Original Research Article

Predictors of Breast Milk Macronutrient Composition in Filipino Mothers

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Objectives: There is increasing evidence that breastfeeding has long-term effects on offspring biology and health, which has heightened interest in understanding the extent of variation in breast milk composition and its underlying determinants. Here, we report variation in milk macronutrient composition in a well-characterized cohort of young Filipino mothers and test underlying maternal predictors of this variation.

Methods: Morning breast milk samples, anthropometrics, dietary recalls, and other interview data were collected in 102 Filipino young breastfeeding mothers (age range 24.6–25.4 years) living in Cebu City, Philippines. Milk samples were analyzed for protein, fat, sugar, and milk energy density. Regression models were used to test associations between milk macronutrient composition and maternal diet, body composition, breastfeeding duration, and feeding frequency.

Results: Consistent with past studies, milk fat and energy increased with duration of breastfeeding; there were no associations between maternal diet or percent body fat and milk composition with the exception of a modest, inverse association between maternal adiposity and milk sugar content.

Conclusions: The relative lack of associations between maternal diet or body composition and milk composition at Cebu is consistent with past studies and suggests that milk composition may be buffered against fluctuations in maternal dietary intake or nutritional status. We speculate that the tendency for milk composition to vary between populations faced with different nutritional ecologies, but to show minimal responsiveness to intake during lactation, may enhance the reliability of milk composition as a stable intergenerational cue of typical local environmental quality. Am. J. Hum. Biol. 00:000–000, 2012.

INTRODUCTION

The benefits of breastfeeding for infant health include reduced infectious disease morbidity and mortality (Duijts et al., 2009) and improved cognitive and physical development (Daniels and Adair, 2005; Isaacs et al., 2009). In addition, there is increasing evidence that breastfeeding is associated with improved long term health outcomes for infants such as reduced risk of diabetes, obesity, cardiovascular disease, and other adult-onset metabolic disorders (Grummer-Strawn and Mei, 2004; Koletzko, 2006; Owen et al., 2003, 2006). These findings raise questions about the biological basis for these differences. In particular, the nutrient and hormonal composition of breast milk has emerged as a likely influence on individual physiological development (Koletzko et al., 2009; Riva et al., 2004).

Although once considered relatively homogenous (Hall, 1979), the variation within human milk has recently received more research attention (Jensen, 1999; Prentice, 1995; Wojcik et al., 2009). Table 1 summarizes milk macronutrient composition from past studies in human populations. Average fat content of human milk across populations varies from 2.8 to 4.78 g/dl, with women in the same population showing equal, if not greater amounts of variation than different populations (Prentice, 1995; Wojcik et al., 2009). Carbohydrate and protein content of milk show less variance, with carbohydrates generally ranging from 6.5−8.0 g/dl and protein constrained from 0.9–1.5 g/dl in mature milk (Table 1). Colostrum, the milk produced in the first week postpartum, has more protein but much lower carbohydrate and fat content when compared with mature milk (Macy, 1949).

Although maternal factors such as diet and body composition are plausible influences on this variation, most studies find weak or modest associations between maternal characteristics and the nutrient composition of the mother's milk. For instance, milk macronutrient composition appears to be largely independent of maternal diet (Coward et al., 1984; da Cunha et al., 2005; Dewey, 1998; Villalpando and del Prado, 1999) and anthropometric measures of maternal body composition have inconsistent associations with milk composition. Milk protein is usually independent of body composition while the majority of studies report a modest association between body composition and milk fat, sugar, and total energy (Allen et al., 1991; Barbosa et al., 1997; Brown et al., 1986; Forsum and Lonnerdal, 1980; Prentice et al., 1981a; Villalpando and del Prado, 1999). Associations between body fat and milk fat, if present, are usually positive, whereas milk sugar

Contract grant sponsor: National Science Foundation (Doctoral Dissertation Improvement); Contract grant number: 0726231; Contract grant sponsor: National Science Foundation; Contract grant number: BCS-0746320.

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Received 10 October 2011; Revision received 7 February 2012; Accepted 9 February 2012

DOI 10.1002/ajhb.22266

Published online in Wiley Online Library (wileyonlinelibrary. com).

