

## A Paradigm Shift in Fortification for Preterm Newborns

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### Introduction

Human milk is the preferred form of nutrition for nearly all infants. Human milk is uniquely specific for the nutritional needs of newborns, improves digestibility, provides unique immunological components, and is associated with a reduced incidence of sepsis. For preterm infants, however, human milk may not meet protein and mineral requirements. As a result, human milk fortifiers are available and are often added to increase the nutritional density of human milk.

Human milk is highly variable between mothers, at varying points throughout the day, and over time. In order to optimize the growth patterns and clinical outcomes of preterm infants, recent research has suggested that it is necessary for human milk to be fortified based on the composition of an individual mother's milk. Infrared analysis has been proposed as a viable option for analyzing the macronutrient composition of human milk.

In the present study, we analyze protein, fat, lactose, and caloric content of human milk from mothers of very low birth weight infants using mid-infrared technology. In addition, we determine the potential significance of the use of such technology in the clinical setting.



Figure 1. Calais Milk Analyzer

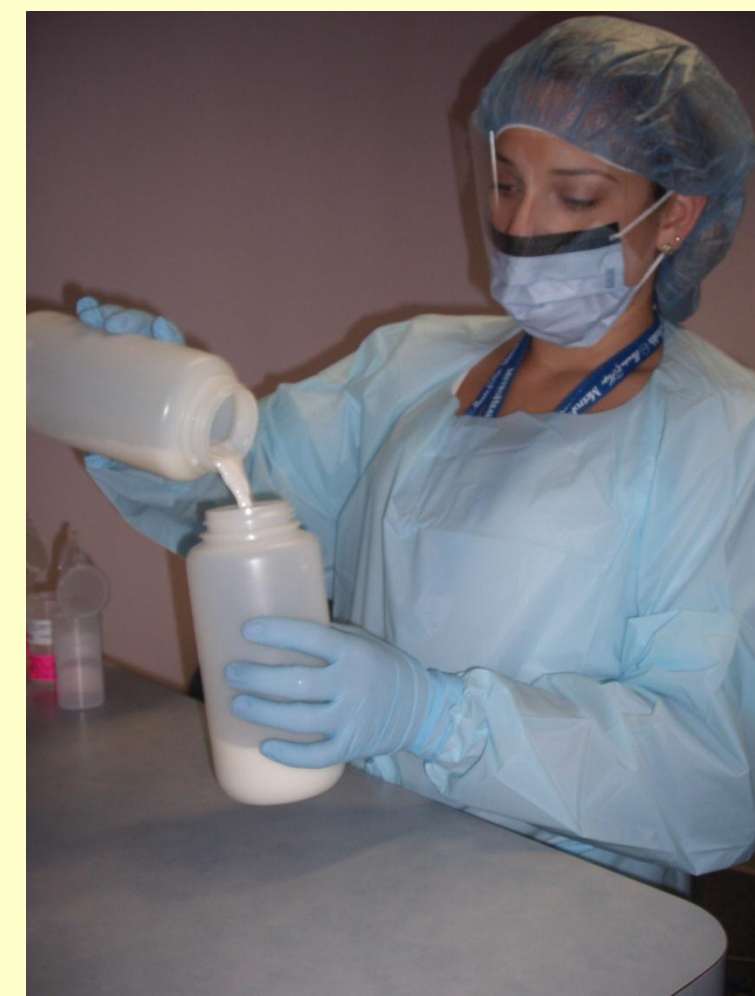


Figure 2. Homogenization procedure

### Methods

This prospective study was approved by the MetroHealth IRB. Subjects were recruited from the neonatal intensive care unit (NICU) at MetroHealth Medical Center in Cleveland, OH. Inclusion criteria for the study encompassed non-minor mothers of infants weighing <2000 g who were pumping breast milk.

