Open Access

Recent Progress in Nutrition

LIDSEN Publishing Inc.

Original Research

The Fat Level in the Breast Milk and Its Association with Dietary Intake and Body Mass Index of Breastfeeding Mothers in Central Ethiopia, 2017

Haile Bikila [1,*], Solomon Shiferaw [2], Tariku Tesfaye Bekuma [1]

1. Department of Public Health, Institute of Health Science, Wollega University, Nekemte, Ethiopia; E-Mails: haile.bikila@gmail.com; tarii2007@gmail.com
2. Department of Public Health Nutrition and Dietetics, School of Public Health, Institute of Health Science, Addis Ababa University, Addis Ababa, Ethiopia; E-Mail: solomonshiferaw@gmail.com

* **Correspondence:** Haile Bikila; E-Mail: haile.bikila@gmail.com

Academic Editor: Cristiano Capurso

Special Issue: Diet Quality, Nutrition during Pregnancy and Infant Offspring Health

Recent Progress in Nutrition
2024, volume 4, issue 4
doi:10.21926/rpn.2404021

Received: July 24, 2024
Accepted: December 03, 2024
Published: December 24, 2024

Abstract

Human milk is not static; it is an invariant fluid that fluctuates in micro and macronutrient composition during feeding. The fat in human milk contributes above half the energy the infant demands. Moreover, fat is one of the most essential nutrients for brain tissue development during infancy. However, the effect of maternal nutritional status, including dietary intake, on human milk fat levels is not well investigated, particularly in developing nations. This study aimed to assess the level of human milk fat and its association with dietary consumption and BMI of the lactating mother in Burayyu Town, Oromia Region, Ethiopia. A community-based cross-sectional study was conducted among 206 lactating mothers with infants aged between 2-9 months in Burayu Town, Oromia Region, Ethiopia. Data collection was completed from April 1-April 28, 2017, using the multistage sampling technique. Data were collected using a structured questionnaire and standard measurement procedures, which included, among other things, nutritional status, BMI, and human milk, and repeated multiple pass 24-hour dietary recalls. The human milk sample was transported to the Holeta laboratory center by ice bag and stored at -20°C until Laboratory analysis. EPI data was applied for data entry, and 24-hour food recall was processed using Excel, Nutrition Survey, and IMAPP software. Then, it was exported to STATA version 14 for statistical analysis. A multivariable linear regression model was applied to determine the association between human milk fat level, dietary intake and BMI of lactating mothers by controlling other confounding factors. The mean (±SD) energy intake of lactating mothers was (1820.99 ± 399.98) Kcal, while the mean (±SD) of fat, protein, and carbohydrate intake of lactating mothers was found to be 43.49 ± 18.6 g/day, 89.95 ± 38 g/day and 275.58 ± 62.8 g/day respectively. The lactating mother's mean (±SD) human milk fat level was (3.03 ± 1.03) g/dl. This study did not find any significant association between human milk fat level with energy (β = 0.0002, 95% CI: -0.0004-0.0007), fat (β = -0.004, 95% CI: -0.018-0.009), protein (β = -0.0036, 95% CI: -0.011-0.0037), and carbohydrate intake (β = 0.003, 95% CI: -0.0003-0.006) respectively. Moreover, cesarean section delivery (β = -0.55825, 95% CI: -1.02954 - -0.08696) was inversely associated with human milk fat level. However, human milk fat level was positively associated with breastfeeding frequency (β = 0.074, 95% CI: 0.008-0.139), frequency of meat eating (β = 0.22123, 95% CI: 0.04681-0.39566), and early initiation of human milk (β = 0.19987, 95% CI: 0.03223-0.36751) respectively. Conclusion: Human milk fat content is pretty in the constant range (2-5 g/10 ml) and is not affected by maternal nutrition of energy intake, fat, carbohydrate, or BMI of the mother. It has a significantly positive association with the frequency of eating meat and feeding frequency. The mean human milk fat level is lower than that reported in other countries; however, it would fulfill most infant energy requirements.

