DETERMINATION OF SEASONAL FLUCTUATIONS OF FAT IN DONATED HUMAN MILK.

by

Stephanie Garver and Madelyn Wilson

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Project Approved:

Gina Jarman Hill, Ph.D.
Department of Nutritional Sciences
(Supervising Professor)

Mary Anne Gorman, Ph.D.
Department of Nutritional Sciences

Ellen Broom, Ph.D.
Department of Psychology

TABLE OF CONTENTS

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INTRODUCTION

Human milk is a unique substance that provides the perfect amount of nutrients for infants. Additionally, it contains hormones and antibodies that cannot be replicated in formula solutions. Breast milk composition changes continuously and is distinctive to each mother. Fluctuations in composition begin when the initial colostrum begins to change to become mature milk and even from the initiation of a particular feeding to the conclusion. Fore milk, which is what human milk mostly consists of once a feeding has begun, has a higher level of protein and water, whereas hind milk which is more concentrated at the end of the feeding, has more fat.

The composition alterations in breast milk may be attributed to the age and stage of nursing, the mother's diet, or seasonal changes. Available research, though limited, has shown that fat content is typically higher in milk produced during the warmer months of the year and lower in the cooler ones (Gao, Zhang, Wang, Li, Man & Song, 2011). Researchers sought to confirm the relationship between fat content and seasonal changes.

In collaboration with the Mothers' Milk Bank of North Texas (MMBNT), researchers were able to collect and analyze over 1,000 donated human breast milk samples to determine the impact of external temperature on macronutrient content. The following thesis will describe how a significant relationship was discovered between fat, protein, and caloric content with average or high external temperatures.

Research Objective

The primary objective of this research was to identify the relationship between fat content in human milk and average or high monthly temperature.

LITERATURE REVIEW

The benefits of breastfeeding are well established. The cells, hormones and antibodies that are within a mother's milk cannot be replicated by infant formulas and, in fact, help to protect the baby from illness. Those babies that are fed infant formula have a higher incidence of ear infection and diarrhea ("Womenshealth.gov," 2011). Not only does a mother's milk facilitate a defense for the baby's immune system, it is actually custom made for each individual and even changes to match the baby's stage of development. Research also shows that babies who are formula-fed are at a higher risk for necrotizing entercolitis, respiratory infection, asthma, obesity and type 2 diabetes ("Womenshealth.gov," 2011).

Milk banking is the process of pasteurizing human milk for use by infants who do not have their mothers' own milk. Milk banking provides a method for preterm babies to get all of the nutrients and antibodies contained in human milk (Texasmilkbank.org). Mothers of premature infants are often unable to provide breast milk due to illness, medications, or other complications. In these cases, donor human milk is the next best option (Texasmilkbank.org). Exclusively human milk-based diet reduces the risks of infants developing necrotizing enterocolitis (NEC) (Ghandehari, Lee & Rechtman, 2012). Breast milk is very interesting in that its composition changes when the mother's milk shifts from the initial colostrum, which is for a newborn baby, to mature milk as the baby grows older. Breast milk also varies from the initiation of a single feeding to the conclusion. The foremilk has more protein, vitamins, minerals, and water whereas the hindmilk has more fat. Typically, the fat content is highest in the mornings and lower at night. Additionally, fat content may vary by season. Research also shows that pre-term infants who were enterally fed human milk were more developmentally advanced at 18 months compared to those who received formula by tube feeding (Myer, 2006). Additionally, the cholesterol content in human milk is higher than that found in formula. Cholesterol promotes brain growth and provides the building blocks of hormones, vitamin D, and intestinal bile (Stoppler, 2011).

Though there are many environmental factors that can alter the lipid content of human or bovine milk, research shows that fat composition is typically higher in the warmer months of the year and lower in the cooler ones. Among Chinese women, researchers identified that those living in three different geographical regions had significant different fatty acid composition (Gao, Zhang, Wang, Li, Man & Song, 2011). The three regions likely vary in seasonal temperatures, resulting in different fat content of human milk.

