Human Milk Analysis Using Mid-Infrared Spectroscopy

Sharon Groh-Wargo, PhD, RD1,2; Jennifer Valentic, BS1; Sharmeel Khaira, MD1; Dennis M. Super, MD, MPH1; and Marc Collin, MD1

Abstract

Background: The composition of human milk is known to vary with length of gestation, stage of lactation, and other factors. Human milk contains all nutrients required for infant health but requires fortification to meet the needs of low-birth-weight infants. Without a known nutrient profile of the mother’s milk or donor milk fed to a baby, the composition of the fortified product is only an estimate. Human milk analysis has the potential to improve the nutrition care of high-risk newborns by increasing the information about human milk composition. Equipment to analyze human milk is available, and the technology is rapidly evolving. This pilot study compares mid-infrared (MIR) spectroscopy to reference laboratory milk analysis. Methods: After obtaining informed consent, we collected human milk samples from mothers of infants weighing <2 kg at birth. Duplicate samples were analyzed for macronutrients by MIR and by reference laboratory analysis including Kjeldahl for protein, Mojonnier for fat, and high-pressure liquid chromatography for lactose. Intraclass correlation coefficients, Bland-Altman scatter plots, and paired t tests were used to compare the two methods. Results: No significant differences were detected between the macronutrient content of human milk obtained by MIR vs reference laboratory analysis. Conclusions: MIR analysis appears to provide an accurate assessment of macronutrient content in expressed human milk from mothers of preterm infants. The small sample size of this study limits confidence in the results. Measurement of lactose is confounded by the presence of oligosaccharides. Human milk analysis is a potentially useful tool for establishing an individualized fortification plan. (Nutr Clin Pract. 2016;31:266-272)

Keywords

neonates; enteral nutrition; human milk; newborn infant

Human milk is the preferred feeding for all infants, including low-birth-weight infants. Studies of preterm infants have established a wide range of advantages of human milk over formula, including better feeding tolerance, faster progression to full enteral feedings, reduced rates of necrotizing enterocolitis (NEC) and late-onset sepsis, and improved neurodevelopmental outcomes.1-4 The nutrient composition of human milk varies depending on the length of gestation,5 stage of lactation,6 and time of day7; nutrient composition also varies within a feeding8 and with various dietary intakes.9 Current clinical practice assumes uniform composition when calculating nutrient intake of infants in the neonatal intensive care unit (NICU) who are fed expressed human milk.

Despite its many advantages, human milk does not meet all nutrient needs of the rapidly growing preterm infant. Specifically, protein intake from human milk is substantially below the protein requirement of preterm infants.10 Commercial human milk fortifiers and/or preterm formulas are routinely added to increase the nutrient density of human milk.11,12 Without a known nutrient profile of the expressed human milk fed to a baby, the composition of the fortified product is only an estimate. Nutrient analysis of human milk would allow an individualized approach to fortification.

Infrared spectroscopy is commonly used in the dairy industry for milk analysis.13 Equipment to analyze human milk is available and currently under study by many researchers.14 The technology needs to be validated with human milk and adapted to the needs of the newborn infant. The purpose of this pilot study was to compare the macronutrient composition of human milk as measured by mid-infrared (MIR) spectroscopy vs reference laboratory analysis.

Materials and Methods

Samples

Mothers who had low-birth-weight infants and who were expressing breast milk provided samples of milk for macronutrient analysis. Mothers were eligible if they were >18 years old, had an infant of any gestational age but with a birth weight...
<2 kg, were pumping and willing to provide a complete 24-hour collection of their milk, and signed an informed consent. The study was approved by the MetroHealth Medical Center’s Institutional Review Board.