Keywords

BMI of the mother; human milk; feeding frequency; maternal diet intake; fat level

1. Introduction

Human milk contains significant amounts of lipids, including triglycerides, cholesterol esters, retinyl esters, and other lipophylic nutrients surrounded by the milk fat globule membrane. The energy and nutrient intake of most lactating mothers in the developing world falls well below the Recommended Dietary Allowance (RDA) [1, 2]. Human milk composition is dynamic and variable among lactating mothers; variations within a breastfeeding mother on the level of human milk are less well understood, with human milk fat content shown to be influenced by the lactating mothers' dietary intake and BMI. Fat is the primary energy source of human milk, which fulfills 50% of the infant's energy demand and is the most changeable of the proximate constituents [3]. Human milk fat is an essential nutrient for babies' growth and development, which can be affected by factors like one's diet. It is a complex mixture of lipids, carbohydrates, proteins, and vitamins that ought to provide the infant with a nutritionally adequate diet [4].

Human milk generally contains 87% water, 3.8% fat, 1.0% protein, and 7% lactose [5, 6]. Fat is a critical component of human milk, providing energy and nutrients that are key to the development of the central nervous system, and it cannot be synthesized in the beginning by the infant [7, 8]. Evidence suggests that a high-fat (H-F) diet increases human milk fat concentration and content by

13% and 15%, respectively, when compared to a high-carbohydrate (H-CH, O) diet, which does not affect protein or lactose [9]. On the other hand, during low-fat diet consumption, the milk fat is maintained by mobilizing body fat; the maternal fat stores throughout pregnancy are more straightforward to mobilize during lactation than other fat stores, which may limit milk fat during exhaustion if it's shallow [10].

There are various perspectives on the significance of maternal diet for human milk fat levels. There is a scarcity of evidence linking human milk fat levels to maternal diet and body mass index, with conflicting evidence about the effect of maternal diet and BMI on total human milk fat levels. Studies on human milk have shown different evidence about the human milk fat level; therefore, this study aimed to assess the level of human milk fat content and its association with dietary intake and BMI of lactating mothers in central Ethiopia.

2. Materials and Methods

2.1 Study Area

The study was conducted in Burayyu Town, located 10 kilometers west of Addis Ababa, the capital city of Ethiopia. The town has 6 kebeles with 92,937 total population of 92,937, of which 47,122 are female. Of the total females, 8779 (18.63%) were in the reproductive age group, while 3.47% were breastfeeding mothers in urban and rural areas. The town's main sources of income were trading, government workers, daily labor, and agriculture. Most of the communities around the area consume teff, barley, wheat, maize, bananas, potatoes, and different green vegetables.

2.2 Study Design and Period

A community-based cross-sectional study was conducted from April 1 to April 28, 2017, in Burrayu town of Finfinne special zone, Oromia regional state, Ethiopia.

2.3 Study Population

Breastfeeding mothers with 2-9 months of age infants were selected by a systematic random sampling method from a sample frame of the source population.

Inclusion criteria: The study included mothers who did not take the drug for any medical problems during data collection and those who breastfed continuously without interruption.

Exclusion criteria: Mothers with confirmed pregnancy who were not breastfeeding for more than a day during the data collection were excluded.

2.4 Sample Size and Sampling Procedure

The sample size (n) required for the study was calculated using the single mean population formula with the following assumption:

For the first objective:

Confidence interval; 95%

D = margin of error (precision) = 0.2

$$n = \frac{(z_{\alpha/2})^2 * (SD)^2}{d^2}$$

Where

n = -------------- The desired sample size

SD = ------------- standard deviation (1.41)

$Z_{\alpha/2}$ = ----------- critical value at 95% CI (1.96)

d = the margin of error between sample and the Population mean, Calculation of the sample size

$$\mathrm{n} = \frac{(1.96)^2 * (1.41)^2}{(0.2)^2} = 190.93 \approx 191$$

with the assumption of standard deviation (SD) = 1.41 confidence level = 95% ($Z_{\alpha/2}$ =1.96), D = the margin of error between sample and the Population mean of 2%, and the calculated sample size using a single mean population formula was: $\mathrm{n} = \frac{(1.96)^2 * (1.41)^2}{(0.2)^2} = 191.$

Considering a 10% non-response rate, the required sample size was 210 lactating mothers, which was taken as a final sample size. A multistage sampling technique was used to select lactating mothers. First, the kebeles were stratified into urban and rural areas, and two kebeles from each area were selected using simple random sampling. A list of breastfeeding mothers was prepared in consultation with health extension workers. After preparing a sampling frame based on the list of lactating mothers between 2-9 months in selected kebeles, the study subjects were selected using simple random sampling from all levels of households located in the selected kebeles. Accordingly, 206 breastfeeding mothers were chosen for the study. Two hundred six breastfeeding mothers were interviewed using a pretested interviewer-administered structured questionnaire with height and weight measurements. For laboratory analysis, we took 50% of the calculated sample size for breast milk collection. Accordingly, 103 lactating mothers were selected by simple random sampling for the breastmilk sample collection, from which 98 (95.12%) mothers provided their breastmilk for analysis. The sample was taken after mothers were interviewed for dietary assessment and body mass index measurement.

