Original Research Article

Infant Sex Predicts Breast Milk Energy Content

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ABSTRACT During human evolutionary history, and for many around the world, breast milk is the primary source of nutritional energy for infants. Variation in breast milk quality might logically have important effects on infant health, growth, and development, yet the sources of this variation remain largely unelucidated. We quantified nutrient and energy content of breast milk from 25 healthy, well-nourished Massachusetts mothers with infants aged 2–5 months. We examined several potential sources of variation in milk quality, particularly feeding patterns, infant sex, and maternal breast growth during pregnancy. After controlling for time since last feeding, a known correlate of milk composition, we found that mothers of male infants produced milk that had 25% greater energy content than mothers of female infants ($P < 0.001$). Change in maternal bra cup size during pregnancy was associated with 16.17 kcal/100 ml greater energy content of milk ($P = 0.009$), but was not significant after taking infant sex into account. Greater nutritional investment in sons may account for the greater observed growth rates in male compared to female infants. Am. J. Hum. Biol. 22:50–54, 2010. © 2009 Wiley-Liss, Inc.

Milk caloric content is known to vary greatly between individual human mother–infant pairs, but the source of much of this variation is unexplained (Allen et al., 1990; Michaelsen et al., 1994; Mitoulas et al., 2002; Prentice et al., 1981a,b). During pregnancy, under the influence of estrogen, prolactin, progesterone, and other hormones, breast glandular tissue undergoes a period of glandular proliferation and differentiation (Gardner and Shoback, 2007).

Breast milk energy content may differ between individual mothers based on genetic differences or because of differences in the proliferation and differentiation of milk epithelial cells during pregnancy under a specific hormonal milieu. This may be associated with maternal nutritional status, innate characteristics, or fetal cues. Prior research has shown that milk caloric content fails to respond to maternal dietary intake even among malnourished women (Prentice et al., 1983). Exercise similarly does not affect the caloric content of breast milk (Dewey et al., 1994). There is some evidence that the size of maternal fat stores is associated with milk caloric content in poorly nourished populations, but it is unclear whether this relationship holds up in well nourished populations. Differences in fat stores alone do not explain most of the variation in milk caloric content between human mothers (Barbosa et al., 1997; Brown et al., 1986; Nommsen et al., 1991; Perez-Escamilla et al., 1995; Prentice et al., 1981a). Despite the fact that changes in the breast during pregnancy are responsible for breast milk production capacity postpartum, breast size and change in breast size during pregnancy has seldom been examined in studies of milk composition.

Differences in milk energy content between mothers may alternatively be responsive to infant demand, given that prolactin, the hormone which promotes the production of milk is exquisitely sensitive to the suckling stimulus. It is well established that milk fat content changes over a feeding and is related to the fullness of the breast, but it has not been shown that an infant's level of demand is associated with changes in milk composition or caloric content. We designed a study to determine whether differences in milk composition were associated with maternal characteristics, breast development, and infant characteristics reflective of energy requirement.

SUBJECTS AND METHODS

We conducted a cross-sectional study of well-nourished mother–infant pairs recruited from Massachusetts. Eligible mothers were exclusively breastfeeding their 2-to 5-month-old infants. We considered mothers to be exclusively breastfeeding if their infants relied on breast milk for all of their caloric needs, but we did not exclude mothers whose infants had received formula on a rare occasion (1–3 times in life). Both stay-at-home mothers who nursed their infants at the breast, and working mothers who pumped while away from their infants and fed their infants at the breast while at home, could participate. We recruited mothers and their infants from Harvard University Health Services OB/GYN Department in Cambridge, Massachusetts, The Center for Breastfeeding in Sandwich, Massachusetts, and mothers groups in Watertown, Arlington, and Boston, Massachusetts through flyers, emails, or through clinic staff. All procedures involving human subjects were approved by the Committee on the Use of Human Subjects at Harvard University, Cambridge, Massachusetts and we obtained written informed consent from participating mothers.