Recruited mothers brought their 24-hour supply of fresh breast milk daily or whenever they visited. The work area was cleansed with a sterilization cloth and a gown, bonnet, mask, and gloves were put on by the investigator. The total volume of the supply was summed. The 24-hour supply was then poured into a mixing bottle. The supply was homogenized by pouring the milk between two mixing bottles five times. A 15-ml aliquot was removed from the supply and poured into a subject-coded, labeled container. The aliquot was frozen and the remaining breast milk was repackaged and refrigerated or frozen per the nurse's request.

The aliquots were analyzed on a weekly basis using mid-infrared technology (Calais Milk Analyzer, Metron Instruments, Solon, OH. See Figure 1). The analyzer was calibrated to breast milk values. Fat, lactose, total protein, true protein, and caloric data for each aliquot were reported. Bioavailable Protein (BAP) was calculated using the following formula:

$$\text{Total Protein} \times 0.825 = \text{Bioavailable Protein}$$

If the mother had over 200 ml of milk for five consecutive collection days, a one time 60 ml sample was taken for future reference chemistry analysis.

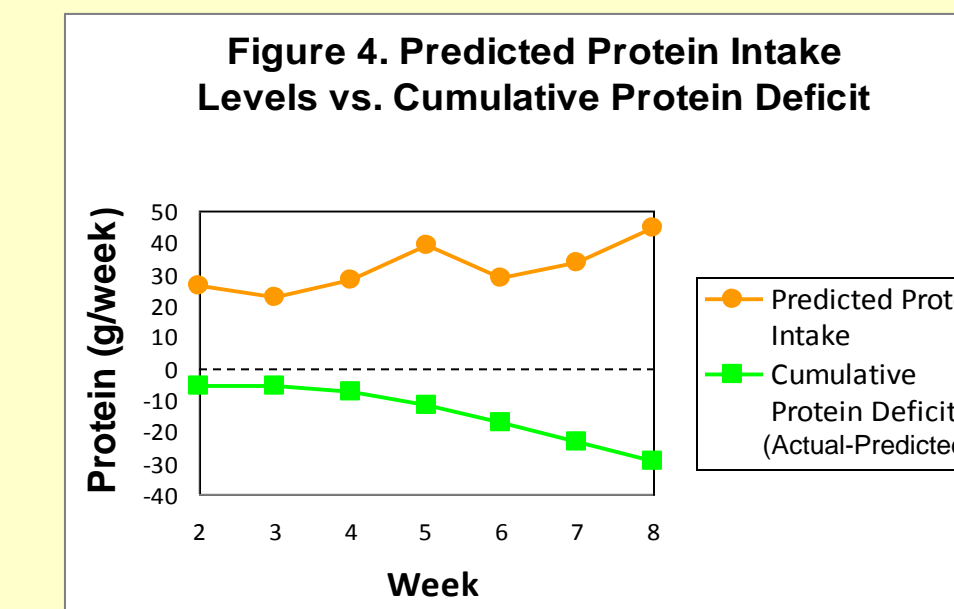
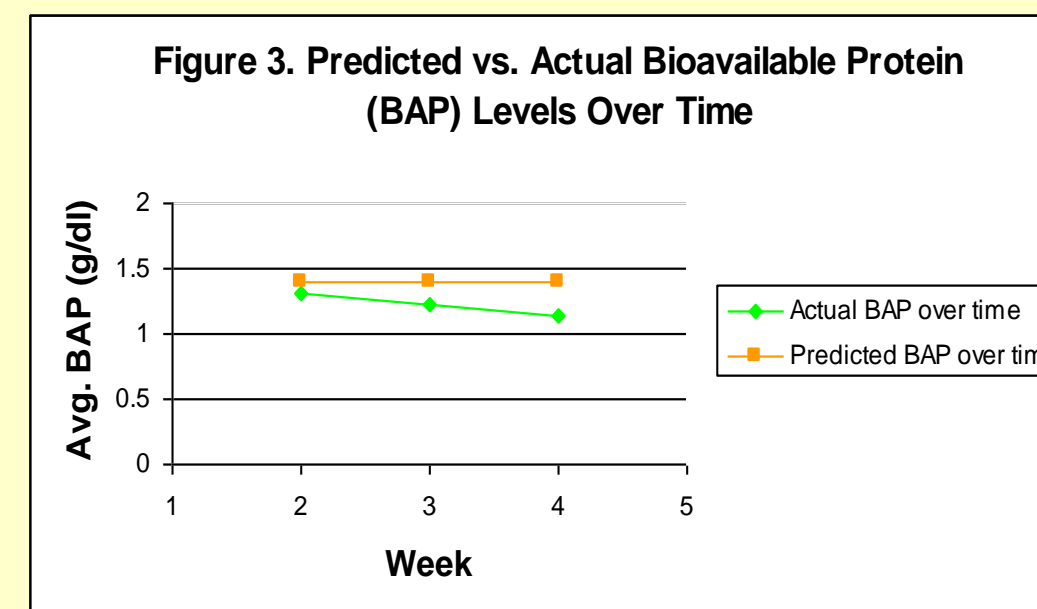
Demographic, growth, and milk composition information was collected (Tables 1 and 2). Statistical analysis was performed using SPSS (Windows version 13; SPSS Inc., Chicago, IL). Descriptive statistics, Friedman test, paired T-tests and Pearson Correlations are reported.

