

Consistent Circadian Variations in Creamatocrit over the First 7 Weeks of Lactation: A Longitudinal Study

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ABSTRACT

Objective: The objective of this study was to test the hypothesis that fat content of expressed human milk from mothers of preterm infants is higher in samples expressed in the evening than in the morning during the first 7 weeks of lactation.

Methods: The authors collected samples of expressed human milk obtained from 22 mothers of growing preterm infants, born at 26 to 31 weeks gestation, who routinely expressed all their milk every 3 hours using breast pump from the beginning of the second week to the seventh week after delivery. One aliquot was obtained from the first morning expression and the second from the evening expression. The entire aliquot was collected and mixed, and creatinocrit (CMT) was measured in a capillary. Results are expressed as mean $\pm$ standard deviation (SD), and analyses were by repeated measures analysis of variance.

Results: Mean CMT was significantly higher in evening than morning samples during the whole lactation period, week after week ($p < 0.0001$). Neither CMT values nor the morning-evening difference in CMT values correlated with gestational age, birth weight, or week of lactation. Morning CMT correlated significantly with evening CMT ($R^2 = 0.28$, $p < 0.0001$).

Conclusions: Circadian variations in CMT are consistent during the first 7 weeks of lactation. The authors speculate that if higher caloric content expressed human milk is needed in a specific preterm infant, evening samples should be used preferentially, if available.

INTRODUCTION

LITTLE IS KNOWN about circadian variations of fat content of expressed human milk from mothers of preterm infants. The authors have previously described that on the second week of lactation there are circadian variations in creatinocrit (CMT).¹ It is unknown whether such variations are present after that time.

Thus the authors designed the following prospective study to examine whether circadian variations observed on the second week of lactation persist in the following weeks. Specifically, the hypothesis that fat content of expressed human milk from mothers of preterm infants is higher in samples expressed in the evening (after three daily meals) than in the morning (after a night-long fast) during the first 7 weeks of lactation was tested.

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MATERIAL AND METHODS

Sample collection

Samples of expressed human milk were obtained from 22 consecutive mothers of hospitalized growing preterm infants, recruited between January 2004 and August 2004, with a gestational age at birth ranging from 26 to 31 weeks, who routinely expressed all their milk every 3 hours during the day, just before bed, and as soon as they woke up, using a commercial breast pump (Medela AG, Baar, Switzerland). The data of the second week of lactation (first sample of this longitudinal study) of 15 of the 22 infant-mother pairs of this study were used in a cross-sectional study published previously.¹ Milk samples were collected weekly, on the first day of each week, from the second to seventh weeks of lactation. One sample was obtained from the first morning expression (6–9 AM) and the second one from an evening expression (9 PM to midnight). Each mother in the study contributed one single morning and one single evening sample (obtained on the same day) every week. Each mother was instructed to ensure that her breasts were thoroughly emptied, that is, to stop pumping whenever there was a feeling of emptiness, as well as 2 to 3 minutes of pumping without any milk expression.

Laboratory methods

The entire sample was collected and homogenized by hand shaking for 30 seconds, as suggested by Jensen.² Fat content was esti-

TABLE 1. MATERNAL AND NEONATAL CHARACTERISTICS

	Mean $\pm$ SD	Range
Maternal age (years)	30.7 $\pm$ 5.3	20–45
Pre-pregnancy weight (kg)	60.9 $\pm$ 12.8	46–100
Pre-pregnancy BMI* (kg/m ²)	22.4 $\pm$ 4.0	18–34.2
Gestational age (weeks)	28.8 $\pm$ 1.7	26–31
Birthweight (grams)	1198 $\pm$ 373	566–2060
1-Minute Apgar score	6.6 $\pm$ 1.9	3–9
5-Minute Apgar score	8.2 $\pm$ 1.3	5–10

*BMI = body mass index.

mated using the CMT method, as described.^{3–8} Briefly, 75- μ L aliquots were poured into glass capillary tubes, which were sealed at one end and spun in a hematocrit centrifuge for 5 minutes at 9000 rpm. The CMT was read to the nearest 0.5 mm and expressed as a percentage of the length of the milk column in the tube. Each reading was performed in duplicate, in a blinded manner, by an investigator who was not aware of the origin and time of sampling. The results of the two readings were averaged. This method is extremely precise, reproducible, and has an intra-assay and inter-assay coefficient of variation of less than 1%. The CMT value highly correlates with total lipid biochemical measurements,⁹ making it an “accurate, inexpensive and useful technique for estimating lipid concentration” of expressed breast milk.⁹

Statistical analyses

Results are expressed as mean $\pm$ SD. Repeated ANOVA was used to study the circadian differences, while taking into account ges-

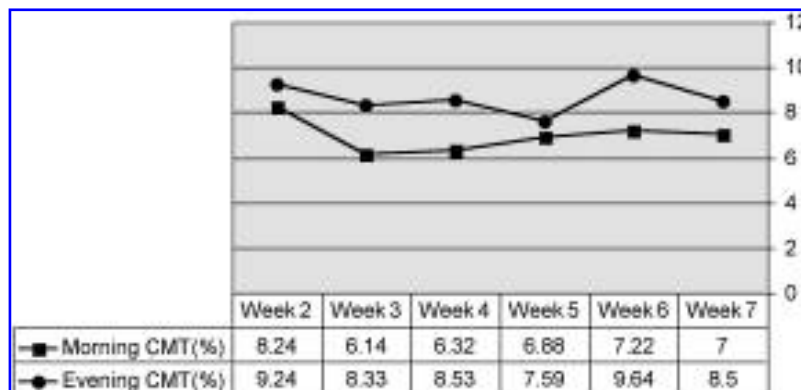


FIG. 1. Mean morning and evening creatocrit values (%).