TABLE 1. Variation in milk macronutrient content in a sample of populations using similar or overlapping methodologies

Population	Fat	Lactose	Protein	Reference
Philippines (Manila)	3.93	7.31	0.85	WHO, 1985
The Gambia	3.78	7.74	1.09	Prentice et al., 1981a
Australia	3.74	6.14	0.92	Mitoulas et al., 2002
Bangladesh	2.66	8.08	1.00	Brown et al., 1986
Sweden	5.69	6.70	0.83	WHO, 1985
Guatemala	2.40	8.00	0.94	WHO, 1985
Zaire	3.30	6.30	1.30	WHO, 1985
USA (DARLING)	3.80	7.40	1.10	Nommsen et al., 1991
Mean	3.66	7.21	1.00	

Macronutrients are presented as grams per 100 ml and calculated where necessary using a nitrogen to protein conversion factor of 6.38.

and maternal adiposity typically show an inverse relationship. Milk composition has also been shown to change over the course of lactation (Lubetzky et al., 2007; Mandel et al., 2005; Mitoulas et al., 2002; Nommsen et al., 1991), possibly reflecting developmental maturation of the lactating breast or differences in nursing frequency as the infant ages. These generally weak and inconsistent relationships between maternal characteristics and milk composition may be evidence for physiological buffering of milk composition. Evidence for marked variation in milk composition among individuals that does not relate to current diet or body composition would point to the likely importance of other factors, such as early life nutrition or growth history of the mother (Lammers et al., 1999; Van Amburgh et al., 1998), as potentially important influences on milk composition.

In this study, we describe the extent of variation and maternal predictors of the energy density and macronutrient composition of breast milk collected from 102 Filipino mothers participating in a longitudinal birth cohort in Cebu City, Philippines. These women represent all original cohort members who were still participating in the study in 2007 and were currently lactating at the time of interview. Although the mothers in this study were drawn from a longitudinal birth cohort, they were recruited into the study at different time points in their lactations, resulting in a cross sectional sampling of milk composition and infant age. Breastfeeding behaviors vary by infant age with older infants generally nursing less than younger infants, particularly following the introduction of complimentary foods. The decline in nursing frequency and bout duration will result in a decrease in milk production. However, in Cebu, there is only a modest decline in breastfeeding frequency for mothers nursing older infants, and infant age and nursing frequency were tested as independent predictors of milk macronutrient and energy content. Other groups have reported age related differences in milk macronutrients and energy without consideration of age related changes in nursing frequency (Michaelsen et al., 1990; Mitoulas et al., 2002). We hypothesized that milk fat and energy would increase while sugar would decline with increasing infant age in this sample, following trends previously described in the literature. Building from evidence for maternal buffering of milk composition reported in other populations, we predicted that there would be few or no significant associations between current maternal diet or body composition and milk composition (Brown et al., 1986; Nommsen et al., 1991; Prentice et al., 1981a, 1994; Villalpando and del Prado, 1999; Villalpando et al., 1992, 1998).

MATERIALS AND METHODS

Subjects

Participants were drawn from the Cebu Longitudinal Health and Nutrition Survey (Cebu Study) an ongoing, longitudinal study of a one year birth cohort (1983–1984) from Cebu City (see Adair et al., 2011). At its inception, 3,327 pregnant women were enrolled in this study and they and their offspring have been followed longitudinally at regular intervals until the present. The infants born in 1983–1984 are now adults, and the majority of have started their own reproductive careers. During a tracking survey conducted from September 2007 to March 2008, 848 of the original female cohort members were identified and interviewed. Any female participant who was currently lactating or was in their third trimester of pregnancy was invited to participate in the present study. The majority of women from the Cebu Study who have given birth since 1998 have breastfed (96%), with prematurity the most common reason why women elected not to breastfeed. All eligible women agreed to participate, resulting in an initial sample size of 131. Five women were later removed from analyses because of non-nutritive suckling or concurrent pregnancy giving a sample of 126 women. For the purposes of these analyses, only women who had been nursing for less than 18 months were included, resulting in a final sample size of 102 mothers and infants. The sample was truncated at 18 months as breastfeeding becomes increasingly erratic in this population after 18 months, with the majority of infants nursing very few times a day with extended periods between bouts. Simple t-tests for unequal sample sizes were used to test for between-group differences in maternal characteristics between those women nursing for less than 18 months and those women nursing for longer than 18 months. There were no significant differences in education, household assets, dietary energy, or maternal body fat or weight (all $P > 0.10$) between women nursing for less than or greater than 18 months in this sample. Infant characteristics of length and weight were significantly different between the two groups, as expected given that one group was considerably older than the other.