BACKGROUND

In 1985, HMBANA was created to establish uniform policies, provide professional and public education as well as to facilitate communication between milk banks within the United States. This allows for babies whose mothers' are unwilling or unable to produce breast milk to receive the benefits that breast milk can offer through donated human milk. Specifically, mothers of premature infants are often unable to provide breast milk due to illness, medications, or other complications. In these cases, donor human milk is the next best option (texasmilkbank.org). Exclusively human milk-based diets reduce the risk of infants developing necrotizing enterocolitis (NEC) (Ghandehari, Lee & Rechtman, 2012). NEC is the most common cause of gastrointestinal related mortality and is seen most often in premature infants (Gephart, McGrath, Effken & Halpern, 2012). In a systematic review done in 2003, infants who received donor milk were three times less likely to develop NEC and four times less likely to have confirmed NEC than those who received infant formula (McGuire & Anthony). The same study also found that 7-15% of preterm infants fed formula develop NEC, while less than 1.5 % of preterm infants fed human milk developed the disease (McGuire & Anthony). The mortality rate of infants who develop this disease is 67% and accounts for 3,000 to 4,000 infant deaths a year. If an infant is able to survive NEC the child can have additional complications, which can have life-long affects.

Because breast milk changes so much as the baby ages and also from feeding to feeding, it is important to know what those changes are in order to understand what nutrients the baby is receiving or needs to receive. The colostrum is the first stage of breast milk, which lasts for several days once the baby is born and is high in protein, fat-soluble vitamins, minerals, and immunoglobulins (American Pregnancy Association). This milk then transitions into mature milk about two weeks postpartum. Mature milk is 90% water, which is important to keep the baby hydrated while the other 10% is comprised of carbohydrates, proteins, and fats which are necessary for both growth and energy (American Pregnancy Association). Breast milk also varies within a single feeding. The foremilk has more protein, vitamins, minerals, and water whereas the hindmilk has more fat, which helps the child to feel full and satiated once the feeding is complete.

METHODS

This study was approved by the Department of Nutritional Science Research Review Committee. Human milk donated to the Mothers' Milk Bank of North Texas (MMBNT) served as the samples for analysis in this study. The MMBNT follows the guidelines set forth by the HMBANA. The milk used was raw milk, expressed by the donors. The milk is received at MMBNT in five to six ounce bags in a frozen state with pump dates recorded on them from donors. Each of the bags was logged into the MMBNT system then placed in the lab at MMBNT. There they were poured into different three ounce samples, chosen with different pump dates intentionally so that various months were represented for the analysis. The sample bottles were labeled with both the donor ID number and the pump date. If the sample was poured more than three days before being analyzed by researchers, then it was frozen. If not, the sample was kept in a refrigerator waiting for analysis. The frozen or cooled milk samples were slowly thawed at room temperature and then placed in a warm water bath to reach a temperature of 35 - 41°C. Samples were then analyzed using the York Dairy Analyzer.

The York Dairy Analyzer is calibrated to detect and measure human milk macronutrients including fat, protein, lactose, and calories using mid-range infrared spectroscopy. Calibrations are periodically tested against the industry standard for these components by the USDA office in Carrollton, Texas**.** The instrument is calibrated with bias and slope as well as automatic linear regression. The accuracy of the York is as follows: Fat <0.03 grams/Liter against Rose Gottlieb, protein <0.03 grams/liter against Kjeldahl, lactose 0.03 against Polarimetry. The Fat C 6-channel output filter allows accurate prediction of high fat content in dairy.

Gloves and closed toe shoes were worn while handling milk samples. The machine was "zeroed" to ensure that there was nothing in the lines that would affect the analysis. Researchers then ran a pilot test with whole cow's milk to make certain the machine was measuring and analyzing the samples accurately. Samples were at least 20 ml or more as required for accurate analysis. Once a sample was heated, it was removed from the water bath and gently agitated by flipping the container upside down twice to ensure a homogenous sample. Then the pipette on the York Analyzer was placed into the sample of milk and set on the stand. A thermometer within measured the milk temperature to ensure that the sample was within 35 – 41°C range. Samples were aspirated into the York through the front pipette. The sample was heated, further homogenized, and analyzed. Results were displayed on the linked computer display and recorded by researchers. The analysis took less than 60 seconds/sample. Each sample was analyzed for fat, protein, lactose and kcals twice, resulting in an average with reduced error. Upon completion, a rinse was performed to clear the sample milk from the milk analyzer.

