

# Maternal Characteristics Associated with Milk Leptin Content in a Sample of Filipino Women and Associations with Infant Weight for Age

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## Abstract

**Background:** Human milk contains many metabolic hormones that may influence infant growth. Milk leptin is positively associated with maternal adiposity and inversely associated with infant growth. Most research has been conducted in populations with higher leptin levels; it is not well understood how milk leptin may vary in lean populations or the associations that reduced leptin may have with infant size for age. It is also largely unknown if associations between maternal body composition and milk leptin persist past 1 year of age.

**Objectives:** We investigated the association between maternal body composition and milk leptin content in a sample of lean Filipino women and the association between milk leptin content and infant size for age.

**Methods:** Milk samples were collected at in-home visits from 113 mothers from Cebu, Philippines. Milk leptin content was measured using EIA techniques; anthropometric data, dietary recalls, and household information were also collected.

**Results:** Mean  $\pm$  standard deviation (SD) milk leptin in this sample was  $300.7 \pm 293.6$  pg/mL, among the lowest previously reported. Mean  $\pm$  SD maternal percentage body fat was  $24.8\% \pm 3.5\%$ . Mean  $\pm$  SD infant age was  $9.9 \pm 7.0$  months, and mean  $\pm$  SD weight for age z-score was  $-0.98 \pm 1.06$ . Maternal percentage body fat was a significant, positive predictor of milk leptin content. Milk leptin was a significant, inverse predictor of infant weight and body mass index z-scores in infants 1 year old or younger.

**Conclusion:** The association between maternal body composition, milk leptin, and infant growth persists in mothers with lean body composition. Milk leptin is not associated with growth in older infants.

## Keywords

breastfeeding, human milk, infant feeding, leptin

## Well Established

*It is well known that human milk contains bioactive available leptin. Milk leptin is correlated with maternal body mass index (BMI) and plasma leptin. Milk leptin is inversely associated with infant weight and BMI.*

## Newly Expressed

*Maternal body fat is a stronger predictor of milk leptin than BMI, in a lean population with low milk leptin. In mothers breastfeeding older infants, there is no association between milk leptin and infant weight or BMI for age z-score.*

## Background

It has been suggested that some of the hormones contained in human milk, especially metabolic hormones such as leptin

and adiponectin, may be protective against the later development of metabolic and cardiovascular diseases (CVD). It has been hypothesized that breastfeeding may reduce the risk of

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**Table 1.** Sample Size, Population, and Study Design for Prior Studies Looking at the Association between Maternal Body Mass Index (BMI) and Human Milk Adiponectin.<sup>a</sup>

Population	No.	Study Design	Infant Age	Milk Leptin, ng/mL	Maternal BMI, kg/m <sup>2</sup>	Reference
Spain	34	Single point	< 5 days	1.35 (0.16)	26.3 (0.9)	Casabiell et al <sup>10</sup>
Spain	28	Longitudinal	1-9 months	0.16 (0.04)	21.6 (0.5)	Miralles et al <sup>23</sup>
Turkey—obese	17	Cross-sectional	< 4 months	0.27 (0.40)	25.6 (4.1)	Uysal et al <sup>21</sup>
Turkey	33	Cross-sectional	< 4 months	0.37 (0.40)	26.1 (4.6)	Uysal et al <sup>21</sup>
Swiss	24	Longitudinal	< 6 weeks	0.92 (0.12)	25.7 (0.7)	Bielicki et al <sup>15</sup>
Italy	40	Single point	10 days	0.50 (0.10)	25.7 (4.2)	Zanardo et al <sup>14</sup>
Turkey	43	Combination	< 6 months	1.60 (0.18)	26.0 (1.0)	Ilcol et al <sup>13</sup>
Germany	766	Longitudinal	< 6 months	0.28 (0.38)	23.5 (4.0)	Weyermann et al <sup>36</sup>
Germany	23	Longitudinal	< 6 months	0.18 (0.15)	21.4 (2.6)	Schuster et al <sup>16</sup>
United States	19	Single point	1 month	0.92 (0.47)	26.6 (6.6)	Fields and Demerath <sup>20</sup>
Italy	41	Cross-sectional	1-6 months	2.34 (5.73)	27.5 (4.9)	Savino et al <sup>28</sup>
Cebu	120	Cross-sectional	1-24 months	0.28 (0.29)	20.1 (3.4)	This study