TABLE 2. MORNING AND EVENING CREAMATOCRIT VALUES (%)

	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Morning CMT (%)						
Mean $\pm$ SD	8.24 $\pm$ 3.24	6.14 $\pm$ 2.31	6.32 $\pm$ 2.21	6.88 $\pm$ 2.47	7.22 $\pm$ 2.81	7.0 $\pm$ 1.0
(Range)	(4–17)	(3–13)	(4–12)	(4–12)	(4–14)	(4–10)
Evening CMT (%)						
Mean $\pm$ SD	9.24 $\pm$ 3.24	8.33 $\pm$ 3.38	8.53 $\pm$ 2.52	7.59 $\pm$ 2.81	9.64 $\pm$ 4.16	8.5 $\pm$ 2.30
(Range)	(5–20)	(3–16)	(4–14)	(2–12)	(5–18)	(5–14)

CMT, creatinocrit.

Data are expressed as mean $\pm$ SD (range).

tational age, birth weight, and week of lactation (time factor). Regression analysis was used to study the correlation between morning and evening CMT. A p -value of <0.05 was considered significant.

RESULTS

Demographic and maternal characteristics of the participants in this study are presented in Table 1. Mothers were healthy, had not suffered from hypertensive disorder of pregnancy or diabetes, and all were omnivorous.

Repeated measures ANOVA demonstrated significantly higher CMTs in the evening than morning samples ($p < 0.0001$), as shown in Figure 1 and Table 2, whereas during the week of lactation the gestational age and birth weight did not influence the CMT values. On average, morning CMT was lower than evening CMT by $1.65\% \pm 2.8\%$. Morning CMT values correlated significantly with those in the evening ($R^2 = 0.28$, $p < 0.0001$) (see Fig. 2).

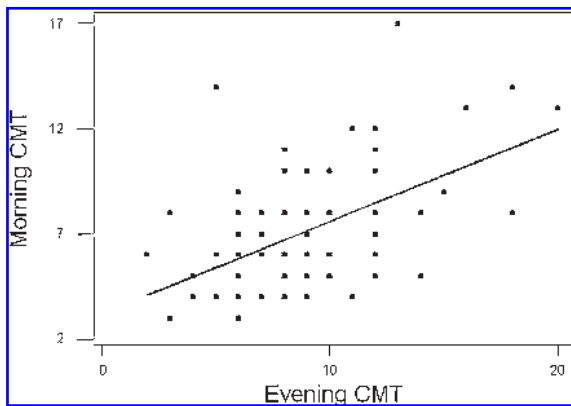


FIG. 2. Correlation of morning and evening creatinocrit values (%).

DISCUSSION

In a previous, cross-sectional study, it was demonstrated that human milk expressed by mothers of preterm infants during the second week of lactation¹ undergoes circadian variations in fat content. In the present longitudinal study, it was demonstrated that circadian variations in CMT occur consistently over the first 7 weeks of lactation in human milk expressed by mothers of preterm infants. These variations in CMT most likely represent variations in fat and energy content, as CMT is closely related to both.^{4,8} It was also demonstrated that average CMT does not undergo significant changes over the first 7 weeks of lactation.

The mechanism by which morning human milk appears to be lower in fat and energy than evening milk is unclear. A likely possibility is that breast fullness (higher in the morning than in the evening)¹⁴ may be influential, in particular in view of the fact that total fat content is lower in foremilk than in hind milk,¹⁵ and in spite of the fact that mothers were asked to pump their breast as fully as possible. Indeed, in their important study, Daly et al. showed that 41% to 95% of the variance in fat content of human milk is explained by the degree of breast emptying.¹⁴ In another study, Daly et al. showed that changes in rate of milk synthesis were directly associated with the degree of emptying of the breast, results that indicate the importance of degree of breast emptying in the short-term control of human milk synthesis.¹⁶ Thus, the morning–evening difference in CMT values might reflect a simple dilutional effect. A limitation of the present study is that maternal milk volumes were not prospectively obtained for each pumping for which creatinocrit was tested. If it was found that more milk

was removed in the morning pumping, this would have supported the theory that morning milk is more "dilute." An alternative explanation would be that the observed circadian rhythm in human milk fat content is dependent on circadian variations in maternal diet. Indeed, most dietary intake of adults is diurnal, with night fasting. Thus, if maternal fat intake was influential on human milk fat content, it would be possible that circadian changes of fat content in milk parallel the dietary habits of the mother. However, most studies, whether performed in developing or developed countries, have failed to demonstrate a clear relationship between maternal energy intake and milk fat content.^{10–12} In one single study by Insull et al., daily fat milk production was affected by dietary manipulations, and correlated with high energy and fat intake by the mother.¹³ Another study conducted in poorly nourished lactating women found that dietary habits were influential on milk fat content.¹⁷

A potential clinical application of the present findings is that, whenever a high-energy content milk is needed in preterm infants, evening-collected samples could be used preferentially. However, whenever this is done, one should make sure that protein intake is not sacrificed for calories. Additional studies are required to determine whether it is possible, through maternal dietary manipulations, to enrich the fat and energy content of human milk.

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