**Procedures**

Mothers were provided with a hospital-grade electric breast pump and with containers that were sterile, airtight, and leak-proof; were made of polypropylene; had an attached flip-top lid; and were designed for milk collection (Capital Vial, Inc, Auburn, AL). When a 24-hour collection of fresh milk was received at the hospital, it was placed in a plastic bin marked for the study and refrigerated immediately. Within a few hours of receipt, the milk containers constituting a 24-hour time period were pooled. The work surface was cleansed with a sterilization cloth (Sani-Cloth Plus, PDI Inc, Orangeburg, NY). All containers constituting the 24-hour supply were poured into a clean mixing bottle large enough to accommodate the entire supply without completely filling the bottle. The volume of the 24-hour supply was summed and recorded. The 24-hour supply was thoroughly mixed by gently pouring the milk between 2 mixing bottles 5 times. If a mother had >200 mL of milk per day for 5 consecutive collection days, a 60-mL sample and a 15-mL sample were removed from that mother’s expressed human milk. The 2 samples were placed in 70-mL food-grade polypropylene containers that were subject-coded (top and side) (NeoMed, Inc, Woodstock, GA) and placed in a freezer at –20°C (–4°F). The remainder of the pooled sample was returned to the original continents and refrigerated or frozen depending on the anticipated need of the baby. To minimize the effects of freezing on milk composition, only fresh milk that had never been frozen was sampled for the study, ensuring that the milk that was analyzed had only been exposed to one freeze-thaw cycle.15

The 15-mL aliquot of milk was analyzed using a MIR analyzer (Calais Milk Analyzer, North American Instruments, LLC, Lake Oswego, OR, and Metron Instruments, Inc, Solon, OH). MIR milk analysis was performed on the premises of the manufacturer, which was located within 10 miles of the medical center, and all analyses were performed by one designated technician. After receipt, milk was thawed overnight in a refrigerator. To solubilize and distribute the fat in the milk, the sample was first inverted several times to mix well and then was warmed to 40°C (104°F) in a water bath to ensure that a representative sample was presented to the analyzer pipette. The Calais has an internal “ball and seat” high-pressure (3500 psi) mechanism that homogenizes the sample before analysis is performed.14

The MIR analyzer was calibrated by the manufacturer to human milk values. To ensure a wide range of nutrient content, the calibration set included 6 samples of donor milk and 6 samples from mothers of preterm infants recruited for the current study. Calibration samples were not used in the analysis comparing MIR spectroscopy to laboratory values. MIR spectroscopy exposes a very thin film of milk to infrared radiation.13 The amount of energy absorbed at these macronutrient-specific wavelengths is proportional to the concentration of the macronutrient but also depends on the specific product being analyzed (eg, cow’s milk vs human milk). Unique calibration coefficients are used to calculate the macronutrient concentration for each product and are stored in a file in the analyzer. Additional details of MIR spectroscopy are described elsewhere.16,17 Concentrations of fat (grams), lactose (grams), total protein (grams), and true protein (grams) were reported per deciliter, and energy (kilocalories) was calculated at 9 kcal/g for fat, 4 kcal/g for protein, and 4 kcal/g for lactose. Total protein refers to total nitrogen × 6.25, and true protein is total protein minus 24% for nonprotein nitrogen.18 Total protein, as reported by the MIR analyzer, was converted to bioavailable protein for data analysis using the following equation: total protein (grams) × 0.825.18

The 60-mL sample was packed in dry ice and sent overnight to Eurofins DQCI Services (Mounds View, MN; ISO 17025), where reference laboratory tests including Mojonnier for fat analysis, Kjeldahl for protein, and high-pressure liquid chromatography (HPLC) for lactose were performed within 2 weeks of analysis by MIR spectroscopy.19 Volumes required for tests were 1–5 mL, 2–4 mL, and 5 mL, respectively. Relative standard deviations for tests were as follows: fat (0.512%), total protein (0.504%), true protein (1.051%), and lactose (0.05%) (personal communication, Bruce Kueghle, Chemistry Laboratory Supervisor, Eurofins DQCI, January 23, 2015).

**Analysis**

Statistical analysis to assess the difference between MIR spectroscopy analysis and reference laboratory tests was performed using IBM SPSS Statistics Version 20 (IBM Corporation, Armonk, NY). Correlation and concordance between MIR spectroscopy analysis and reference laboratory analysis were also compared using the paired t test. Interval-level data are reported as the mean ± standard deviation. Statistical significance was defined a priori as P < .05 (2 tailed).