<sup>a</sup>Values are reported as mean (standard deviation) based on published values in the referenced studies.

these conditions,<sup>1,2</sup> although the evidence is somewhat mixed, especially for rapidly urbanizing populations.<sup>3</sup> Animal models have also provided support: rat pups supplemented with subcutaneous or oral doses of leptin have decreased risk of CVD and obesity in adulthood compared to control pups.<sup>4-6</sup> Early exposure to leptin through milk was hypothesized to explain this decreased risk of overweight and obesity seen in breastfed infants when compared to formula fed infants,<sup>7,8</sup> possibly through changes to postnatal metabolism and appetite regulation.<sup>9</sup> Additional evidence from rat models showed that leptin from mother's milk survived digestion in the immature stomach and was detectable in neonatal circulation.<sup>6,10,11</sup>

Prior studies have reported positive correlations between milk leptin levels and both maternal body composition, typically reported as body mass index (BMI), and plasma leptin levels.<sup>12,13</sup> However, data on milk leptin content between populations are quite limited (Table 1),<sup>14-16</sup> with the majority of research conducted in well-nourished, primarily Western populations. Only a handful of populations with low levels of body fat and/or reduced BMIs have been studied so far. In these few populations, the correlations between maternal BMI, maternal adiposity, and milk leptin appear consistent. However, although the correlation between BMI and milk leptin levels is consistent, there appears to be greater sensitivity of milk leptin to changes in maternal adiposity at the lower end of the distribution.<sup>17-19</sup>

There is some evidence that the content of leptin in human milk contributes to the regulation of infant weight velocity. Infants receiving milk with more leptin have lower body weights and BMI.<sup>20,21</sup> In longitudinal studies, milk leptin content is positively associated with infant weight gain but not length gain in the period of time between measurements (these can be variable from 1-12 months).<sup>16,22,23</sup> Other metabolic hormones have also been shown to predict aspects of

infant growth, with particular emphasis on the roles of ghrelin and adiponectin.<sup>24,25</sup> Additional metabolic hormones, such as insulin, obestatin, and resistin, are also found in milk, although their association with infant growth is not yet fully elucidated.<sup>26-28</sup>

These metabolic hormones in human milk have been hypothesized to contribute to infant developmental plasticity, specifically altering metabolic development<sup>7,8</sup> and possibly contributing to the decreased risk of obesity, type 2 diabetes, and related metabolic conditions commonly reported for breastfed infants.<sup>29</sup> By comparison, infants fed commercial infant formula will not be receiving these hormones.<sup>30</sup> The differences in early exposure to metabolic hormones, especially leptin, have been hypothesized to contribute to the risks of obesity. However, this is often presented as a dichotomous present or absent scenario and ignores the within- and between-population variability in milk leptin levels—and the possible implications that this may have for infant metabolic development. If increased milk leptin predicts decreased infant growth, heavier mothers, who will have more milk leptin, should have infants with slower weight gain. Conversely, lean mothers should have milk with less leptin, and greater postnatal infant growth.