Interviews and anthropometrics

Mothers were interviewed and measured in their homes between 6 am and 10:30 am. A Cebuano speaker who was familiar to the participants (FL) interviewed all mothers. Standard anthropometric measures (height, weight, triceps, biceps, subscapular, and suprailiac skinfolds, MUAC) were collected on all mothers and infants using standard techniques. All measurements were done in triplicate and had minor variation between measurements. Dietary information was collected using a single dietary recall with measuring cups to prompt portion sizes. Recalled dietary information was then converted to energy and macronutrient units using reference values for common Filipino foods available from the Food and Nutrition Research Institute of the Philippines (FNRI, 1997). Maternal BMI was calculated from weight and height measurements. Percent body fat was calculated using the four-site skinfold (triceps, biceps, subscapular, suprailiac) formula of Durnin and Womersley (1974), which has been validated for use in several related Asian populations (Deurenberg et al., 2002). Nursing frequency and duration were self-reported by mothers for the prior 24 h.

Milk collection and analysis

Milk samples were collected by hand expression following the protocols of Ruel et al. (1997). Each infant was allowed to suckle for 2–3 min. At this time, the infant was switched to the opposite breast to continue nursing and the mother hand expressed milk from the initial breast. Mothers were asked to express 10 ml of milk into a sterile polypropylene cup (Philips AVENT Via commercial cup). The samples were sealed, placed on freezer packs, and transported within 2 h to the University of San Carlos, where they were vortexed to assure uniform mixing, aliquoted, and stored at −35°C. Samples were shipped frozen on dry ice to the United States for compositional analysis.

Milk composition of each sample was analyzed at the Nutrition Laboratory at the Smithsonian National Zoological Park using standard methodology (Hinde et al., 2009; Milligan et al., 2008; Oftedal, 1984; Power et al., 2002). Total dry matter, fat, protein, and sugar were determined for all samples, and milk energy density was calculated from these values. Milk fat was extracted using a micro Rose-Gottlieb procedure with sequential extractions by ethanol, diethyl ether, and petroleum ether (Power et al., 2008). Dry matter was determined gravimetrically by weighing a 20 μl sample of milk to the nearest 0.001 mg. These were dried for 3 h in a forced air oven at 100°C. Dried samples were reweighed to determine dry matter, followed by combusted in a CHN elemental analyzer (Model 2400, Perkin-Elmer, Norwalk, CT) to measure total nitrogen. The values obtained from combustion were converted to crude protein by multiplying the total nitrogen by 6.38. Milk sugar was measured using the phenol-sulfuric acid method (Barnett and Tawab, 1957; DuBois et al., 1951); values may be slightly higher than those reported in other populations measuring lactose, as this method quantifies total milk sugars rather than specifically lactose. All samples were run in duplicate or triplicate, any sample with a CV greater than 10% for fat, and 2% for nitrogen or sugars was rerun. Total milk energy was determined from these proximate macronutrient measures using conversion factors of 9.25 kcal/g for fat, 5.86 kcal/g for protein, and 3.95 kcal/g for sugar (Garza et al., 1985). Although this formula may slightly overestimate the energy content of milk compared with other measures such as bomb calorimetry (Garza and Butte, 1986; Hinde et al., 2009; Oftedal, 1984), this formula was recently shown to provide values identical to results from bomb calorimetry for rhesus macaque milk measured on the same equipment (Hinde et al., 2009).