This process was repeated over the course of several months until all samples were analyzed (n=1030). Fat, protein, lactose, and kcal content as determined by the analysis were recorded in an excel spreadsheet. Using the MMBNT database, donor ID numbers, pump date, location, and when available the baby's date of birth and the baby's birth term (full term, premature) were identified and recorded. Researchers used the National Oceanic and Atmospheric Administration website (http://www.noaa.gov/) to determine average and high monthly temperatures for each city in which the milk was from. These temperatures represent average and high temperatures over a ten year period. Data were then coded into SPSS Statistics software version 21 and analyzed for descriptive statistics and bivariate correlations using Pearson correlation coefficient (p). A $p \leq 0.05$ was considered significant.

RESULTS

The average fat content of the entire set of samples (N=1029) was 3.26 g/dL ± 1.50 g/dL (mean ± SD), average protein content was 1.19 g/dL ± 0.33g/dL, and the average lactose content was 7.58 g/dL ± 0.68 g/dL. The average caloric content was 20.09 kcal/dL ± 4.48 kcal/dL. Average seasonal temperatures ranged from 22.4°F to 92.5°F and the average high temperature was 80.2°F ± 13.20. Analysis revealed a significant positive correlation between fat content with both average temperature ($r = +0.08$, $p \leq 0.01$) and high temperature ($r = +0.07$, $p \leq 0.05$). Fat content was significantly positively associated ($r = +0.95$) with caloric density ($p \leq 0.01$). A significant positive correlation was found between caloric density with both high temperature ($r = +0.08$, $p \leq 0.05$) and average temperature ($r=+0.09$, $p \leq 0.01$). Fat content was negatively correlated ($r = -0.09$) with lactose content ($p \leq 0.01$) and positively correlated ($r = +0.46$) with protein content ($p \leq 0.01$). There were no significant correlations found between either content of protein or lactose with average or high temperatures. The data were then filtered and the same statistical tests were run to include only those sample with a fat content of 2.4-4.0 g/dL (N=536), which is the typical range reported in the milk banking industry. The average fat content of the samples was 3.15 g/dL ± 0.45 g/dL, average protein content was 1.20 g/dL ± 0.32g/dL, and the average lactose content was 7.55 g/dL ± 0.52 g/dL. The average caloric content was 19.72 kcal/dL ± 1.58 kcal/dL. Average seasonal temperatures ranged from 35.2°F to 92.5°F and the average high temperature was 79.8°F ± 13.4. Neither fat content nor caloric content were significantly correlated with either average or high temperature. However, protein content was significantly negatively correlated with both average temperature (r=-0.11, p≤0.05) and high temperature (r=-0.11, p≤0.01). Caloric content was significantly positively correlated with fat content (r = +0.83, p≤0.01), protein content (r= +0.54, p≤0.01), and lactose content (r=+0.40, p≤0.01).

Analysis of the entire set of data revealed average seasonal temperatures ranged from 22.4°F to 92.5°F and the average high temperature was 80.2°F ± 13.20. The average fat content for the entire set of samples (N=1029) was 3.26 g/dL ± 1.50 g/dL (mean ± SD). For the entire set of data there was a significant positive correlation between fat content with both average temperature (r = +0.08, p≤0.01) and high temperature (r = +0.07, p≤0.05). Fat content was significantly positively associated (r = +0.95) with caloric density (p≤0.01). A significant positive correlation was found between caloric density and both high temperature (r = +0.08, p≤0.05) and average temperature (r=+0.09, p≤0.01). There were no significant correlations found between either protein or lactose content with average or high temperatures.

