Clinical Nutrition 34 (2015) 465–476

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Rapid measurement of macronutrients in breast milk: How reliable are infrared milk analyzers?☆

Gerhard Fusch, Niels Rochow, Arum Choi, Stephanie Fusch, Susanna Poeschl, Adelaide Obianuju Ubah, Sau-Young Lee, Preeya Raja, Christoph Fusch*

Division of Neonatology, Department of Pediatrics, McMaster University, Hamilton, ON, Canada

ARTICLE INFO

Article history:
Received 5 November 2013
Accepted 5 May 2014

Keywords:
Fat
Protein
Lactose
Validation study
Freeze thaw cycle
Stability

SUMMARY

Background & aims: Significant biological variation in macronutrient content of breast milk is an important barrier that needs to be overcome to meet nutritional needs of preterm infants. To analyze macronutrient content, commercial infrared milk analyzers have been proposed as efficient and practical tools in terms of efficiency and practicality. Since milk analyzers were originally developed for the dairy industry, they must be validated using a significant number of human milk samples that represent the broad range of variation in macronutrient content in preterm and term milk. Aim of this study was to validate two milk analyzers for breast milk analysis with reference methods and to determine an effective sample pretreatment. Current evidence for the influence of (i) aliquoting, (ii) storage time and (iii) temperature, and (iv) vessel wall adsorption on stability and availability of macronutrients in frozen breast milk is reviewed.

Methods: Breast milk samples ($n = 1188$) were collected from 63 mothers of preterm and term infants. Milk analyzers: (A) Near-infrared milk analyzer (Unity SpectraStar, USA) and (B) Mid-infrared milk analyzer (Miris, Sweden) were compared to reference methods, e.g. ether extraction, elemental analysis, and UPLC-MS/MS for fat, protein, and lactose, respectively.

Results: For fat analysis, (A) measured precisely but not accurately ($y = 0.55x + 1.25$, $r^2 = 0.85$), whereas (B) measured precisely and accurately ($y = 0.93x + 0.18$, $r^2 = 0.86$). For protein analysis, (A) was precise but not accurate ($y = 0.55x + 0.54$, $r^2 = 0.67$) while (B) was both precise and accurate ($y = 0.78x + 0.05$, $r^2 = 0.73$). For lactose analysis, both devices (A) and (B) showed two distinct concentration levels and measured therefore neither accurately nor precisely ($y = 0.02x + 5.69$, $r^2 = 0.01$ and $y = -0.09x + 6.62$, $r^2 = 0.02$ respectively). Macronutrient levels were unchanged in two independent samples of stored breast milk (−20 °C measured with IR; −80 °C measured with wet chemistry) over a period of 14 months.

Conclusions: Milk analyzers in the current configuration have the potential to be introduced in clinical routine to measure fat and protein content, but will need major adjustments.

1. Introduction

International guidelines on nutrition of preterm infants recommend breast milk for enteral nutrition. Its unique combination of essential nutrients leads to a wide range of benefits for the health, growth, immunity, and development of the infant [1,2]. However, feeding breast milk does not guarantee a sufficient nor standard nutrient intake due to inter- and intra-individual variation in the nutrient content of human breast milk, particularly with regards to protein and fat [3–6]. Caloric and macronutrient content of breast milk samples can vary by a factor of up to four and is influenced by many factors such as: frequency of breastfeeding, time of day, early vs. late lactation, parity, maternal diet and age,

Abbreviations: UPLC-MS/MS, ultra performance liquid chromatography-tandem mass spectroscopy; EA, Elemental analyzer.

☆ **Previous presentations**: Rochow N, Fusch G, Choi A, Fusch C. Human Lactoengineering (II): Validation of Breast Milk Analyzers. Poster presentation at the Annual Meeting of the Pediatric Academic Societies, 2012, Boston, USA, Abstract: E-PAS2012:4512.147.

* Corresponding author. Division of Neonatology, Department of Pediatrics, McMaster University, 1280 Main Street West, Room HSC-4F5, Hamilton, ON L8S 4K1, Canada. Tel.: +1 (905) 521 2100x75721; fax: +1 (905) 521 5007.
E-mail address: fusch@mcmaster.ca (C. Fusch).

http://dx.doi.org/10.1016/j.clnu.2014.05.005

and the technique used to pump milk [7–9]. To meet the nutritional requirements, breast milk is commonly enriched with a standard milk fortifier. However, this approach is not able to compensate for nutritional deficiencies in cases where native breast milk composition is below average. Newer fortification methods aim to reduce macronutrient deficits in breast milk by individually adjusting protein and/or fat content [10,11].

To make the target fortification strategy feasible in the NICU, a simple and rapid method to measure macronutrient content at bedside is required. Several commercially-available point-of-care milk analyzer devices have been adopted from their use in dairy industry (Unity, Miris, Esetek, Calais, and Advanced Instruments). However, due to differences between cow milk and human milk, IR analyzers must first be validated for analysis of human milk before being implemented in clinical routine. Some of the differences between cow's milk and human milk include: changes in the ratio of casein to lactalbumin concentration, absence of oligosaccharides in cow milk and different fatty acid profiles. Since milk analyzers use infrared analysis, which is highly influenced by the matrix of milk, differences between the matrices of cow milk vs. human milk may impact the accuracy and precision of the device. Thus, in order to account for variations in the matrix of human milk, validation needs to cover the whole range of available milk samples (i.e. fore, mid, and hind milk; early/late lactation period; various gestational ages). Additionally, the matrix is determined not only by macronutrients but also by pre-analytical treatment such as the degree of homogenization, a factor that has been paid very little attention so far.