Limitations of the study design

This study relied on a single sample of human milk, collected between 6 am and 10 am, as discussed above. This time has been shown elsewhere to be the most representative of 24 milk averages (Ruel et al., 1997). A 10 ml sample of milk was collected after 2 min of nursing, reflecting an early to mid-feed sample of milk. This reflects a compromise between the ideal sampling method of a full mammary expression and concerns about the risks such a sampling method would impose on mothers and infants. We were also unwilling to give a portion of the sampled milk back to the infant in a bottle, both out of concern for reinforcing bottle feeding and limited access to appropriate storage conditions for the milk. Small samples, either paired before and after the infant nurses (Kent et al., 2006; Mitoulas et al., 2002) or mid-feed samples (Xiang et al., 2005), have been used elsewhere. Neville et al., (1984), comparing fore, mid, and pooled breast milk samples reported less than 13% coefficient of variation for residuals of protein, lactose, and several micronutrients between samples, and suggested that mid-feed samples could be an alternative to full mammary expression for analyses of these specific components. However, milk fat does vary significantly across a single feeding (Daly et al., 1993; Neville et al., 1984) and fat measures may be biased accordingly. It has been shown that milk composition varies across a single feed (Daly et al., 1993), across a day (Daly et al., 1993; Kent et al., 2006; Ruel et al., 1997) and even between mammary glands (Daly et al., 1996; Mitoulas et al., 2002). There is some possibility that some of the associations reported here may be the product of differences in sampling protocols. Kent et al. (2006) have shown that while milk fat content is independent of nursing frequency, it related to time of day with morning samples tending to be lower in overall fat. Pooled 24 h samples reflect the gold standard for collection; however, the nutritional impact and disruption of normal nursing behaviors makes them problematic for field use. Ruel et al. (1997), as previously described, has validated samples collected between 6 am and 8 am as the best time for a single sample approximation of milk composition, particularly for fat and energy, although again, here we present early to mid-milk samples rather than a full mammary expression as done in the original study.

Statistical analyses

All statistical analyses were performed using Stata 10.0 (College Park, TX). Analyses first included evaluating distributions and descriptive statistics, which allowed comparisons between the sample and values derived from other human populations. A series of multivariate linear regression models were then used to test for associations between the outcome variables (human milk fat, protein, sugar, and total energy) and several candidate predictor variables suggested as predictors of milk composition in prior studies (mother's BMI, percent body fat, dietary energy and macronutrient intake, and infant age). Regression models testing for an association between milk composition and maternal parity and infant sex found no significant association between these predictors and milk macronutrients, which were not included in the models reported below.

RESULTS

Sample characteristics are shown in Table 2. Most participants were in the average BMI range with relatively low percent body fat. Because the sample was drawn from a one year birth cohort, all mothers were within 1 year of age when measured (age range 24.6–25.4 years). Infants averaged 8.4 ± 5.5 months of age (range 2 weeks to 18 months). Almost a quarter of mothers were primiparous, 39% were on their second child, 23% were on their third child, and 14% had had four or more children (range 4–6). The majority of multiparous women had previously breastfed (94.8%). The majority of infants were receiving foods in addition to breast milk (82%) with formula first being introduced at an average age of 2.5 ± 4 months and solids at 5.9 ± 2.4 months. Over half the mothers (54%) had resumed menstruation at the time of sample collection.

Milk energy, fat, protein, and sugar were within the ranges reported for other human populations (Picciano, 2001; Prentice, 1995; Wojcik et al., 2009). Average milk energy for this population was 0.72 ± 0.15 kcal/g of milk, with fat representing 3.81 ± 1.5%, protein 1.34 ± 0.48%, and total sugars 7.31 ± 0.58% of total weight. Regression models linking maternal characteristics with milk energy, fat, protein, and sugar are shown in Tables 3–6. There were significant changes in some milk macronutrients with increasing duration of lactation (age of nursing infant). As duration increased, milk fat increased while milk sugar declined, resulting in a moderate increase in caloric density of milk. There was no association between lactation length and protein content.