2.5 Data Collection Tools

Data was collected through a house visit using an interviewer-administered questionnaire that included socio-demographics, dietary history, and the BMI of a lactating mother. Dietary intake was collected in multiple passes, i.e., it was performed by quickly listing all the foods they remembered consuming in the previous 24 hours of food recall, providing details on each food consumed, including portion size, checking for any missing items, reviewing the foods reported as consumed, and double-checking no items were missed for the lactating mothers by using every other day for three days by considering every other day exist within a week.

2.6 Data Collection Procedure

During the data collection period, data collectors used a food weighing scale and home utensil material for the estimation of portion size. A sample of human milk was taken from the mothers by revisiting them after data on height, weight, and 24-hour food recalls were collected. During the human milk sample collection, the mother was asked to wash her hands and express her human milk. The sample was finally collected in a sealed tube labeled with a code, saved in a vaccine carrier with ice bags, and later stored in a deep freeze until laboratory analysis.

The subject's height was measured barefoot with arms hanging freely at the sides and recorded to the nearest 0.5 cm using a steel tape measure and at 90^0 angles with a wall. Body weight was measured with a beam balance of the nearest 0.1 kg on the subject's barefoot while wearing light clothing. Standardization was done with data collectors to minimize measurement errors before measuring the actual height and weight. Respondents were asked to describe all foods and beverages consumed, including cooking methods and recipes, during multiple 24-hour food recalls. All 206 participants (breastfeeding mother) were invited to stay for three days, including weekends. Using the software, nutrient data from food composition tables in Ethiopia, Tanzania, and the United States were combined to calculate dietary intake.

2.7 Human Milk Sample Collection

The human milk sample was collected during regular feeding time, two hours after the previous nursing, for self-expression by the mothers. Before expressing human milk, the mother washed her hands with soap. A 15-ml human milk sample was collected into a rigid plastic container with an airtight seal. After that, it was transported by ice bag to the health center and stored at an appropriate temperature till it transported to Holota laboratory center for analysis. The human milk sample was stored at -20°C [9] until fat extraction was done at Holota laboratory center. The primary test used to determine the fat content of human milk was a Gerber method test.

2.8 Method of Human Milk Fat Content Analysis

The primary test used to determine the fat content of milk and milk products is the Gerber method test. Routine methods for testing milk are now utilized in central laboratories and large processing centers. Milk fat exists as an emulsion that is stabilized by phospholipids and proteins. The theory of the Gerber method is based on the fact that the fat globules are demulsified by adding 90% sulphuric acid (H_2SO_4). Free fat, with a lower density than the surrounding medium, may be separated rapidly by centrifugal force. The sample and H_2SO_4 were mixed and centrifuged for 5 minutes at 1100 rpm. When amyl alcohol is added to it, a clearer dividing line on the butyrometer scale between the fat layer and the other material is formed.

2.9 Statistical Methods

EPI data version 3.1 was used for data entry, and Excel nutrition survey software and IMAPP were applied for a food processor. In the end, it was exported to STATA version 14 for data analysis. A 24-hour food recall was analyzed by preparing a food composition table and introducing it into the nutrition survey software, which was analyzed using Excel. The analyzed data in Excel was averaged, and the mean value was exported to STATA for final analysis. Descriptive statistics like mean and standard deviation were used in the analysis of the dietary intake of lactating mothers. The mean human milk fat was compared with maternal diet intake, feeding frequency, and BMI. All variables were tested by linear regression concerning human milk fat level. In the bivariate analysis; the age of the mother, BMI, early initiation of breastfeeding after birth, feeding frequency, habitual high meat diet intake, energy intake, carbohydrate intake, normal delivery, wealth index, and alcohol consumption were included. Those variables that had a P-value < 0.25 were entered into the multivariable linear regression model. Linear and multivariable regression models were employed for the candidate variables selected with a statistical significance of ($p < 0.25$). Variables are significant at $p < 0.05$, and 95% CI were employed for the final multivariable models to assess the association between human milk fat content, maternal dietary intake, and other variables.

2.10 Operational Definition

Inadequate energy intake - If the mother consumes less than 2000 kcal/day [10].