The filtered data (N=536) revealed a fat range of 2.4-4.0 g/dL that is the typical range reported in the milk banking industry. Average seasonal temperatures ranged from 35.2°F to 92.5°F and the average high temperature was 79.8°F ± 13.4. The average fat content of the samples was 3.15 g/dL ± 0.45 g/dL. Neither fat content nor caloric content were significantly correlated with either average or high temperature. However, protein content was significantly negatively correlated with both average temperature (r=-0.11, p≤0.05) and high temperature (r=-0.11, p≤0.01). Caloric content was significantly positively correlated with fat content (r = +0.83, p≤0.01), protein content (r= +0.54, p≤0.01), and lactose content (r=+0.40, p≤0.01).

Table 1: Results After Analysis of Data Collected

	All samples (N=1029)		Filtered Data (N=536)	
	Range	Mean ± SD	Range	Mean ± SD
Fat (g/dL)	0.99-14.58	3.26± 1.50	2.40-4.00	3.14± 0.45
Protein (g/dL)	0.53-2.80	1.19± 0.33	0.63-2.80	1.20± 0.32
Lactose (g/dL)	3.75-12.03	7.58± 0.68	4.16-9.98	7.55± 0.52
Calories (kcal/dL)	9.10-53.40	20.09± 4.48	15-23.90	19.72± 1.58
Average Monthly Temperature (^{o}F)	22.40-92.50	69.17± 13.65	35.2-92.50	68.80± 13.71
Average High Monthly Temperature (^{o}F)	41.30-104.20	80.17± 13.19	47.50-104.20	79.80± 13.40