TABLE 2. Descriptive characteristics for the sample

	Mean (SD)	Range
Maternal characteristics (n = 102)		
Age (years)	23.8 (0.3)	23–24
Height (cm)	150.5 (5.9)	138–163.8
Weight (kg)	46.3 (8.6)	29.1–74.5
BMI (kg/m^2)	20.4 (3.5)	14.5–35.9
Body fat (%)	24.9 (3.9)	9.6–34.8
Primiparous (%)	24.5%	
Resumed menstruation (%)	53.9%	
Education (yrs)	9.8 (2.6)	2–15
Maternal dietary intake		
Dietary energy (kcal/day)	1,411.3 (720.1)	257.7–3,478.1
% kcal from fat	19.3% (13%)	2–57%
% kcal from carbohydrates	50.0% (10%)	22–66%
% kcal from protein	19.3% (5%)	11–38%
Infant characteristics		
Age at milk collection (days)	253.0 (165.4)	9–548
Feeding frequency (bouts/day)	12 (3.6)	4–24
Infant weight (g)	7,036 (1,823)	3,170–10,215
Infant length (cm)	65.8 (8.4)	46.9–79.7
Infant BMI	15.9 (1.5)	12.9–19.5
Infant sex	51% Male	
Birth weight (g)	3,037 (668)	1,364–4,545
Milk composition		
Fat (% wt)	3.8 (1.5)	0.98–9.37
Protein (% wt)	1.3 (0.48)	0.84–3.24
Sugars (% wt)	7.3 (0.58)	4.96–8.91
Total energy (kcal/gram)	0.72 (0.15)	0.43–1.24

Self-reported feeding frequency, a known predictor of milk fat (Daly et al., 1993), has also been shown to decrease with breastfeeding duration, as older infants nurse less frequently than younger infants. We evaluated whether a mother's self-reported nursing frequency predicted milk fat and other milk nutrients (Table 7). Both infant age and nursing frequency were independent predictors of milk fat content, with opposite relationships: as nursing frequency increased milk fat decreased as expected. Breastfeeding frequency was not associated with infant age except at 6 months when mothers reported nursing frequency decreasing from 13 bouts per day to 11 bouts after which nursing frequency remained relatively stable through 18 months postpartum.

Subsequent models (Models 1–3) evaluated whether other maternal characteristics predicted each milk compositional outcomes (Tables 3–6) independent of any duration-related changes. Maternal dietary energy or sources of calories were not significant predictors of any milk macronutrient in this sample. The mother's BMI was inversely associated with milk sugar but not significantly associated with fat or energy content of milk. Adjustment for infant age led to minor changes to these coefficients. It may be that differences in milk composition predicted by BMI were not simply secondary to differences in infant age (a result of women postpartum weight loss) but independent predictors of milk composition. When we tested whether maternal BMI changed significantly with infant age, BMI and infant age were not significantly correlated in this sample. This supports the interpretation that these characteristics have independent associations with milk composition. Milk energy, however, will not be independent of milk macronutrient content, and differences in milk energy in the absence of milk volume measurements will reflect differences primarily in milk fat and sugar.

To clarify relationships with adiposity, models were repeated with percent body fat replacing BMI as the primary predictor. There were no significant associations between percent body fat and milk macronutrients or energy in this sample. Similarly, there were no associations between maternal dietary energy or percentage of dietary calories from fat, protein, or carbohydrate and any milk macronutrient or total milk energy.

ANCOVA was used to test for differences in milk macronutrient content by maternal parity and resumption of menstruation. Neither were significant predictors of any milk macronutrient or total energy in this sample. Models 1–3 were rerun using milk macronutrients expressed as milligrams per kilocalorie. There were no differences in the results between the two sets of models.

TABLE 3. Regression models testing associations between maternal predictors and milk energy content

	Unadjusted B (SE)	$R2$	Model 1	Model 2	Model 3
Infant age (months)	0.005 (0.003)*	0.03	0.005 (0.003)*	0.005 (0.003)*	0.005 (0.003)*
BMI (kg/m^2)	−0.009 (0.004)**	0.04	−0.009 (0.004)		
Body fat (%)	−0.004 (0.004)	0.01		−0.002 (0.004)	
Energy intake (kcal)	−0.034**0.028)	0.02			−0.036 (0.029)
% kcal from fat	−0.038 (2.068)				0.113 (2.043)
% kcal from protein	0.131 (1.546)				0.193 (1.527)
% kcal from carbs	−0.142 (2.907)				0.023 (2.872)
Model R^2			0.079	0.039	0.055

Models 1–3 are adjusted for breastfeeding duration, measured in months.
*$P < 0.1$, **$P < 0.05$.