Adequate energy intake - If the mother consumes over 2000 kcal/day [10].

24-hour dietary recall - This is a structured interview intended to capture detailed information about all foods and beverages consumed by the mother in the past 24-hours, most commonly by periods like midnight to midnight.

Estimated food records - This quantifies foods and drinks estimated by household measures such as cups or spoons, food photographs, and food models rather than weighing.

Multiple passes - Refers to the steps involved during the interview to allow revisiting and checking dietary information.

Mean fat content of human milk - the average fat in human milk above and below the mean value studied.

Inadequate fat content - If the level of fat content is less than 2 g/10 ml [11].

Adequate fat content - If the fat level in the human milk is greater than or equal to 2 g/10 ml [11].

2.11 Ethics Clearance

The ethics clearance was obtained from the ethics review committee at the School of Public Health and the Institution Review Board (IRB) of the College of Health Sciences, Addis Ababa University. All participants’ right to self-determination and autonomy was respected. Before the interview, written informed consent was taken from each participant.

3. Results

3.1 Socio-Demographic Characteristics

Of the 210 lactating mothers, 206 completed the anthropometric and dietary data assessment (98. 1% response rate). Among the 103 lactating mothers selected for breastmilk sample collection, 98 mothers (95.12% response rate) had completed the data. The respondents' mean (±SD) ages were (25.7 ± 4.6) years. About 91 (44.17%) of lactating mothers are in the age range between (25-29) years. The participant mothers were predominantly housewives, 159 (77.18%), followed by a farmer, 17 (8.25%). Of the participants, 90 (43.96%) mothers attended primary school (Table 1).

Table 1 Socio-demographic characteristics of the lactating mother in Burrayu town, Oromia region, Ethiopia, 2017.

Socio demographic characters	Frequency	Percent (n = 206)
Age of the mother (n = 206)		
15-19	12	5.83
20-24	65	31.55
25-29	91	44.17
30-34	26	12.62
35-45	12	5.83
Religion of the mother (n = 206)		
Orthodox	73	35.44
Muslim	63	30.58
Protestant	68	33.01
Other specify	2	0.97
Ethnicity		
Oromo	104	50.49
Amhara	28	13.59
Tigire	2	0.97
Gurage	47	22.82
Other	25	12.14
Educational states of the mother (n = 206)		
No education	35	16.99
Primary	90	43.96
Secondary	48	23.30
More than secondary	33	15.75
Occupational states (n = 206)		
House wife	159	77.18
Farmer	17	8.25
Merchant	10	4.85
Government employ	15	7.28
Daily labors and other	5	2.43
Socio demographic characters	**Frequency**	**Percent (n = 98)**
Age of the mother (n = 98)		
15-19	3	3.06
20-24	33	33.67
25-29	42	42.86
30-34	12	12.24
35-45	8	8.16
Religion of the mother (n = 98)		
Orthodox	35	35.71
Muslim	34	34.69
Protestant	28	28.57
Other specify	1	1.02

Ethnicity		
Oromo	49	50
Amhara	9	9.20
Tigire	2	2
Gurage	24	24.49
Other	14	14.29
Educational states of the mother (n = 98)		
No education	18	18.37
Primary	46	46.93
Secondary	25	25.51
More than secondary	9	9.8
Occupational states (n = 98)		
House wife	73	74.53
Farmer	13	12.27
Merchant	5	5.10
Government employ	6	6.12
Daily labours and other	1	1.02

3.2 Maternal Dietary Intake Status

The mean (±SD) daily energy intake of lactating mothers was (1820.99 ± 399.98, n = 206) kcal/day. The mean (±SD) energy intake of lactating mothers included in the biological study was (1830.3 ± 387.28, n = 98) kcal per day. The lactating mother's protein and fat mean (±SD) daily intake was (89.95 ± 38.14 and 43.5 ± 18.6, n = 206) grams per day, respectively. The daily mean (±SD) protein intakes were above the estimated average requirement (39.2 g/day) per day. Based on the EAR, only (1.46%, n = 206) of the lactating mothers had protein inadequacy. The mean (±SD) daily protein and fat intake for the breastmilk study group were (85.8 ± 28.24, 43.24 ± 15.7, n = 98) grams per day, respectively. The prevalence of fat inadequacy cannot be evaluated with the EAR of lactating mothers like other nutrients. The general recommendation of fat is based on its contribution to daily energy: 15-30% of the power should come from fat, keeping in mind that saturated and trans fatty acid consumption should be kept as low as possible with a nutritionally adequate diet. Based on this, the study found that the daily fat intake of lactating mothers showed that fat contributes (15.8%-25.2%, n = 206, 17.18%-23.92%, n = 98) respectively, to the daily average energy intake for both groups (Table 2).