However, little is known about how maternal adiposity may predict milk leptin in populations with lean body composition and comparably low energy intakes. It is also uncertain how leptin subsequently may be associated with infant weight gain in populations at or below the 25th percentile of weight for age by the 2011 World Health Organization standards. Equally uncertain is if the association between milk leptin and infant weight may persist in infants breastfeeding more than 1 year, as all prior studies have not included breastfeeding toddlers, despite global recommendations for breastfeeding to 2 years or more postpartum. Here, we investigate the associations between maternal body composition

and diet and milk leptin levels in a sample of 113 Filipino women and between milk leptin levels and infant weight and BMI for age. Two specific hypotheses were investigated followed by 2 secondary hypotheses generated by the initial findings. The primary hypotheses were, (1) Is maternal body composition (BMI and/or percentage body fat) and dietary energy positively associated with milk leptin? and (2) Is milk leptin a significant predictor of infant weight or BMI in this sample? Following the results of hypothesis 2, we tested for age and sex-specific associations between milk leptin and offspring growth in males compared to females and infants 1 year old or younger compared to toddlers up to age 3.

## Methods

Mothers in this study were drawn from the Cebu Longitudinal Health and Nutrition Survey (CLHNS), an ongoing birth cohort study run and managed by the Office of Population Studies Foundation (OPS) at the University of San Carlos in Cebu City, Philippines. The CLHNS began as a 1-year birth cohort study of 3000+ children delivered from May 1, 1983, to April 30, 1984, in 33 communities within the large Cebu metropolitan area and has been described in depth elsewhere.<sup>31</sup>

During a bi-yearly survey conducted from September 2007 to March 2008, we identified 132 currently breastfeeding women, offspring of participants from the original CLHNS birth cohort. All eligible women agreed to participate. Mothers were 24 to 25 years of age at the time of recruitment; offspring age ranged from 10 days to 36 months. Breastfeeding rates in this population were high, with more than 93.7% of women initiating breastfeeding—measured by asking if the mother ever gave the child breast milk (unpublished data); women from Cebu were more likely to initiate breastfeeding, compared with the national average of 87%.<sup>32</sup> Exclusive breastfeeding durations were quite short, with the mean  $\pm$  SD age for the introduction of other foods (solids, semi-solids, other milks) at  $3.2 \pm 2.7$  months, with 59.3% of infants first supplemented with formula or other milk; national exclusive breastfeeding durations at that time were estimated at 24 days.<sup>32</sup>

Mothers were visited in their homes between 6 and 10 AM, as prior work has identified this as an optimal time for a nutritionally representative sample of milk.<sup>33</sup> A 10-mL sample of milk was collected from a single breast after allowing the infant to nurse for 2 minutes, following widely used protocols for optimizing sample collection while minimizing the nutritional disruption to the infant.<sup>34,35</sup> Mothers collected samples using manual expression of the breast into a sterile polypropylene cup. Milk samples were transported on freezer packs, aliquoted within 2 hours, frozen, and stored prior to shipment on dry ice to the United States. All protocols were approved by the institutional review boards at OPS, Northwestern University, and Washington University in St Louis.

Mothers were also interviewed about their health, infant health, feeding practices, and general child care behaviors. One-day dietary recalls were collected on both mothers and infants using survey mechanisms historically used by the CLHNS. Briefly, mothers were asked to list, by meal, each food item consumed and its cooking preparation and to estimate the weight of each food eaten using reference items and measuring cups. Energy and macronutrient content of foods was calculated using nutritional databases from the Food and Nutrition Research Institute of the Philippines.<sup>36</sup> Anthropometric measurements were also collected in triplicate by the same researchers (FL, EAQ) on mothers and included height, weight, mid-upper arm circumference, and thicknesses of the biceps, triceps, suprailiac, and subscapular skinfolds. Offspring measurements collected were recumbent length, weight, head circumference, mid-upper arm circumference, and measurement of the abdomen, suprailiac, subscapular, calf, thigh, bicep, and tricep skinfolds. Maternal percentage body fat was calculated using the 4-site formula of Durnin and Womersley,<sup>37</sup> previously validated in this population.