**Results**

Each of the samples for the analysis came from unique mothers whose babies ranged in birth weight from 641 g to 1620 g. Birth gestational ages ranged from 23 6/7 to 32 3/7 and, when the analysis was performed, postconceptional ages ranged from 25 1/7 to 36 5/7. Postpartum day of life when the samples
were collected ranged from 7 to 43 with a median of 28. When the values obtained from the reference laboratory methods were compared with those obtained from the MIR analyzer, the results were highly correlated with intraclass correlation coefficients (95th percentile confidence intervals) of 0.989 (0.955–0.997) for fat, 0.987 (0.948–0.997) for protein, 0.848 (0.504–0.960) for lactose, and 0.982 (0.931–0.996) for energy ($P < .001$). The regression coefficients (intercept, slope) for the macronutrients were as follows: fat (0.036, 0.998), protein (0.126, 0.881), lactose (1.482, 0.776), and energy (1.37, 0.978). As expected, fat and energy were highly correlated ($r^2 = 0.981$, $P < .001$) with the following linear equation: Energy (kcal) = 27.70 + (9.59) (Grams of Fat). No significant differences were observed between the macronutrient values obtained from the MIR analyzer and those values obtained from reference laboratory tests were detected by paired $t$ tests (Table 1). Bland-Altman plots for energy and each of the macronutrients were constructed (Figure 1). The means and standard deviations of the differences between MIR spectroscopy and reference laboratory analysis reflect the bias and the precision of the comparison, and the 95% limits of agreement ± 2 standard deviations reflect the range of the mean differences (Table 1).

**Table 1.** Analysis of the Macronutrient Content of Human Milk: Comparison of 2 Methods (N = 10).

<table>
<thead>
<tr>
<th>Macronutrient</th>
<th>MIR Spectroscopy, Mean ± SD (Range)$^a$</th>
<th>RL, Mean ± SD (Range)</th>
<th>Difference (MIR-RL), Mean ± SD</th>
<th>95th Percentile Confidence Interval Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, g/dL</td>
<td>1.18 ± 0.12 (1.01–1.4)</td>
<td>1.21 ± 0.14 (1.03–1.44)</td>
<td>−0.02 ± 0.03</td>
<td>0.04, −0.074</td>
</tr>
<tr>
<td>Fat, g/dL</td>
<td>3.10 ± 0.87 (1.24–3.98)</td>
<td>3.13 ± 0.89 (1.46–4.23)</td>
<td>−0.03 ± 0.18</td>
<td>0.333, −0.393</td>
</tr>
<tr>
<td>Lactose, g/dL</td>
<td>6.23 ± 0.32 (5.78–6.79)</td>
<td>6.12 ± 0.35 (5.41–6.62)</td>
<td>0.11 ± 0.19</td>
<td>0.475, −0.257</td>
</tr>
<tr>
<td>Energy, kcal/dL</td>
<td>57.6 ± 8.2 (40.6–67)</td>
<td>57.5 ± 8.2 (42.1–67.8)</td>
<td>0.12 ± 1.54</td>
<td>3.155, −2.866</td>
</tr>
</tbody>
</table>

MIR, mid-infrared; RL, reference laboratory.

$^a$Paired-samples $t$ test between MIR spectroscopy and RL methods; $P = .098$ for protein, .621 for fat, .098 for lactose, and .810 for energy; not significant.

**Discussion**

The primary goal of this study was to assess the accuracy of human milk analysis by MIR spectroscopy vs reference laboratory methods. Specifically, we sought to determine the energy and macronutrient content of expressed human milk taken from 24-hour collections of unique mothers whose low-birth-weight infants varied in birth weight and gestational age and who were cared for in the NICU. Good agreement was observed between the macronutrient values obtained from the MIR analyzer and from reference laboratory testing. The small sample size may mask possible linear trends indicating systematic deviations for protein and possibly lactose.

Analysis of cow milk has been routine in the dairy industry for many years. The technologies for analyzing human milk composition mainly come from the dairy world and are evolving rapidly to be standardized for human milk. Methods to analyze milk include traditional reference laboratory analysis and, more recently, infrared transmission spectroscopy, ultrasound, and centrifugation.