TABLE 4. Regression models testing associations between maternal predictors and milk fat content

	Unadjusted B (SE)	R^2	Model 1	Model 2	Model 3
Infant age (months)	0.063 (0.026)*	0.05	0.065 (0.026)*	0.061 (0.027)*	0.064 (0.027)*
BMI (kg/m^2)	−0.068 (0.042)	0.03	−0.073 (0.041)**		
Body fat (%)	−0.029 (0.040)	0.01		−0.012 (0.040)	
Energy intake (kcal)	−0.280 (0.298)	0.02			−0.302 (0.292)
% kcal from fat	0.051 (21.354)				1.844 (20.871)
% kcal from protein	2.024 (15.971)				2.834 (15.602)
% kcal from carbs	−0.995 (30.020)				1.169 (29.334)
Model R^2			0.082	0.055	0.073

All models are adjusted for breastfeeding duration, in months.
*$P < 0.05$, **$P < 0.1$.

TABLE 5. Regression models testing associations between maternal predictors and milk protein content

	Unadjusted B (SE)	R^2	Model 2	Model 3	Model 4
Infant age (months)	−0.004 (0.009)	0.01	−0.004 (0.009)	−0.004 (0.009)	−0.006 (0.009)
BMI (kg/m^2)	−0.010 (0.014)	0.00	−0.010 (0.014)		
Body fat (%)	0.001 (0.013)	0.05		0.000 (0.013)	
Energy intake (kcal)	−0.075 (0.093)				−0.073 (0.093)
% kcal from fat	−4.316 (6.674)				−4.496 (6.696)
% kcal from protein	−1.994 (4.992)				−2.071 (5.006)
% kcal from carbs	−5.359 (9.383)				−5.565 (9.412)
Model R^2	0.002		.007	0.002	0.0547

All models were adjusted for breastfeeding duration, in months. There were no significant associations between any maternal predictors and milk protein content in this sample.

TABLE 6. Regression models testing associations between maternal predictors and milk sugar content

	Unadjusted B (SE)	R^2	Model 2	Model 3	Model 4
Infant age (months)	−0.018 (0.010)*	0.03	−0.016 (0.010)**	−0.022 (0.010)†	−0.015 (0.010)
BMI (kg/m^2)	−0.046 (0.016)*	0.08	−0.044 (0.016)*		
Body fat (%)	−0.026 (0.015)**	0.03		−0.032 (0.015)†	
Energy intake (kcal)	−0.102 (0.113)	0.07			−0.098 (0.112)
% kcal from fat	5.696 (8.09)				5.266 (8.056)
% kcal from protein	1.616 (6.051)				1.432 (6.022)
% kcal from carbs	6.640 (11.374)				6.148 (11.323)
Model R^2			0.099	0.067	0.0844

Models were adjusted for breastfeeding duration in months.
*$P < 0.01$, **$P < 0.1$, †$P < 0.05$.

TABLE 7. Regression models relating self-reported daily breastfeeding frequency to milk composition

	Unadjusted B (SE)	R2	+ BF Dur.	R^2
Energy	−0.009 (0.004)	0.05	0.008 (0.004)	0.07
Fat	−0.109 (0.040)**	0.07	−0.097 (0.040)*	0.11
Protein	0.009 (0.013)	0.004	0.008 (0.013)	0.00
Sugars	0.010 (0.016)	0.004	0.006 (0.016)	0.03

Unadjusted models for self reported daily feeding frequency. Models were then adjusted for breastfeeding duration. As shown in other studies, both breastfeeding duration and feeding frequency had independent, significant associations with milk fat.
*$P < 0.05$, **$P < 0.01$.

DISCUSSION

In this sample of Filipino women living in the metropolitan area of Cebu city, the strongest predictor of variance in breast milk macronutrients and energy was breastfeeding duration. Few maternal characteristics predicted the composition of milk that a woman produced. Current maternal diet was not associated with milk composition, while BMI was significantly inversely associated with sugar. No maternal predictors investigated here predicted milk protein. The generally weak or lack of association between most milk macronutrients and current maternal diet and nutritional status is in line with prior findings in other populations (Lönnerdal et al., 1987; Nommsen et al., 1991; Prentice et al., 1981a; Villalpando and del Prado, 1999; but see also Brown et al., 1986; Butte et al., 1984) and is consistent with the notion that milk composition tends to be buffered against fluctuation in maternal dietary intake or nutritional status.