## Milk Leptin Analysis

Milk samples were analyzed for leptin in the Biomarkers & Milk Laboratory at Washington University in St Louis using a modified enzyme-linked immunosorbent leptin Duo Set from R&D Systems (catalog no. DY398; R&D Systems, Inc, Minneapolis, Minnesota, USA), previously validated for use on human milk.<sup>38</sup> Milk samples were skimmed prior to analysis by spinning at  $2000 \times G$  for 20 minutes at 4C. A spatula was used to remove the fat pellet. Skimmed milk samples were run without dilution, given the sensitivity of the assay and the low levels of leptin commonly identified in human milk.

## Statistical Analysis

The sample size presented here includes 113 mothers with complete data. Nineteen mothers from the original sample were excluded from analyses because of inadequate milk volume/non-nutritive suckling ( $n = 9$ ), offspring age of greater than 36 months ( $n = 6$ ), or concurrent pregnancy or mastitis ( $n = 4$ ). Sample exclusion reflected known or anticipated changes to milk leptin because of maternal condition (mastitis or pregnancy) or infrequent breastfeeding by older infants.

Statistical analyses were conducted using Stata/IC 10.1 (College Station, Texas, USA). The majority of predictors were not normally distributed and were log transformed for analysis, including maternal and infant body composition measures. The 2 primary outcome measures, milk leptin and infant weight for age z-score, were also not normally distributed and were log transformed. Comparisons by infant age and sex for predictor variables, as shown in Table 2, were done using Mann-Whitney *U* tests. Linear regression was

**Table 2.** Descriptive Characteristics for the Sample.<sup>a</sup>

Variable	All		≤ 1 Year		> 1 Year		P Value <sup>b</sup>
	N = 113		n = 71		n = 42		
	Mean	SD	Mean	SD	Mean	SD	
Leptin, pg/mL	300.8	292.9	302.5	256.4	296.0	349.5	.91
Infant age, mo	9.9	7.0	5.5	3.9	17.5	4.5	.00
Infant weight, g	7228	1920	6338	1773	8819	1002	.00
Infant weight for age z-score	-0.99	1.05	-0.70	1.03	-1.53	0.89	.00
Infant length, cm	67.0	9.4	62.0	8.1	75.4	4.1	.00
Infant length for age z-score	-0.36	1.11	-0.08	1.09	-0.85	0.95	.00
Mother weight, kg	45.7	8.2	34.8	4.6	36.2	3.9	.06
Mother height, cm	67.7	2.8	150.8	5.6	149.7	5.4	.34
Resumed menstruation, %	58		38		95		
Mother BMI	20.1	3.3	20.2	3.1	20.4	4.1	.40
Mother % body fat	24.8	3.6	25.2	3.4	24.7	4.1	.45
Nursing frequency, per 24 h	11.9	3.8	12.3	3.6	10.9	3.9	.03
Percentage calories from fat	20.7	13.7	20.3	13.5	21.6	16.1	.64
Daily dietary energy, kcal	1475	806	1437	778	1551	885	.48
Mother's education, y	9.8	2.7	10.2	2.5	9.3	2.8	.07
Living in urban area, %	64.3		64.7		60.0		.62

Abbreviations: BMI, body mass index; SD, standard deviation.

<sup>a</sup>Data are presented for the full sample and then stratified by infant age into categories of younger than 366 days and older than 365 days. *t* tests were used to test for differences between younger and older offspring. As expected, younger infants were lighter and shorter, although both weight for age and length for age z-scores were greater. Milk leptin did not differ between groups.

<sup>b</sup>P value for difference between infants (≤ 1 year of age) and toddlers (>1 year of age).

**Table 3.** Multivariate Linear Regression Models Testing the Association between Log-transformed Maternal Body Fat and Milk Leptin Content, Testing for an Association with Additional Possible Predictors.

