Original Article

Mothers' Milk Feedings in the Neonatal Intensive Care Unit: Accuracy of the Creamatocrit Technique

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OBJECTIVE:
To establish the accuracy of the creamatocrit (CRCT) for estimating lipid and calories in a heterogeneous sample of own mothers' milk (OMM) in the neonatal intensive care unit (NICU), using a hematocrit reader, rather than fine vernier calipers.

STUDY DESIGN:
In this blinded study, CRCT techniques were performed on 32 fresh OMM samples (7 foremilk, 12 hindmilk, 13 composite milk) that were also analyzed for total lipid and caloric concentration.

RESULTS:
Mean lipid and caloric concentrations for the OMM samples were 50.87 g/l (28.3–86.5) and 703.96 kcal/l (477.2–1183.6), respectively. Results revealed a stronger linear relationship between CRCT and total lipid ($r = 0.94$; $p < 0.001$) than between CRCT and caloric density ($r = 0.76$; $p < 0.001$).

CONCLUSION:
The CRCT, using a hematocrit reader, is an accurate, inexpensive, and useful technique for estimating the lipid and caloric concentration of individual OMM samples in the NICU. The variability in lipid and calories in these 32 OMM samples underscores the utility of this technique.
Journal of Perinatology (2002) **22,** 646–649 doi: 10.1038/sj.jp.7210825

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The unique immunologic and nutritional benefits of own mothers' milk (OMM) for premature infants have been delineated in original research and summarized in recent reviews.[1–7] This evidence has formed the basis for lactation programs in the neonatal intensive care unit (NICU), and for the feeding of OMM to even the smallest premature infants.[8–10] However, individual OMM collections provided by mothers can vary markedly in lipid concentration and caloric density, depending upon the time of day, completeness of breast emptying, and the techniques used to express milk.[11–15] Researchers control for this variability by analyzing and reporting compositional data for *24-hour* OMM collections.[11–14] However, in most NICUs, infants are fed *individual* OMM collections, which often differ significantly from the 24-hour composite values, especially with respect to lipid and calories. We speculated that the creamatocrit (CRCT), a technique that has been used in numerous clinical, laboratory, and field studies,[16–27] would be useful in the NICU to estimate the lipid and calories in these individual OMM specimens.

Although several studies have demonstrated high correlations between the CRCT and standard laboratory analyses for OMM lipid and caloric concentrations,[12,23–27] these OMM samples were collected almost exclusively from mothers of healthy term infants — often in the early days postbirth when colostral lipid concentrations are lower than for mature milk.[12,13] No previous research has established the accuracy of the CRCT for use with high-lipid, postmilk ejection hindmilk, which can be used to achieve accelerated short-term weight gain in small premature infants.[7,19,20,28] Additionally, CRCT measures in previous studies were performed exclusively with fine vernier calipers, which have limited practical utility in the NICU setting, where they are easily dropped, broken, or lost. The purpose of this study was to examine the accuracy of the CRCT when performed on a heterogeneous sample of OMM collections that are commonly encountered in the NICU, using a standard hematocrit reader rather than vernier calipers.

METHODS

Thirty-two freshly expressed OMM specimens were collected from 17 mothers of NICU infants over a 6-month period. All mothers used a hospital-grade electric breast pump with a double collection kit to express milk. Replicate OMM samples from the same mother reflected either specimens collected at least 3 days apart ($N = 7$), mothers who provided both foremilk (premilk ejection, low-lipid OMM) and

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hindmilk at the same milk expression ($N=6$), or mothers who provided separate OMM specimens from the right and left breasts ($N=2$). To ensure heterogeneity in lipid and calories, of the 32 specimens, 7 were foremilk, 12 were hindmilk, and 13 were composite milk (OMM from a complete milk expression, which includes both the foremilk and hindmilk). All composite and hindmilk samples were taken from OMM expressions that were used for infant feedings.

For each OMM collection, one investigator (P.M.) aliquoted a 5-ml sample into a polypropylene collection container. From this container, 1 ml was withdrawn to perform the CRCT and the remainder was immediately frozen at -70 °C. All CRCT techniques were performed and recorded by the same investigator (P.M.) within 30 minutes of milk expression, using a standardized technique as described by Lucas et al.[24] Care was taken to thoroughly mix OMM specimens at each step of the handling to ensure representative lipid sampling. When all 32 specimens had been collected, total lipid and caloric concentrations were determined using gravimetric[29] and bomb calorimetry[30] analyses, respectively. Investigators who performed CRCT (P.M.) and laboratory analyses (R.S.) were blind to the others' measures. This project was approved by the Institutional Review Board at Rush-Presbyterian St. Luke's Medical Center, Chicago, IL.

Data were analyzed using the Statistical Package for the Social Sciences (SPSS Base 10.0) software for windows.[31] Descriptive data were analyzed as described by Engstrom.[32] Correlational analyses were performed using Pearson correlation coefficients. Regression analyses were performed using linear and nonlinear analyses to determine the lines of best fit. Mean differences between estimated and predicted lipid and caloric concentrations were compared with Wilcoxon signed-rank tests.