We met with each subject pair once at either their home or one of two clinical settings by the subject's choice. Mothers were asked to pick a time at which their infant would not typically be feeding if possible. We collected data on maternal characteristics, infant characteristics, and feeding behaviors by self-report. Participants completed a survey which requested information about their

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age, race, parity, and breastfeeding history with previous infants, pre-pregnancy weight, pregnancy weight gain, pre-pregnancy and current bra size, age at menarche, and medical conditions. Participants were also asked to complete a 24 h dietary recall. Mothers also reported their infant's birth weight, birth date, due date, typical feeding schedule, medical conditions, and whether they fed on one breast more than the other. They were asked to complete a breastfeeding/pumping log from the past 12 h.

Infant weight was measured in pounds using the Health-O-Meter Grow with Me Teddy Bear Baby to Toddler Scale (Jarden Corporation, Rye, NY). Infant length in inches was measured using the Seca 210 Infant Measure Mat for Infants and Small Children (Seca, Hamburg). We measured maternal weight with the Tanita HD317 Digital Scale with Memory Function (Tanita Corporation, Arlington Heights, Illinois). Maternal height was measured using a 6-foot long ruler. Maternal body fat was measured using the Omron HPF 306 Body Fat Tracker (Omron Healthcare, Kyoto, Japan), which uses bioelectrical impedance to determine body fat percentage.

A milk sample was collected from each participant at the study meeting using an electric breast pump. Mothers were permitted to use their own pumps, but if they chose to or if they did not own a pump, they used the Ameda Elite (Hollister, Liberty, IL) hospital grade breast pump with a sterile collection kit. Mothers were instructed to pump one breast until it was empty. Milk samples were given a random number prior to analysis.

Samples were placed on ice before freezing and freeze drying. Total nitrogen was determined using the Kjeldahl procedure (Pierce and Haenisch, 1947). Crude protein (CP) was estimated as (Total Nitrogen) × 6.25. The concentration of free simple sugars (FSS) was determined using the sulfuric acid-phenol method (Dubois et al., 1956; Strickland and Parsons, 1972). Lipids were extracted from dried breast milk using a method modified from the Association of Official Analytical Chemists (Association of Official Analytical Chemists, 1984). Ash was measured by heating sample to 500°C overnight in a muffle furnace, and the weight of the residue was measured (Association of Official Analytical Chemists, 1984). Total nonstructural carbohydrates (TNC) were estimated as the percent fat, percent ash, and percent crude protein subtracted from 100%. Milk caloric density was calculated by using the fractions of TNC, lipid, and CP. Lipid was assumed to have the physiological fuel value of 9 kcal/g, TNC and CP were assumed to have 4 kcal/g (National Resource Council, 1980).

Maternal and infant ages were calculated from the birth dates reported on the questionnaire using an age calculator website (Pon, 2000). Corrected infant age (corrected for gestational age) was calculated using the same calculator, but by plugging in the due date of the infant instead of the birth date (Pon, 2000). Self reported prepregnancy and current bra sizes were scored by cup size. Size *A* was given a score of 1 and each ascending size was given the next ascending number: $B = 2$, $C = 3$, etc. Change in cup size was taken as pre-pregnancy size subtracted from current size. Lactation history was calculated for each mother as the total number of months spent breastfeeding children in her lifetime. Time spent nursing two infants at the same time was counted twice. The average number of feedings per 24 h was calculated as: *day feedings + night feedings*. The average number of minutes spent feeding per day was calculated as: *Minutes feeding per day = (Number of daytime feedings) × (Average length of daytime feedings) + (Number of nighttime feedings) × (Average length of nighttime feedings)*. Infant weight gain was calculated as the birth weight subtracted from the current weight. We used pounds and ounces for the units of infant weight rather than grams because mothers were better able to recall their infants birth weights in pounds and ounces.

We conducted an exploratory statistical analysis to determine which of the maternal and infant characteristics that we measured were correlated with milk energy density. We calculated Pearson's correlations between continuous maternal and infant characteristics. Student's *t*-tests (two-tailed) were used to compare the characteristics and feeding behaviors of male infants with those of female infants, the characteristics of the mothers of male infants to those of the mothers of female infants, the composition of the milk of mothers with male infants to the composition of the milk of the mothers with female infants.