Final Model	β (SE)	P Value	R <sup>2</sup>
Log body fat	2.293 (0.498)	.000	0.157
Infant age, mo	-0.006 (0.010)	.541	
Parity	-0.080 (0.070)	.251	
Breastfeeding frequency	-0.018 (0.019)	.343	
Constant	-1.573 (1.604)	.329	

Abbreviation: SE, standard error.

used to test for an association between maternal BMI, body fat, and milk leptin. After the best fit maternal body composition measure had been selected, multivariate linear regression was used to test for an association between secondary predictors and milk leptin. To test for an association between infant weight for age z-score and milk leptin, the sample was divided into infants less than or equal to 1 year (*n* = 71; female = 34; male = 37) and toddlers older than 1 year of age (*n* = 42). As prior studies have focused only on children younger than 1 year, this seemed like a natural division, allowing for comparison of the sample with existing studies. The sample of older infants includes individuals from 366

days to 36 months. Regression models were adjusted for infant age, maternal BMI, and infant sex.

## Results

The sample descriptive characteristics are presented in Table 2. Milk leptin levels ranged from 46.06 to 2098 pg/mL, with a mean ± SD of 300.8 ± 292.9 pg/mL for the full sample. Only 5% of mothers were overweight; 30% were underweight at the time of measurement. Undernutrition was amplified by relatively low average dietary energy intakes (1476 kcal/d). Although these estimates seem low, they are within the range reported in prior survey rounds with this sample.

In regression models, most measures of maternal body composition were significant predictors of milk leptin content (Table 3). Both BMI and body fat were significant predictors of milk leptin content. Maternal BMI explained 9% of the variation in milk leptin, with each log unit of BMI predicting a 1.59 log unit increase in milk leptin, or approximately 18 ng/mL of leptin per 1 unit increase in maternal BMI. Maternal percentage body fat was a stronger predictor of milk leptin content, explaining 16% of the variation. Each increase in a log unit of body fat predicted a corresponding increase of 2.52 log units of leptin, or 23.6 ng/mL of leptin. Maternal weight, sum of skinfolds, and individual skinfold thicknesses from 4 sites were also analyzed but were less

**Table 4.** Regression Models Testing the Association between Maternal Measures of Body Composition and Milk Leptin Content.<sup>a</sup>

	Individual Regression Models			
	Log BMI	Log Weight	Log Body Fat	Sum Skinfold
β (SE)	1.593 (0.446)	1.270 (0.394)	2.252 (0.481)	1.296 (0.279)
P value	.001	.002	.000	.000
Constant	0.558 (1.337)	0.488 (1.503)	-1.897 (1.544)	0.431 (1.056)
R <sup>2</sup>	0.096	0.078	0.160	0.158

Abbreviation: BMI, body mass index; SE, standard error.

<sup>a</sup>In this sample, the mother's percentage body fat was the strongest predictor of milk leptin content.

robust measures of the association between maternal body composition and milk leptin content.

Subsequent analyses used percentage body fat as the primary predictor of milk leptin and tested 2 sets of secondary predictors: infant and maternal level predictors. Infant predictors were infant age, daily breastfeeding frequency, and parity (Table 4). Maternal predictors tested in model 2 were years of education, living in an urban environment, daily energy intake, and percentage of calories from fat.

As shown in Table 3, infant age, daily breastfeeding frequency, and parity were not significant predictors of milk leptin content. Maternal dietary energy intake and percentage calories from fat also had no association with milk leptin, nor did household wealth or mother's education.

In the full sample of all infants from 10 days to 36 months, there was no association between milk leptin and infant size for age z-score. However, when the analyses were stratified into younger infants (1 year or younger) and older infants/toddlers (12-36 months), milk leptin content was a significant predictor of infant weight for age and BMI for age z-scores (Table 5). In the sample of infants 1 year or younger, regression analyses were rerun as sex-specific models. When stratified by sex, milk leptin was an inverse but significant predictor of both weight and BMI for age z-scores in female but not male infants (Figure 1). Together with infant age and maternal BMI, milk leptin explained 37.1% of the variation in infant weight z-score in female infants, with each 1 log unit increase in leptin predicting a 0.57 decrease in infant weight for age z-score. There were no significant associations between milk leptin and weight or BMI for age z-scores in male infants.