Infant age was the strongest predictors of milk composition; in accordance with prior studies. Infant age was positively associated with milk fat and energy, and had a moderate inverse association with milk sugar as predicted. Past studies have conflicted on whether infant age predicts milk fat and energy, with some studies reporting a decline in milk fat with increasing infant age (Mitoulas et al., 2002) while others have reported an increase, particularly after 12 months (Boediman et al., 1979; Mandel et al., 2005; Prentice et al., 1981a). The moderate, inverse association with milk sugars noted at Cebu is consistent with similar decreases in milk total carbohydrates to 6 months documented elsewhere (Coppa et al., 1993; Saarela et al., 2005), although increases have also been reported (Allen et al., 1991; Brown et al., 1986). At Cebu, there was no association between infant age and milk protein content. Although previous studies have reported a decline in the protein content of milk with increasing infant age (Brown et al., 1986; Mitoulas et al., 2002), it should be noted that the largest decrease occurs during early lactation with the transition from colostrum to transitional and then to mature milk (Allen et al., 1991; Saarela et al., 2005).

Some of the increase in milk fat with infant age may be related to changes in breastfeeding behavior, as fat has been shown to vary based on feeding frequency, feed duration, and the length of time between feeds (Daly et al., 1993; Jackson et al., 1988; Kent et al., 2006). Similar to findings elsewhere (Kent, 2007), feeding frequency was a significant inverse predictor of total energy at Cebu, reflecting decreasing fat content with higher frequency feeding. In this sample, mothers of young infants (<6 months) reported nursing 13 times each day, while older infants (6–18 months) were nursed less, averaging 11 bouts per day. Adjusting for nursing frequency in models reduced the strength of the association between infant age and milk fat, supporting a role of changing feed frequency as an influence on the age-related milk compositional changes.

We found no evidence for associations between intake of any maternal dietary macronutrient and milk composition in this sample. This is consistent with past studies, which show that milk macronutrient composition appears to be primarily independent of maternal dietary intake (Boniglia et al., 2003; Forsum and Lonnerdal, 1980; Mohammad et al., 2009; Rakicioğlu et al., 2006) even under conditions of maternal malnutrition (Hambraeus et al., 1978; Jelliffe and Jelliffe, 1978a; Lonnerdal et al., 1976; Villalpando et al., 1992). This absence of an effect of maternal diet is evident in both observational studies and experimental designs in which nutritional supplements were shown to result in minimal or negligible changes to milk macronutrients (Prentice et al., 1983). Short term fasting, such as during Ramadan, or dieting during lactation, has also been shown to have no influence on milk macronutrient composition (Lovelady et al., 1990; Rakicioğlu et al., 2006). It has been suggested that compensatory physiological mechanisms help maintain a relatively constant milk macronutrient composition during periods of nutritional fluctuations in the maternal diet (Tigas et al., 2002). Milk volume, however, may be more sensitive to maternal condition (energy intake and adiposity), as demonstrated in both observational (Ettyang et al., 2005) and experimental (Strode et al., 1986) studies. These changes may be independent of composition, resulting in compromised nutrition for the infant.

The effect of maternal body composition on milk composition was evaluated using both BMI and percent of body fat estimated from skinfold measurements obtained at four sites. In some (Brown et al., 1986; Barbosa et al., 1997) but not all past studies, maternal adiposity has been shown to be a positive predictor of milk fat and energy. We failed to find a similar relationship in Cebu. Indeed, the women at Cebu with lower BMIs tended to produce milk with higher fat content than women with higher BMIs. The significant inverse association between milk fat and BMI suggests that BMI may be indexing lean mass in this sample. This interpretation is supported from the finding that direct measures of maternal adiposity, such as her skinfolds or percentage body fat, were unrelated to milk fat. Although past research has at times reported associations between adiposity and milk fat content, these relationships are often limited to overweight or obese women (Barbosa et al., 1997; Nommsen et al., 1991; Villalpando and del Prado, 1999; Villalpando et al., 1992, 1995). Elsewhere, it has been suggested that increasing maternal adiposity may be associated with impaired milk lactose synthesis, and that the increase in milk fat reflects this decrease in sugar (Rolls et al., 1986).