Linear regression procedures were used to create models for milk energy density (kcal/100 ml) which included maternal and infant variables associated with milk energy density. Goodness of fit was checked by examining residuals. All analyses were performed using Statistical Package for the Social Sciences (SPSS).

RESULTS

We collected data from 25 mother–infant pairs between September 2005 and February 2006. The characteristics of the mothers, the infants, and their feeding behaviors are summarized in Table 1. The liquid energy density of milk was highly variable between mothers. Kilocalories per 100 ml ranged from 32.9 to 116.1 with a mean of 68.48 ± 18.87. Figure 1 shows the distribution of milk energy densities in our sample. Milk energy density was not significantly correlated with maternal age, maternal parity, maternal pre-pregnancy weight, maternal pregnancy weight gain, maternal current weight, maternal body fat percentage, maternal bra size (pre or post pregnancy), lactation history, maternal weight, or household income. Milk energy density was not significantly correlated with infant age, age corrected for gestational age, infant (birth or current) weight, infant (birth or current) length, infant weight gain, infant feeding frequency, or minutes feeding per day.

Among 25 mothers, the liquid energy density of milk produced by mothers of male infants was 75.56 kcal/100 ml versus 60.811 kcal/100 ml of milk produced by mothers of female infants (*t*-test, $P = 0.049$). To control for the fullness of the breast, which can affect the milk fat content, and thus caloric content of the milk, we controlled for time since last feeding (Daly et al., 1993; Jackson et al., 1988; Prentice et al., 1981a). The effect of infant sex was statistically significant after adjustment for time since last feeding (multiple regression, $P < 0.001$) (Table 2). After this adjustment in multiple regression analysis, mothers of male infants in our sample had a milk energy concentration that was 24.68 kcal/100 ml greater than milk energy of mothers of females. Based on a milk consumption volume of 788 ml per 24 h (Daly et al., 1993), the difference in composition between milk consumed by infants of different sexes would result in a difference of 194.5 kcal per day in infant intake. The sex difference in milk composition is sufficient to account for differences in

TABLE 1. *Characteristics of mothers and infants*

Subject characteristics (N = 25, unless noted)	Mean (95% confidence interval), unless noted.
Maternal	
Age, years	33.3 (31.3–34.7)
Parity	1.28 (1.0–1.5)
Pregnancies	1.88 (1.5–2.3)
Pre-pregnancy weight, kg	64.7 (59.6–69.8)
Pregnancy weight gain, kg	14.9 (13.2–16.6)
Pre-pregnancy bra size, score	3 (2.4–3.6)
Current bra size, score[a]	4.36 (3.7–5.1)
Current breast size, cm[b]	16 (14.3–17.7)
Lactation history, months	8.77 (4.2–13.4)
Weight, kg	67.6 (62.7–72.5)
Height, cm	164 (161.7–166.3)
Body fat percentage	28.6 (26.1–31.1)
Maternal race	
Caucasian, No. (%)	23 (92)
Asian, No. (%)	2 (8)
Ethnicity	
Hispanic/Latino, No. (%)	2 (8)
Not Hispanic/Latino, No. (%)	23 (92)
Household Income	
150% or below FPL, No. (%)	2 (8)
250–151% FPL, No. (%)	0 (0)
400–251% FPL, No. (%)	3 (12)
Over 400% FPL, No. (%)	20 (80)
Infant	
Age, weeks	16.7 (14.7–18.7)
Corrected Age, weeks	15.8 (13.8–17.8)
Birth weight, kg	3.42 (3.2–3.7)
Birth length, cm	50.8 (49.7–51.9)
Current weight, kg	6.77 (6.3–7.2)
Current length, cm[c]	64.1 (62.6–65.6)
Weight gain, kg[c]	3.35 (3.0–3.7)
Male sex, No. (%)	13 (52)
Feeding Behaviors	
Feedings per 24 h, no. of times	8.64 (7.9–9.4)
Minutes feeding per 24 h, min	135.1 (104.8–165.4)
Time since last feeding, h[c]	3.46 (2.8–4.1)
Fed on demand, No. (%)	23 (92)
Never had formula, No. (%)	15 (60)