Table 2 Energy and macronutrient intake of lactating mothers in Burayyu Town, Oromia region, Ethiopia, 2017 (n = 206).

Indicators	Mean ± SD daily intake of mother	EAR	Prevalence of women with inadequate intake
Energy (kcal/d) (206)	1820.99 ± 399.98 kcal/day	2000 kcal/day	**73.3%**
Protein (g/d) (n = 206)	89.95 ± 38.14 g/day	39.2 g/day	**1.5%**
Fat (g/d) (n = 206)	43.49 ± 18.6 g/day	15-30% from total calorie	20.4%

Carbohydrate (g/d) (n = 206)	275.58 ± 62.8 g/day	230 g/day	**21.8%**
Energy (Kcal/d) (n = 98)	1830.329 ± 387.3 g/day	2000 kcal/day	**71.5%**
Protein (g/d) (n = 98)	88.45 ± 33.5 g/day	39.2 g/day	**1.4%**
Fat (g/d) (n = 98)	43.24 ± 15.7 g/day	15-30% from total calorie	**19%**
Carbohydrate(g/d) (n = 98)	279.7 ± 65.2	230 g/day	**20.5%**

EAR - Estimated average requirement.

3.3 The Association Between Maternal Nutrition and Human Milk Fat Level

The mean (±SD) of the human milk fat content was 3.03 ± 1.03 g/dl. 52 (53.06%) lactating mothers had human milk fat levels above 3.03 g/dl. However, many lactating mothers had adequate (68.39%) human milk fat levels. The human milk fat level, which is less than 2%/10 ml, is assumed to be inadequate. In this study, 16.33% of the lactating mothers had a human milk fat level of less than 2%, which is considered to be an inadequate fat level in human milk (Table 3).

Table 3 Bivariate linear regression analysis to determine the association between macronutrient intake and human milk fat level of a lactating mother in Burrayu Town, Oromia, Ethiopia, 2017.

Macronutrients	B	P-value	Significance (95% CI of β)
Energy kcal/day	0.00016	0.562	-0.00038-0.00069
Carbohydrate g/day	0.00312	0.051	-0.00001-0.00626
Fat g/day	-0.00432	0.520	-0.01760-0.00896
Protein g/day	-0.00363	0.329	-0.01099-0.00372

The energy intake of lactating mothers had no significant association (β = 0.0002, 95% CI: -0.0004-0.0007, $P < 0.562$) with human milk fat level.

3.4 Maternal BMI with Human Milk Fat Level

The average BMI was within the normal body mass index range (22.44 ± 2.45). In this study, 7.14%, 75.51%, and 17.35% of the study subjects were underweight, normal-weight, and overweight, respectively. The mean human milk fat level of the mothers with BMI > 25 was (3.01 ± 0.12) g/dl, while those with BMI < 18.5 were (2.7 ± 0.45) g/dl. The mean (±SD) of different nutritional status categories of the lactating mothers has not shown a statistically significant association as tested by one-way ANOVA analysis of variance (F statistics = 0.65, p-value = 0.53).

3.5 Predictors of Human Milk Fat Level

Age of the mother, CHO intake, alcohol consumption, BMI of the mother, chronic consumption of meat per day, cesarean section delivery, early initiation of breastfeeding, and feeding frequency were selected for multivariable linear regression. The inter model method was used to examine the association.

In multivariable linear regression, breastfeeding frequency (β = 0.07386, 95% CI: 0.00775-0.13998, p < 0.029) and frequency of meat intake (β = 0.22123, 95% CI: 0.04681-0.39566, p < 0.014), showed significant association with the human milk fat level, while energy intake and BMI of the mothers had no significant association. In this study, as breastfeeding frequency increased by one unit, the human milk fat level increased by 0.74 unit points. Similarly, it was found that for each unit increase in the frequency of meat intake, the human milk fat level increased by 0.217 unit points (Table 4).

Table 4 Multivariable linear regression analysis to determine the variable associated with human milk fat level of lactating mothers in Burrayu town, Oromia, Ethiopia, 2017.