This pattern of findings suggests that milk composition within lean women is largely buffered against negative fluctuations in maternal adiposity or diet, but that additional fat may be added to milk under conditions of plentiful nutrition and abundant fat stores. In addition, it is important to note that other nutritional and energetic factors not measured in this study have been proposed as potential influences on milk composition, including pregnancy weight gain (Michaelsen, 1997) and physical activity level (Dewey et al., 1994). Because we were not able to collect these measures in the women included in this study, we cannot rule out their influence on milk compositional variation at Cebu.

The predictors used in this study have been investigated elsewhere, as described above. Overall, there are generally very weak associations between these predictors and milk composition, including the significant association between maternal adiposity and milk lactose content. Issues of sample collection, particularly the use of a single mid feed sample, may have obscured associations between potential maternal predictors and milk macronutrients and energy. However, the associations reported for this sample are similar to those reported elsewhere.

Evidence that milk composition is largely insulated from maternal dietary intake or body composition during lactation has potentially important applied and theoretical implications. From the perspective of public health, maternal buffering may help explain why interventions targeting the period of lactation tend to yield only modest changes in milk macronutrient composition, although such interventions may benefit the mother's nutritional status (Prentice et al., 1980). This relative independence of milk macronutrient composition from maternal diet or adiposity may buffer infants from fluctuations in maternal nutrition, including periods of severe maternal malnutrition (Brown et al., 1986; Prentice et al., 1981b), and points to the importance of milk as a preferred food source under even strained nutritional conditions.

This buffering from short term variation also speaks to the potential role of nutrients and hormones of maternal origin as signals or cues of ecological quality as experienced by the mother. We have argued elsewhere that hormones or nutrients that vary in mean value between populations faced with differing nutritional ecologies, and yet show minimal evidence for responsiveness to short-term fluctuations in maternal intake, might be more effective at tracking longer term and more stable ecological trends (Kuzawa and Quinn, 2009). Although speculative, one example of this may be found in nonhuman primates, where differences in early nutrition have been proposed as possible factors contributing to differences in infant temperament (Hinde and Capitanio, 2010). Increased milk energy is associated with greater behavioral playfulness and confidence in novel situations, which may signal an environment capable of sustaining greater energy budgets. If milk composition and energy are acting as signals of environmental quality, our findings suggest that is likely a relatively robust and stable signal rather than an acutely sensitive signal that fluctuates in response to transient changes in maternal nutrition. A capacity to respond to sustained rather than transient changes in local ecology could be beneficial in highly seasonal environments characterized by varying dietary intakes between seasons or for generating a stable signal even as resource availability fluctuates on short timescales (Kuzawa, 2007). It should be noted, however, that we have only focused on the predictors of milk macronutrient composition, while other aspects of milk nutrition, such as specific fatty acid profiles or hormones, may prove equally important as influences on offspring biology, and may have different associations with maternal nutritional status and diet than those reported here for macronutrients and energy. Overall, human milk macronutrients and energy appear relatively buffered from immediate maternal condition and diet, although there is some evidence from elsewhere that severe malnutrition may compromise milk volume (Strode et al., 1986), suggesting that specific characteristics of human physiology, such as increased fat stores, may provide humans with capacities for buffering milk composition (Pond, 1977). In other mammals with relatively large fat stores, such as seals, lactation can be almost entirely supported by such fat stores (Oftedal, 2000).

In sum, we document considerable individual variation in human milk composition in these young Filipino mothers. Consistent with prior research, we find evidence that composition changes with the age of the infant and that this is likely partially reflecting changes in nursing frequency. Similar to past studies, we also report minimal evidence for associations between the milk nutritional variation and maternal diet or nutritional status as measured during lactation. To the extent that milk macronutrient composition has short or long-term effects on offspring biology and health, our findings support prior arguments that a woman's current diet or nutritional status during lactation will not be an important predictor of this variation in milk macronutrients. In future research, other potential predictors, including early life nutritional or growth conditions (Lammers et al., 1999), the mother's own exposure to breast milk during infancy (Bar-Peled et al., 1997), and breastfeeding intensity, should be considered as potential influences on human milk macronutrient composition.

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