RESULTS

Mean maternal age for the 17 women was 28.2 (18–40) years; 47.1% was African American, 29.4% was Hispanic/Latina, and 23.5% was Caucasian. Mean infant birth weight and gestational age were 1155.5 g (620–2608) and 28.4 weeks (24–37), respectively. For the 32 OMM samples, the mean and median postbirth sampling time points were 19.9 days (7–82) and 18 days, respectively.

Of the 32 OMM samples, one had insufficient volume for caloric analysis. Thus, data are available for 32 measures of CRCT and total lipid, and 31 measures of calories. Results for the CRCT, total lipid, and calories are summarized in Table 1.

A scatterplot (Figure 1) of the relationship between CRCT and total lipid concentration revealed a significant linear association

Table 1 CRCT, Total Lipid, and Caloric Density: Descriptive Statistics

Measure/milk type	*n*	M	SD	Med	Min	Max
CRCT (%)						
Foremilk	7	8.1	1.8	8.8	5.0	10.0
Hindmilk	12	12.4	2.7	10.9	9.5	17.5
Composite milk	13	9.5	2.4	8.8	6.8	15.0
Total	32	10.3	2.9	10.1	5.0	17.5
Total lipid concentration (g/l)						
Foremilk	7	39.7	8.3	45.3	28.3	46.6
Hindmilk	12	60.7	14.2	58.2	47.9	86.5
Composite milk	13	47.8	14.9	44.3	30.4	81.8
Total	32	50.9	15.5	47.9	28.3	86.5
Total calories (kcal/l)						
Foremilk	7	595	121	527	477	747
Hindmilk	11	782	151	796	509	1089
Composite milk	13	697	198	610	486	1184
Total	31	704	177	706	477	1184
Percentage of calories–lipid (%)						
Foremilk	7	60.9	12.7	56.6	49.2	84.9
Hindmilk	11	70.6	14.0	70.2	55.2	96.8
Composite milk	13	61.9	9.9	60.2	47.1	82.0
Total	31	64.8	12.5	61.1	47.1	96.8

n, number; M, mean; SD, standard deviation; Med, median; Min, minimum; Max, maximum.

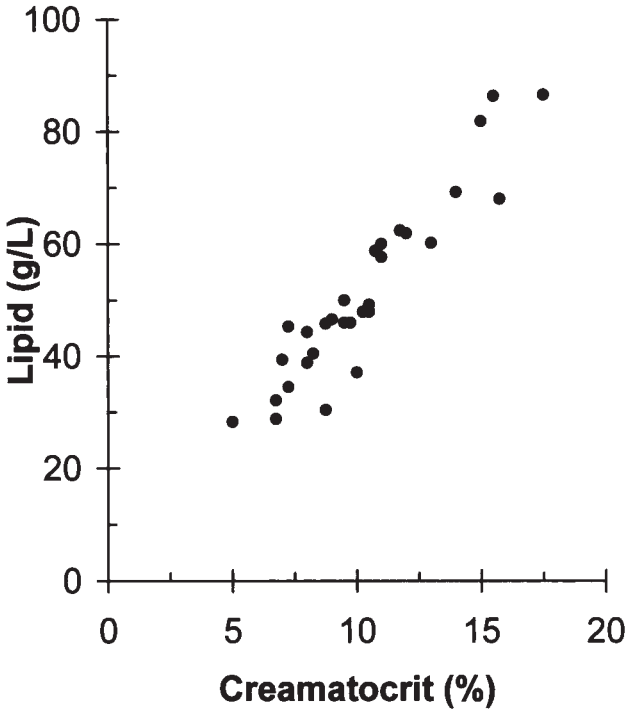

Figure 1. Scatterplot of creamatocrit and total lipid concentration.

($r = 0.94$; $p < 0.001$) across a heterogeneous range of lipid measures (28.3–86.5 g/l). The linear regression equation for this relationship [estimated lipid concentration = −0.238 + (4.97CRCT)] was used to *predict* lipid concentrations for the 32 OMM samples. The mean difference (−0.0045 g/l) between actual and predicted lipid concentrations was statistically similar to zero ($p = 0.81$).

A scatterplot (Figure 2) revealed a significant linear association ($p = 0.76$, $p < 0.001$) between CRCT and caloric density across a broad range of calories (477.2–1183.6 kcal/l). The linear regression equation for this relationship [estimated caloric density = 232.65 + (46.236CRCT)] was used to *predict* caloric density for the 31 OMM samples. The mean difference (0.0026 kcal/l) between actual and predicted caloric density was statistically similar to zero ($p = 0.88$).