Table 2: Average Fat, Protein and Lactose Content in Donated Human Milk Samples

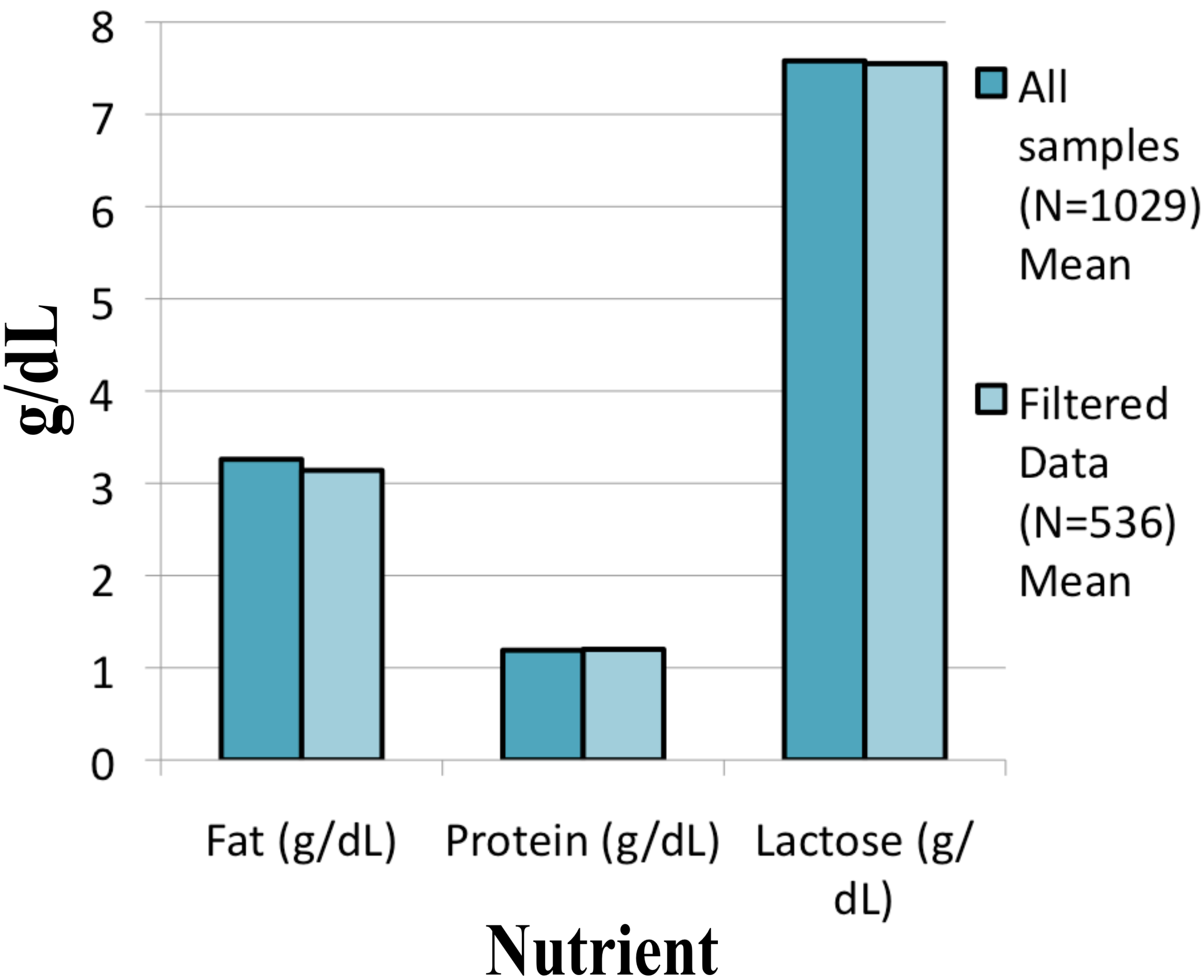
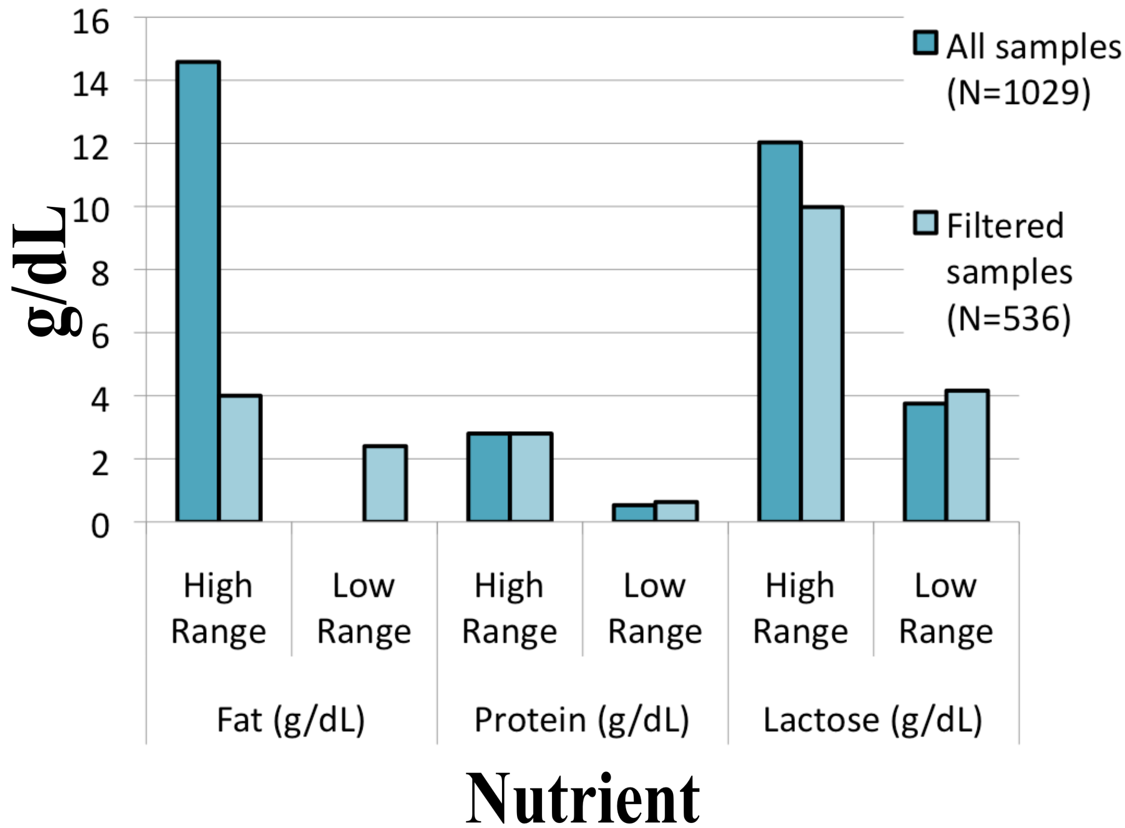