As shown in Table 2, there were no significant differences in the amount of leptin in the milk of mothers breastfeeding older or younger infants (younger mean  $\pm$  SD = 302.4  $\pm$  256.4 pg/mL; older mean  $\pm$  SD = 296.0  $\pm$  349.5 pg/mL;  $P = .45$ ) or male or female infants (male mean  $\pm$  SD = 310.1  $\pm$  335.8 pg/mL, female mean  $\pm$  SD = 289.8  $\pm$  244.4 pg/mL;  $P = .71$ ). As expected, male infants were significantly heavier than female infants (male mean  $\pm$  SD = 7805  $\pm$  2122 grams vs female mean  $\pm$  SD = 7131  $\pm$  2020 grams;  $P = .04$ ), although mean age did not differ between male and female infants in this sample. Mean  $\pm$  SD weight for age z-scores

for the sample were  $-0.93 \pm 1.09$  for males and  $-0.95 \pm 1.07$  for females and were also not significantly different by sex ( $P = .93$ ).

## Discussion

### Maternal Adiposity and Milk Leptin Content

Similar to the reports from prior studies in other, primarily Western populations (Table 1), we found a significant, positive association between maternal body composition and milk leptin (Table 4). The association reported here is slightly different from those reported by earlier studies, as we used percentage body fat, rather than BMI, as our primary measure of maternal adiposity. Compared to BMI, percentage body fat has the advantage of allowing for a more precise measure of adiposity and, as expected, had a stronger correlation with milk leptin.

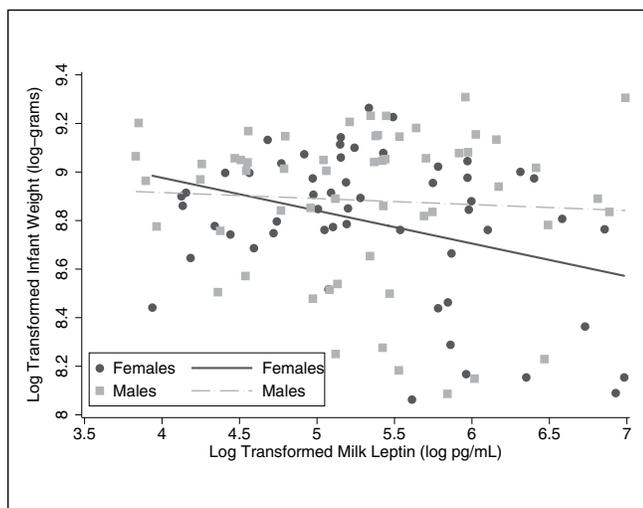
Given the low BMIs and body fat percentages of mothers from Cebu compared to other studied populations, it is not surprising that milk leptin levels were lower than those reported for most other populations (Table 1). Maternal plasma leptin levels are suppressed during lactation.<sup>39</sup> The source of leptin in human milk is uncertain; whereas adipose tissue is generally associated with leptin synthesis for circulating leptin,<sup>40</sup> some researchers<sup>41</sup> have shown that the mammary epithelial cells within the breast also produce leptin. Leptin in milk is likely derived from both adipose and mammary synthesis, although the relative contribution of each remains uncertain.<sup>41</sup> Milk leptin is also correlated with many of the hormones involved in the regulation of lactation; lower levels of prolactin predict decreased leptin transfer to milk, further highlighting the potential role of mammary synthesis.<sup>42</sup> It may be that the low levels of leptin, such as reported by Schuster et al<sup>16</sup> in a small sample of relatively lean German mothers, reflect a stronger association between maternal adiposity and milk leptin content. Although mean leptin is generally higher among populations with higher BMI, the increase is not linear. However, as discussed previously, although widely available and commonly used as a measure of maternal body composition, the association between BMI and milk leptin was much weaker than the