Recently, several validation studies on commercially available milk analyzers have been published, however with inconsistent results [12–16]. The studies used small numbers of samples of selected populations (term or late lactation only), various reference methods, and different sample pretreatments.

Thus, the aim of this study was to validate commercially available milk analyzers using the full spectrum of variation of human milk including early and mature milk from mothers of both preterm and term infants, as well as to investigate the influence of sample homogenization on the analysis of breast milk.

2. Materials and methods

2.1. Study design and sample collection

In the present study, milk analyzers were validated against chemical reference methods for the quantification of macronutrient content (i.e. fat, protein, and lactose) in breast milk. Of all commercially available milk analyzers, only those devices were included that utilized 1 mL of breast milk or less, which is an acceptable amount to obtain in clinical practice.

A total of 1188 breast milk samples were collected on two different occasions in order to cover different time points of lactation and various gestational ages at the time of sample collection. The first set of samples was a cross-sectional collection from 40 voluntary donors (term and preterm pregnancies; gestational age: 31.4 ± 5.6 weeks; days of life: 38 ± 36 days). Each donor provided 5 mL of each fore, middle, and hind milk during a single lactation leading to a total of 120 samples. An experienced lactation consultant assisted in collecting fore, middle, and hind milk according to the following scheme: immediately after the start of pumping (foremilk), after 5 min of pumping (middle milk), and 5 min before the expected end of lactation (hind milk). The second set of milk samples was derived from a longitudinal study of 23 volunteers (preterm pregnancies: gestational age 26.4 ± 1.7 weeks, ClinicalTrials.gov: NCT01305642) as reported recently [17]. Samples (5 mL; $n = 1068$) were taken from daily 12-h feeds that were prepared twice a day over a period of 27 ± 18 days and consisted of pooled aliquots of pumped breast milk (fresh and/or stored). Only mothers, who were producing sufficient amounts of breast milk for their infants, were included in the study. All samples were collected in the NICU (level 3) at the McMaster Children's Hospital in Hamilton, Ontario, Canada. The study protocols were approved by the Research Ethics Board at McMaster University. All mothers gave informed written consent.

2.2. Sample treatment

Initially, all breast milk samples (volume of 5 mL) were homogenized for 3 × 10 s using an ultrasonic vibrator (VCX 130; Chemical Instruments AB; Sollentuna, Sweden) and divided into five 1 mL aliquots. Aliquots were subsequently used for two infrared (IR) methods (Near- and Mid-IR spectroscopy; for fat, protein, and total carbohydrates) and three chemical methods (for fat, protein, and lactose). Near-IR spectroscopy was measured immediately (according to the study protocol) using the fresh milk aliquot. Remaining samples were stored at −80 °C for latter analysis using Mid-IR spectroscopy and chemical analyses. Prior to analysis, the stored milk samples were warmed to 37 °C in a water bath and homogenized for 3 × 10 s to ensure constant quality of the samples. Figure 1 depicts the origin of samples and corresponding chemical and IR measurements.

2.3. Analysis of milk samples using commercially available milk analyzers

The devices used for infrared analysis were SpectraStar (Near-IR, Model 2400 RTW, Unity Scientific, Brookfield, Connecticut, USA) and HMA (Mid-IR, MIRIS, Uppsala, Sweden) using the following software: Unity InfoStar version 3.9.0 for SpectraStar and XMA-SW version 2.0.1 for HMA. The measured IR spectra were recorded in the range from 1200 to 2400 nm (Near-IR) and from 3500 to 9600 nm (Mid-IR) for fat, protein, and carbohydrates. All samples were measured three times for Near-IR and twofold for Mid-IR without replacement. Mean values for fat, protein, and carbohydrates were used for latter analysis. The test–retest variability was 0.10, 0.16, and 0.08 g/dL for Near-IR fat, carbohydrates, and protein; and 0.07, 0.10, and 0.04 g/dL for Mid-IR respectively.

2.4. Near-IR milk analyzer

The homogenized sample (1 mL) was directly poured from the vial into the sample cup and covered with the reflector. The transparent bottom of the sample cup was visually checked to ensure there were no air bubbles. The sample cup was positioned on the instrument and scanned for 60 s. Reference scans were automatically performed to check calibration every 30 min. Daily quality controls were performed as described [18,19].

2.5. Mid-IR milk analyzer

The HMA milk analyzer was operated using the homogenized sample mode according to the manufacturer's recommendations. Prior to analysis, a daily calibration check was performed using the calibration solution (MIRIS check), which was provided by the supplier. The homogenized milk sample (1 mL) was injected into the flow cell and measured 60 s.

2.6. Chemical methods for fat, protein, and lactose in breast milk

Chemical analyses of milk samples were performed using validated micro-methods, which required less than 1.5 mL of sample