Table 3: Range of Fat, Protein and Lactose Content Found in Donated Human Milk Samples



CONCLUSIONS

Research, although limited, has shown that fat content is higher in the warmer months of the year and lower in the cooler ones (Gao, Zhang, Wang, Li, Man & Song, 2011). Results from the analysis of the entire data set confirmed the hypothesis and revealed a positive correlation between environment temperature and the fat content of donated breast milk samples. Results from the analysis of the entire sample set in this study confirmed that as the fat content of breast milk increased, the caloric density increased as well. However, the data showed no significant correlation between lactose content or protein content in regards to average environment temperatures or caloric content. The results changed however, once the samples were limited to the fat content range of 2.4-4.0 g/dL. Rather than fat positively correlating with temperature of the environment, protein negatively correlated with high and average temperature of the environment.

A weakness of this study resulted from a requirement in sampling technique for this methodology. In order to have milk samples that were expressed during different months, it was necessary to use smaller samples that were not homogenized into a large sample pool. Therefore there may have been some samples that were mostly hindmilk or mostly foremilk and not representative of an entire feeding that a baby might receive when directly nursing from his or her mother. Therefore, due to the required methodology and lack of pooling larger volumes of milk, the range of fat in the original smaller sample volumes in the entire sample set was wider than may be typically seen in the milk bank industry. Results reveal that macronutrient content of either fat or protein may change depending on temperature and perhaps point of expression. Using analysis of filtered data with a limited fat range of 2.4-4.0 g/dL, revealed no significant correlation between fat content and average or high temperature. The filtered data represents the typical macronutrient range of donated human milk.

An additional weakness of this study relates to the changes that occur in human milk. As a baby grows the milk produced by his or her mother changes in macronutrient and caloric content. However the age of the baby was not available for the entire sample set. This may have further altered the results.

Further research is needed in a more controlled environment with specific guidelines about when the milk is expressed. Controlling at what point in a feeding the milk is collected would allow researchers to detect the impact of external environment temperature on macronutrient content of human milk.

Suggestions for Further Research

In further research studies, it would be best to have a more controlled pumping environment for the mothers. Because breast milk can be affected by so many factors including diet, and even time of day, it would be best to have most factors controlled so that the one of the only variables would be the weather temperature. Also, a sample size that is more representative of the entire country would also be helpful. Most of the samples from this study were from the Southern states, so it would be helpful to have a sample set that included samples representing different regions.

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ABSTRACT

Although there are many environmental factors that can alter the lipid content of human or bovine milk, the majority of the limited available research shows that fat composition is typically higher in milk produced during the warmer months of the year and lower in the cooler ones. The primary objective of this research was to identify the relationship between fat content in human milk and average or high monthly temperature.

Analysis revealed a significant positive correlation between fat content with both average temperature ($r = +0.08$, $p \leq 0.01$) and high temperature ($r = +0.07$, $p \leq 0.05$). The data were then filtered and the same statistical tests were run to include only those samples with a fat content of 2.4-4.0 g/dL (N=536). This is the typical range reported in the milk banking industry. Analysis of the filtered data revealed protein content was significantly negatively correlated with both average temperature ($r=-0.11$, $p \leq 0.05$) and high temperature ($r=-0.11$, $p \leq 0.01$).

The wide range of fat content in the original sample indicates that the samples tested included substantial amounts of hindmilk or foremilk that were either higher in fat or lower in fat, respectively. Results reveal that macronutrient content of either fat or protein may change depending on temperature and perhaps point of expression.