**Table 5.** Milk Leptin as a Predictor of Infant Weight for Age z-Score.<sup>a</sup>

	All (N = 113)		≤ 1 Year (n = 71)		Females ≤ 1 Year (n = 34)		Males ≤ 1 Year (n = 37)	
	β (SE)	P Value	β (SE)	P Value	β (SE)	P Value	β (SE)	P Value
Unadjusted model								
Log leptin	-0.082 (0.037)	.032	-0.063 (0.157)	.692	-0.106 (0.210)	.617	-0.034 (0.239)	.886
Constant	9.273 (0.202)	.000	-0.288 (0.856)	.737	0.017 (1.159)	.989	-0.497 (1.284)	.701
R <sup>2</sup>	0.033		-0.012		-0.020		-0.027	
Model 2—adjusted								
Log leptin	-0.115 (0.135)	.399	-0.390 (0.159)	.017	-0.566 (0.193)	.006	-0.275 (0.252)	.282
Infant age, mo	-0.038 (0.012)	.002	-0.062 (0.031)	.052	-0.115 (0.041)	.009	-0.028 (0.048)	.555
Mother body fat	0.073 (0.031)	.019	0.137 (0.040)	.001	0.159 (0.045)	.001	0.128 (0.066)	.063
Constant	-1.711 (0.814)	.038	-1.615 (1.079)	.130	-0.958 (0.045)	.438	-2.243 (1.827)	.288
R <sup>2</sup>	0.125		0.195		0.371		0.061	

Abbreviation: SE, standard error.

<sup>a</sup>In the full sample, milk leptin was not significantly associated with infant weight for age z-score. However, leptin was significantly and inversely associated with weight z-score in infants 1 year or younger, although this appeared to be driven by the association in females. Model 1 shows unadjusted regression models for age as a predictor of milk leptin in the full sample, those 1 year or younger, and males and females 1 year or younger. Model 2 has been adjusted for the maternal predictors determined earlier: infant/breastfeeding duration (months) and maternal percentage body fat.

**Figure 1.** Association between Milk Leptin and Infant Weight, by Infant Sex Group.

association between milk leptin and maternal percentage body fat, which is not surprising given that maternal adipose tissue may be a source of milk leptin. At high levels of maternal body fat, the association between milk leptin and circulating leptin levels may not be as strong, possibly reflecting limited capacity to transfer leptin into milk at very high adiposities. This may suggest that milk leptin has greater sensitivity to maternal adiposity among leaner mothers.

Approximately 16% of the variation in milk leptin content was explained by maternal percentage body fat and secondary predictors, well within the range described for other populations. Again, comparative data are rare, as most published reports have reported correlation coefficients and not regression coefficients. The correlation

coefficient for this population was 0.416, within the range reported by other studies.

### Milk Leptin, Maternal Body Composition, and Infant Age

Maternal body fat was an important predictor of milk leptin content in this sample. This may reflect the increased sensitivity to adiposity by percentage body fat compared to BMI. There was no significant linear association between infant age and milk leptin content in this sample, although several prior studies have found significant, inverse associations between infant age and milk leptin content.<sup>43,44</sup> However, these analyses have been limited to mothers breastfeeding infants younger than 1 year of age,<sup>43</sup> and this sample included mothers breastfeeding infants in later lactation (breastfeeding > 1 year). To facilitate comparisons with prior studies, the analyses were subsequently stratified into 2 groups: women breastfeeding infants 1 year or younger and women breastfeeding infants from 1 to 3 years of age. In the 2 groups, infant age was a significant predictor of milk leptin content in the older infants but not the younger infants. Both maternal body fat and milk leptin were higher in mothers breastfeeding younger infants, although neither difference was statistically significant. Mothers breastfed younger infants more frequently than older infants (12.3 vs 10.7 months,  $P = .03$ ). Daily breastfeeding frequency was a significant, positive predictor of milk leptin in older, but not younger, infants (Table 3). These findings—reduced leptin in the milk, decreased body fat, and a significant association between daily breastfeeding frequency and milk leptin—may illustrate increased reliance on mammary leptin synthesis in mothers breastfeeding past 1 year.