Laboratory-based analysis methods are considered the gold standard, but disadvantages include cost and the personnel and equipment requirements. Human milk protein is complex and made up of a variety of fractions including whey, casein, and cellular components. The Kjeldahl method is considered standard for laboratory analysis of protein. The Kjeldahl method is universal, precise, reproducible, and internationally recognized for estimating protein in foods. Fat content in milk is determined by the Mojonnier method. After the lipids are extracted with ether, the fat solids remaining are weighed. Lactose is determined by HPLC. HPLC does not measure the oligosaccharides in human milk. The Kjeldahl, Mojonnier, and HPLC methods are used to reference other analysis technologies when measuring macronutrients in milk.

Spectral analysis occurs across a range from 800–2500 nm (near-infrared) to 2400–6000 nm (MIR). Infrared spectroscopy is widely used to determine organic constituents in a variety of food, including milk. MIR spectroscopy exists as both a filter-based technology and a Fourier transform infrared (FTIR) technology. FTIR collects data from a broader spectrum of wavelengths than filter-based MIR spectroscopy and has been shown to be accurate for the analysis of human milk. FTIR milk analyzers have a larger footprint and significantly greater cost than filter-based MIR analyzers. Early reports of infrared spectroscopy technology for human milk analysis were limited by instruments that were calibrated using bovine milk standards. Several investigators have evaluated the Miris milk analyzer, a filter-based MIR analyzer (Miris Holding AB, Uppsala, Sweden), with variable and conflicting results. The Calais Human Milk Analyzer (North American Instruments, Lake Oswego, OR) used in the present study is a filter-based MIR analyzer but is engineered and sold by a different manufacturer. The current study reports the only data specifically between the Calais Milk Analyzer and reference laboratory methods. Near-infrared spectroscopy technology is also available for human milk analysis but may not correlate well with reference laboratory methods.
Analysis of the carbohydrate component and the calculated caloric density of human milk is confounded by the presence of both lactose and oligosaccharides. There is confusion regarding the extent to which oligosaccharides are included in any of the measurements of human milk carbohydrate. This ambiguity may explain in the present study why the intraclass correlation coefficient was significantly lower, and the bias on the Bland Altman scatter plots slightly higher, for lactose than for fat or protein. Oligosaccharides are not digestible and thus do not contribute to caloric density. MIR spectroscopy analysis appears to remain accurate for fat and protein even when samples are diluted with distilled water prior to analysis.

Other methods of milk analysis include acoustic spectroscopy, or ultrasound, which detects the differences in attenuation and transmission of the constituents within milk, and centrifugation, which separates the fat globules from the aqueous fraction. Table 2 summarizes milk analysis technologies.

The known high variability in human milk composition makes standard calculations of nutrient intake inaccurate, with a risk of underestimation or overestimation of actual energy and macronutrient intake. Sampling expressed human milk from a 24-hour pool reduces nutrient variability. Overall, the validity of milk analysis depends on both the handling of the milk samples and the technology chosen for analysis. The use of analytical equipment that provides information to be used in clinical care of patients is regulated by the Food and Drug Administration (FDA) and requires a 510K designation. None of the milk analysis equipment currently available in the United States has this FDA designation, and therefore the equipment may only be used under an approved research protocol.

Because human milk composition varies and access to human milk analysis may become a reality, interest in an individualized approach to human milk feeding is increasing. The protein content of human milk is known to rapidly decrease over time. Additionally, the caloric density of expressed human milk may often be less than assumed. The lower caloric density may be due to incomplete emptying of the breast, which results in less hind milk and lower caloric density. Other factors thought to be related to inadequate milk expression in mothers of preterm infants include the anxiety and stress related to the NICU hospitalization and the separation of the breastfeeding couple. Many clinicians are anxious to