### Results

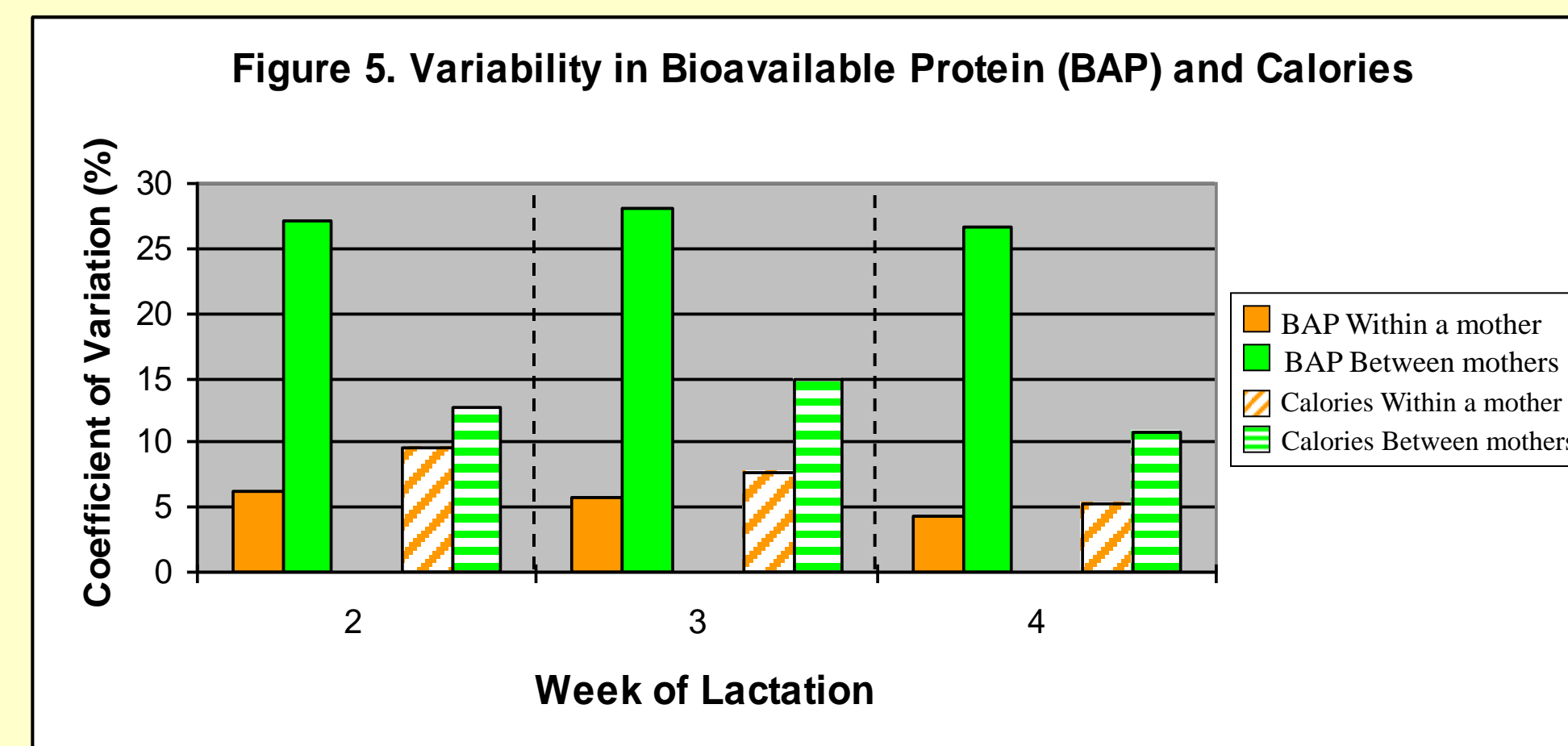
BW, g Mean ± SD	GA, weeks Mean ± SD	Male Gender, n (%)	Race/Ethnicity, n (%)
1249 ± 278	29w4d ± 2w1d	12 (80%)	Hispanic, 8 (53%) Black, 4 (27%) White, 3 (20%)