## Milk Leptin Content and Infant Size for Age z-Score

Milk leptin is hypothesized to contribute to weight regulation in infants, specifically with increasing leptin predicting both decreased weight and weight gain over time.<sup>23</sup> In this sample, milk leptin was a significant predictor of infant weight and BMI for age z-scores for infants 1 year or younger, as reported previously,<sup>18,20-22,45</sup> but not for older infants (Table 5). Upon further analyses, these associations also appeared to be sex specific, with only females showing a very strong inverse association between milk leptin content and infant z-scores (Figure 1). There was no association between weight or BMI for age z-score in male infants, or in older infants of both sexes. This sex-specific association has not been previously reported and may be visible here only because of the large sample size, or it may be an artifact of the reduced growth velocity common in this sample.

It is well established that sex differences in circulating leptin levels are present from birth, with females having high leptin levels at all life stages compared to males,<sup>46</sup> including infancy.<sup>47</sup> Infant leptin levels are highest after birth and then decline over the first few months. Breastfeeding infants typically have initially higher leptin levels than formula fed infants, although this is reversed in later infancy.<sup>45</sup> Trevino-Garza et al<sup>48</sup> recently reported that female infants who are breastfed have a modest but nonsignificant decline in circulating leptin levels, whereas all males and formula fed females have a significant decrease in leptin levels over the first few months of life. Earlier reports have shown that ingested leptin survives digestion<sup>6</sup> and can be detected in circulation<sup>10</sup>; the associations between milk leptin and infant weight velocity have been interpreted as evidence for similar survival of leptin in human neonatal intestines.<sup>48</sup>

As shown in Table 5, despite nearly identical mean ages, male infants were approximately 500 grams heavier than females; these differences were present for birth weight as well. This greater weight in males compared to females may explain the lack of association between leptin and weight, possibly reflecting differences in sex steroids and related hormones in male and female offspring. The recently identified sex-specific effects of breastfeeding on leptin levels in infants may further highlight sex-specific differences in postnatal developmental programming. However, as these findings are novel, we cannot rule out random chance, and additional investigations in other populations are necessary.

## Conclusion

Here, we have reported for the first time sex-specific associations between milk leptin content and infant weight for age. This is also the first study to quantify leptin levels in

human milk from the Philippines, and a significant association was found between maternal adiposity, as indexed by percentage body fat, and milk leptin. Milk leptin levels were quite low in this sample. As with prior studies, both maternal BMI and percentage body fat were significant predictors of milk leptin content. Maternal percent body fat explained 16% of the variation in milk leptin content, within the range reported by prior studies. Evolutionarily, historically, and throughout much of the world today, lean mothers—and reduced leptin content in human milk—were far more normative than the higher levels of milk leptin reported in heavier, primarily Western mothers. Increased knowledge on population variation, especially for lean populations with lower circulating and milk leptin content, is necessary for better understanding of the function and importance of leptin—both milk-borne and that produced by the infant—in long-term developmental programming. We hypothesize that very low levels of leptin in milk, including those below the detectable limit of the assay, may signal information about the quality of the postnatal environment. Low levels may signal that the environment is marginal and thus promote rapid early postnatal growth by the infant during the period when infant growth will be supported by maternal metabolism. Such developmental signaling may be important in maximizing offspring growth and long-term fitness.

Further work in other non-Western populations with low body fat and marginal nutritional intakes is needed to understand how maternal physiology influences milk leptin content and how milk leptin content may contribute to differences in infant weight velocity and later health risks. Although it has been well established in prior studies that increasing milk leptin content predicts a decrease in infant weight and weight gain, we are only beginning to understand the potential role that leptin and other metabolic hormones in human milk have in shaping long-term growth and health of offspring.

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The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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