**Figure 1.** Bland-Altman plots displaying the mean differences and limits of agreement between mid-infrared (MIR) spectroscopy and reference laboratory analysis (N = 10). (a) Energy; (b) protein; (c) lactose; (d) fat.
<table>
<thead>
<tr>
<th>Technology</th>
<th>Name of Device (Manufacturer) Website</th>
<th>Nutrients/Output</th>
<th>Sample Volume, mL</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Limitations include high cost, large sample volume, expertise required, and length of time to receive results</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Filter-based and Fourier transform options available</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Calais Human Milk Analyzer and Delta LactoScope FTIR meet AOAC international standards for milk analysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Limitations include high cost of equipment and lack of FDA approval for use in the clinical area</td>
<td></td>
</tr>
<tr>
<td>Near-infrared spectroscopy</td>
<td>SpectraStar Neonatal Analyzer (Unity Scientific, Columbia, MD) <a href="http://www.unityscientific.com">www.unityscientific.com</a></td>
<td>Protein, fat, carbohydrate; calculated energy</td>
<td>~1</td>
<td>Spectral analysis across 800–2500 nm</td>
<td>31–33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sample volume required is less than that required for mid-infrared</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Limitations include high cost of equipment and lack of FDA approval for use in the clinical area</td>
<td></td>
</tr>
<tr>
<td>Acoustic spectroscopy (ultrasound)</td>
<td>MilkoScope: models Julie Z9 Fulmatic, Julie C8, and Julie Z7 milk analyzers (Scope Electric, Razgrad, Bulgaria) <a href="http://www.scope-electric.com">www.scope-electric.com</a></td>
<td>Protein, fat, carbohydrate; calculated energy</td>
<td>3–12</td>
<td>Measures differences in attenuation and transmission</td>
<td>35,36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower cost than infrared spectroscopy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fat may be more reliably measured than carbohydrate and protein</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Limitation is lack of published comparison to gold standard methods</td>
<td></td>
</tr>
<tr>
<td>Centrifugation</td>
<td>Creamatocrit Plus (Medela Inc, IL) <a href="http://www.medelabreastfeedingus.com/products/328/creamatocrit-plus">http://www.medelabreastfeedingus.com/products/328/creamatocrit-plus</a></td>
<td>Fat and energy</td>
<td>0.2</td>
<td>Separates fat from aqueous fraction</td>
<td>37–42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Affordable</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Limitations include lack of milk protein content data, and possibility for overestimation of fat and energy compared with gold standard methods</td>
<td></td>
</tr>
</tbody>
</table>

AOAC, Association of Analytical Communities; FDA, Food and Drug Administration; HPLC, high-pressure liquid chromatography.
place a “nutrition label” on the human milk fed to hospitalized newborns. Preliminary results from a study comparing growth of preterm infants fed human milk fortified in the traditional way vs growth of preterm infants fed human milk that was analyzed and then individually fortified suggest that targeted fortification may result in growth closer to the growth of preterm infants fed nutrient-dense preterm formula.49

**Conclusion**

The results of this pilot study suggest that the Calais MIR Human Milk Analyzer is a promising device for bedside use but that lactose measurement, and the energy calculations based on it, remain problematic. Human milk analysis by filter-based MIR spectroscopy correlates well with reference laboratory analysis and offers an easy and accurate method to determine macronutrient composition of expressed human milk. Small sample size is a limitation of our study. Larger numbers of samples across a broad range of milk composition may be able to validate MIR spectroscopy and eventually permit its use as a research tool. Validation studies between MIR spectroscopy and laboratory analysis using a larger pool of samples are underway. If approved by the FDA for clinical use, MIR spectroscopy is a potential tool in the nutrition management of low-birth-weight infants in the NICU. Once human milk analysis equipment is sufficiently validated, randomized trials will be needed comparing the growth and body composition of preterm infants managed with information obtained from human milk analysis to the growth and body composition of infants managed with traditional human milk fortification.

**Acknowledgments**

The authors gratefully acknowledge Metron Instruments, Inc, for performing mid-infrared milk analysis.

**Statement of Authorship**

S. Groh-Wargo contributed to the concept and design of the research and the acquisition of the data. S. Groh-Wargo, J. Valentic, S. Khaira, and D. M. Super contributed to the analysis of the data. S. Groh-Wargo, J. Valentic, S. Khaira, D. M. Super, and M. Collin contributed to the interpretation of the data. S. Groh-Wargo drafted the manuscript. S. Groh-Wargo, J. Valentic, S. Khaira, and D. M. Super critically revised the manuscript. All authors read and approved the final manuscript and agree to be fully accountable for ensuring the integrity and accuracy of the work.

**References**