Note: BW is birth weight. GA is gestational age.

Post Natal Day of Life	Mean ± SD			
	Fat (g/dl)	BAP (g/dl)	Lactose (g/dl)	Kcals/Oz.
Median: 20 Range: 7-62	3.72 ± 1.0	1.18 ± 0.34	6.56 ± 0.42	19.35 ± 3.0



A total of 124 samples from 13 mothers were collected and analyzed. Protein content significantly decreased over time ( $p < 0.05$ ) and was less than published, predicted values (Figure 3), while fat, lactose, and caloric content remained constant. Fat and calories were highly correlated ( $r = 0.996$ ) with a linear equation of  $Y = 3.039X + 8.1$ , where  $Y = \text{kcal}$  and  $X = \text{g fat}$ . A paired T-test between predicted and actual protein and calorie intake of the subjects did not reveal a significant p value, however, the cumulative deficit, specifically for protein, becomes disadvantageous over time (See Figure 4). Coefficients of variation were less within a mother than between mothers for protein and calories (Figure 5), as well as for fat and lactose.



### Discussion

The macronutrient composition of human milk differs significantly both between mothers and within a mother's own supply, especially over time. Caloric density stays relatively stable over time, is substantially driven by fat content and is variable between mothers. Milk protein levels decrease over time, creating a cumulative protein deficit. This trend is detrimental and does not support the rapid growth of preterm infants.

It is evident that the variability is significantly less within a mother than between all mothers for all nutrients. Low variability of macronutrient content within a mother's own milk supply suggests that occasional, rather than frequent, analysis could be used clinically to establish an individualized fortification plan. The significant variability between mothers strongly supports the use of milk analysis in the nutritional management of preterm infants.

### Conclusions

- Protein content of preterm milk was less than predicted.
- Actual protein intake was less than predicted, creating a cumulative deficit.
- Fat content and caloric density are highly correlated.
- The variability was significantly lower within a mother than between mothers for all nutrients.
- Analysis of individual mothers' milk will allow for improved fortification regimens.

### Future Considerations

The 60 ml samples were sent to DQCI laboratories (Mounds View, MN). These reference values will be compared to values obtained from the Calais.

### References

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