and Administartion

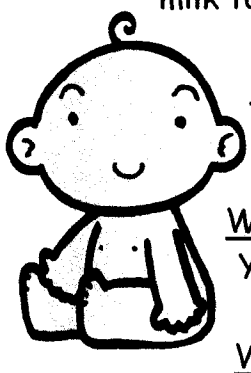
K. Brodeur-Robb

Monday, August 18, 2003

Appendix G: Poster

MOTHERS

I am a nurse in the NICU trying to find the best way to deliver breast milk to babies who need tube feedings. I need your help.



What do I need?

Fifteen, 100 ml samples of fresh breast milk.

What can you do?

You can donate some of your *extra* breast milk for my study.

What happens to the milk?

The milk will be tested for fat content before after pretend four hour milk infusions. This will help us learn which type of feeding tube delivers the most fat to babies.

Who can help?

If you are a mother with more than enough breast milk for your baby you can donate. Before you donate, a lactation consultant will speak with you to make sure you have enough milk for your baby and for the study.

How do I do this?

1. Check with the lactation consultant to see if you have enough milk:
   Erica Kalke, RN, BScN - Lactation Consultant: 477 - 5164
2. Use the electric pump to express just over 3 oz (100 mls) into the bottles marked for this study in the pumping room. Fill milk to the very top.
3. Label the milk with the "study only" label provided.
4. Put the milk in one of the fridges of the common rooms between NICU pods.
5. Call me, Karen at 432-5764 to let me know milk is ready.
6. Only donate once a day.

We are very grateful for your help in trying to improve care for babies. Thank you very much for your interest.
If you have any questions about the study please contact:

Karen Knuth, RNC, MN(C): 432-5764

(Flesch-Kincaid reading level of Grade 4.9)

Appendix H: Consent Form Template

Fat Delivery in Continuous Breast Milk Feeding: Consent Form

Part 1: Research Information		
Principal Investigator: Karen Knuth	Affiliation: University of Alberta, MN Student	Contact information kksmiles@telusplanet.net
Co-Investigator: Lynne Ray, RN, PhD	Affiliation: University of Alberta, Faculty of Nursing	Contact information Lynn.Ray@ualberta.ca

Part 2: consent of Participant		
	Yes	**No**
Do you understand that you have been asked to be part of a research study		
Have you received a copy of the attached information sheet?		
Do you understand the benefits and risks involved in taking part in this research study?		
Have you had an opportunity to ask questions and discuss this study?		
Do you understand that you are free to refuse to participate or withdraw from the study at any time? You do not have to give a reason and it will not affect yours or your child's care.		
Has the issue of confidentiality been explained to you? Do you understand who will have access to your records?		

Part 3: Signatures

I agree to take part in this study.

Signature of Research Participant: _____

Printed Name: _____

This study was explained to me by: _____

Date: _____

Witness (if available): _____

Printed Name: _____

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

Researcher: _____

Printed Name: _____

* A copy of this consent from must be given to the participant. (Flesch-Kinkaid: Grade 7.3)