FPL, federal poverty level.
[a]N = 21.
[b]N = 24.
[c]N = 23.

growth between male and female infants, with male infants gaining an average of 3.72 kg from birth to the time of study and female infants gaining 2.96 kg from birth to the time of study (P = 0.49), despite female infants being slightly older than males at the time of study (Table 3). There was not a statistically significant correlation between milk energy density and absolute infant weight (r = 0.31, P = 0.15).

Each unit increase in maternal bra cup size over pregnancy up until the time of study was associated with a 16.17 kcal/100 ml greater milk energy density (P = 0.009). The effect size increased after adjustment for time since last feeding and the association remained statistically significant (P = 0.029). The association between change in maternal cup size and milk energy density was not significant after taking infant sex into account. This was likely related to mothers of male infants having a larger average increase in cup size (1.35 vs. 1.00), although this result did not reach statistical significance (P = 0.246).

DISCUSSION

This is the first report, to our knowledge, of an infant sex-based difference in breast milk energy content in humans. Our findings, however, are consistent with those of prior animal studies in rhesus macaques and red deer (Hinde, 2007; Landete-Castillejo et al., 2004).

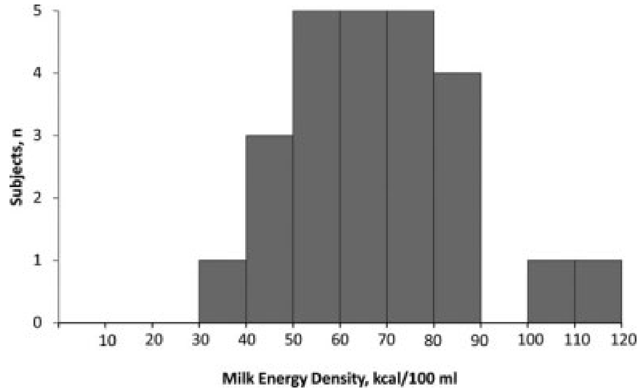

Fig. 1. Milk energy density among 25 Massachusetts mothers.

Increased demand for milk from male infants could feasibly result in an increase in the caloric density of their mothers' breast milk. It has previously been suggested that male infants may consume 8–10% more milk than females (Michaelsen et al., 1994). Consistent with this, male infants (mean = 9.27 times per day) in our study on average fed more times per day than female infants (mean = 7.95 times per day), although this result did not reach statistical significance. Fat content is inversely correlated to the fullness of the breast (Kent et al., 2006). Therefore, at a given feeding frequency, an infant who extracts more volume at each feeding will drive the mean fat content of his mother's milk up. This happens because the infant consumes more of the high fat hind milk at the current feeding and because the breast will not be as full at the subsequent feeding. It has previously been shown that increased feeding from a single breast, initiated by the mother, can increase the mean fat content per feeding, but this maternal behavior change could not increase total infant caloric intake, most likely because total intake is controlled by infant demand (Woolridge et al., 1990).

Taken alone, the increased milk consumption of male infants and milk composition dependence on the fullness of the breast may not explain a difference in average milk caloric content, because prolactin levels are responsive to suckling stimulus and will serve to match milk supply to infant demand if milk demand remains constant. However, faster growing infants logically demand ever increasing milk production from their mothers by emptying a progressively increasing fraction of the milk contained in their mother's breast at each feeding. While the mother's production would increase to match demand, the infant demand would remain ahead of the milk production. Thus, the increased caloric content of milk produced by mothers of male infants may be caused by a greater rate of increase of energy requirement in male infants. This is consistent with the greater weight gain from birth observed among male infants in this study.