DISCUSSION

Our relative differences in lipid and caloric concentrations for the three OMM types are consistent with data reported by Valentine et al.,[28] who also found progressively higher lipid and caloric concentrations in foremilk, composite milk, and hindmilk. However, the heterogeneity in these individual OMM samples, as evidenced by the fact that some women's foremilk contained more lipid and calories than other women's hindmilk, underscores the potential utility of the CRCT in the NICU.

Our data are the first to reveal that the CRCT accurately estimates the lipid concentration for these higher-lipid OMM specimens. The mean total lipid for all 32 specimens in our study was 50.9 g/l (28.3–86.5), and 34% of samples contained >55 g/l. In the classic study of Lucas et al.,[24] only 10 of 59 OMM samples had a lipid concentration >35 g/l, and none was >55 g/l. Similar lipid concentrations were reported by Lemons et al.[25] in their study of 126 OMM specimens from 26 women. Mean lipid concentrations in other examinations of the CRCT revealed 26.6,[27] 28.0,[26] 28.4,[16] 31.5,[17] and 39.0 g/l.[18] Typically, these previous studies have used colostral, transitional, drip, and banked OMM specimens, all of which would be expected to have lower lipid concentrations than mature milk or fractionated hindmilk.

Our linear correlation between CRCT and total lipid concentration (0.94) is slightly lower than those reported by selected other investigators,[12,18,23,24] whose values ranged from 0.97 to 0.99. Although these differences are minor, they probably reflect the fact that our 31 OMM samples contained higher and/or more heterogeneous lipid concentrations than those reported by previous researchers. Additionally, our use of the hematocrit reader — rather than vernier calipers — may partially explain this difference because the hematocrit reader, while simpler to use, does not permit the degree of precision that is possible with vernier calipers.

We found that the linear correlation between CRCT and caloric density, while statistically significant, was weaker than that between CRCT and lipid concentration for the same OMM samples. This result is consistent with those of other investigators whose studies have compared CRCT with both total lipid and caloric density.[25,26] The single exception to this finding is the original study of Lucas et al.,[24] in which the linear correlations between CRCT and both total lipid and caloric density were equally high ($r = 0.99$). However, unlike our study and those of previous investigators, Lucas et al. correlated CRCT with *estimated* measures of caloric density.

The findings from this study are consistent with concerns that have been raised in the scientific literature regarding the quality control of mothers' milk that is used for infant feedings in the

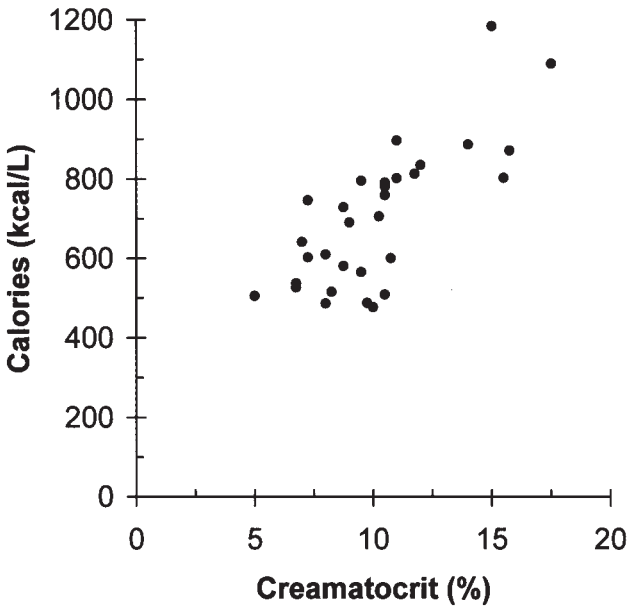

Figure 2. Scatterplot of creamatocrit and total calories.

NICU.[16–18,25] Each of our composite milk and hindmilk samples provided $\geq$50% of the infant's 24-hour intake. This variability in individual OMM samples is most concerning for extremely low birth weight infants during the first weeks of enteral feeding, when the infant's volume requirements are minimal and the mother may produce several times the daily milk volume required by the infant.

For research purposes, the within-mother variability in OMM composition is minimized by collection and analysis of 24-hour samples.[12–14] The results of these analyses have provided compositional standards, which are used for the clinical management of OMM feedings in the NICU.[10] Thus, exogenous oils or commercial formulas are commonly added to modify the ''20-cal'' milk to 27 or 30 cal/oz. Our data, in which lipid represented a mean of 64.8% of OMM calories (with one sample in which lipid was 96.8% of calories), reveal the problem with this management strategy, especially for extremely low birth weight infants.

An accurate, rapid, simple, and inexpensive technique for analysis of individual OMM samples would be useful in the NICU, especially for volume-restricted infants, or for those who gain weight slowly. In a separate study,[19] we demonstrated that the CRCT can be taught to infants' mothers, who perform it as accurately as advanced-practice nurses. Until techniques for more thorough OMM compositional analyses become available in the clinical setting, we conclude that the CRCT, when performed with a hematocrit reader, can provide an accurate and inexpensive measure of lipid and calories in individual OMM samples.

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