To our knowledge, this also is the first report to demonstrate a link between breast enlargement during pregnancy and milk energy content. The growth of breast tissue may more accurately represent the relative mass of structures devoted to milk production and storage. Consistent with this, Neifert et al. (1990) showed that females with less breast enlargement during pregnancy

TABLE 2. Associations between subject characteristics and milk energy density

	Milk energy density					
	Unadjusted			After adjustment for time since last feeding		
Characteristics	β	r^2	*P*-value	β	r^2 (model)	*P*-value
Feeding behaviors						
Time since last feeding	−5.734	0.228	0.021	–	–	–
Feedings per 24 h	−0.1775	0.035	0.370	−1.001	0.238	0.621
Infant						
Sex	14.74	0.159	0.049	24.68	0.603	<0.001
Maternal						
Change in cup size	16.17	0.310	0.009	12.711	0.508	0.029

TABLE 3. Infant and milk characteristics by infant sex

	Male	Female	
Characteristics	Mean (SD)	Mean (SD)	*P*
Birth weight (kg)	3.66 (0.586)	3.18 (0.641)	0.600
Current weight (kg)	7.41 (1.077)	6.12 (0.709)	0.003
Weight gain (kg)	3.72 (0.785)	2.96 (0.961)	0.049
Age (weeks)	16.39 (5.69)	17.13 (4.34)	0.730
Age corr. for gestational age (weeks)	16.02 (6.04)	15.62 (3.79)	0.850
Feedings per 24 h (no. of times)	9.27 (1.63)	7.96 (2.19)	0.101
Time since last feeding (h)	3.92 (1.471)	2.95 (1.73)	0.164
Maternal cup change (size)	1.35 (0.944)	1.00 (0.224)	0.246
Milk energy density (kcal/ml)	75.56 (19.37)	60.81 (15.64)	0.049

No adjustment for time since last feeding.

are more likely to have lactation insufficiency, as measured by infant weight gain. Hytten (1995) suggests that the lower levels of pregnancy breast enlargement in older mothers are associated with their decreased milk production; however, this relationship was not examined directly. Our study advances the results of these prior studies by providing evidence for a direct association between breast enlargement during pregnancy and milk energy content.

The objective of this study was not to determine the mechanism by which milk composition varies among women, thus we cannot rule out alternative mechanisms by which the sex based difference in milk composition comes about, including fetal sex-based differences in hormones secreted by the placenta during pregnancy, which may contribute to breast glandular development. This is supported by our data in that mothers of male infants had greater cup size changes than the mothers of female infants in our study, although this did not reach statistical significance. Once infant sex was taken into account, the association between maternal cup size change and milk energy density was no longer statistically significant, raising the possibility that change in cup size partially mediates the relationship between infant sex and milk energy density. Interestingly a study of 244 Boston women found that pregnant women carrying male infants consume 10% more calories than pregnant women carrying female infants, raising the additional possibility that male offspring place greater demands on their mothers even in utero (Tamimi et al., 2003). These greater demands could contribute to the greater, though nonstatistically significant, cup size change seen in the mothers of male infants.

Of note, the sex-based difference in milk energy content is consistent with the Trivers-Willard hypothesis for sex-specific parental investment (Trivers and Willard, 1973). This hypothesis predicts that when male reproductive success is more dependent on parental investment than female reproductive success, mothers will tend to invest in males and males will be willing to expend more effort to obtain parental investment. Well-nourished mothers may have been selected to invest more energy in their male infants than female infants or male infants may be more aggressive than females in procuring energy from their mothers.

In summary, this study of 25 exclusively breastfeeding mothers is the first report of an association between milk energy density and both infant sex and maternal breast size change during pregnancy in humans. Limitations of this study include the small sample size and the proportion of data collected by self-report. Despite this, the results are intriguing and should be followed with further study. A larger study with prospective data collection should be conducted to verify our results. Despite limitations, the results of our study raise several other possibilities for future research. It remains to be seen whether the association between infant sex and milk energy content will hold up in less energy rich populations more similar to the environment in which humans evolved. A study conducted in a society with scarce energy resources may provide insight into whether the sex-based difference in milk composition is adaptive.

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