Variable	β	P-value	Significance (95% CI of β)
Breastfeeding frequency (within 24 hours)	0.07386	0.029	0.00775-0.13998
Alcohol consumption	-0.53091	0.073	-1.11197-0.05014
Carbohydrate (g/day)	0.00260	0.080	-0.00031-0.00552
BMI of the mother (kg/m^2)	0.02356	0.560	-0.05644-0.10355
Consumption of meat per day	0.22123	0.014	0.04681-0.39566
Early initiation of breastfeeding	0.19987	0.020	0.03223-0.36751
Age of the mother	0.02558	0.264	-0.01966-0.07082
Cesarean section delivery	-0.55825	0.021	-1.02954 - -0.08696

R^2 = 0.3.

4. Discussion

This study aimed to assess the level of human milk fat and its association with dietary intake and BMI in lactating mothers. It found that the mean level of human milk fat is (3.03 ± 1.03 SD) g/dl, which is lower compared to the mean of the reference value (3.52 ± 1.41 SD). The study findings in Brazil were 3.9 g/dl, America 4 g/dl, and Iran 3.8 g/dl [12-15].

This study also found that the human milk fat level ranged from 3.5 g to 4.5 g per 10 mL during lactation. This variation could be due to various reasons, supported by a WHO report that showed lower values of human milk levels in developing countries than in developed ones [16, 17]. The breastfeeding practice of lactating mothers might contribute to the difference in human milk fat levels between different communities. The difference might also be attributed to the differences in human milk sample collection, lipid extraction, and analysis methods.

Our study discovered no link between human milk fat content and mothers' dietary energy intake. However, other studies have shown that diet is the most critical determinant of human milk fat levels [18, 19]. Another study found that even if mothers did not increase their energy intake, they could still produce adequate human milk fat [20]. Lactating mothers' energy requirements are recommended to be higher than those of pregnant women. On the other hand, human milk fat may be independent of energy intake and mobilized from adipose tissue stores regardless of energy intake [21].

Furthermore, this study found that lactating mothers' fat, CHO, and protein intake were not associated with breast fat levels. This contradicts an Iranian study that found a positive correlation between carbohydrate consumption and milk fat content [12]. However, WHO indicates that the lipid content of mature human milk is little influenced by variations in the mothers' nutritional status or dietary intake [16]. The study, conducted on seven healthy lactating mothers randomly for eight days and separately for one to two weeks, found that maternal energy intake did not affect milk volume, lactose, or protein concentrations [22]. This indicates that maternal dietary intake alone does not determine breast fat level in short period of time.

This study shows that breastfeeding frequency has a significant association with the increment of human milk fat level, which is consistent with the study of continuous survey. This study found that breastfeeding frequency has an essential relationship with the increase in human milk fat level, which is consistent with a previous study that found that continuous lactation has a significant role in human milk fat concentration [23]. Feeding frequencies have been suggested to influence the fat content of milk as well [21]. Another study discovered that the amount of fat in human milk varies dramatically during breastfeeding and throughout the day because fat content varies according to breast emptiness (empty breast = high fat, full breast = low fat) [11]. This may be because feeding encourages the mother to use around 700 kcal of energy to produce one liter of milk by mobilizing adipose tissue [3].

There are two experimental theories on the emptiness of the breast. One theory suggests that milk fat globules accumulate on the surface of alveolar membranes and are displaced only when the gland is near empty. The second theory proposes a gradual filtration of the duct wall-adhering fat globules during breast emptying [24-26]. Breastfeeding frequently increases human milk fat production and the mobilization of stored fat, as feeding frequently is often a major initiator of adipose tissue mobilization. This is because frequent breastfeeding makes the breast empty, which in turn increasing the production of human milk fat.

It was also discovered that there is a link between meat consumption and human milk fat level, which is consistent with previous research that found a link between meat consumption and human milk fat level [15]. Another study discovered that a high-protein diet mothers consume may account for approximately 25% of the variation in lipid concentration between mothers' milk [27]. This may be since a high protein diet is associated with increased activity of a hormone called leptin, which is considered an integral part of appetite regulation and energy balance in infant breastfeeding [28, 29]. As a result, if leptin indirectly induces the infant to feed, it increases breastfeeding demand by increasing the infant's self-regulation [19, 30, 31] (The increased breastfeeding frequency caused by the infant's frequent feeding demand may explain why meat is linked to human milk fat level [32].

In this study, no association was found between BMI and human milk fat level. Mothers store fat during their pregnancy and reduce their BMI during breastfeeding; this is the physiological process during pregnancy. However, mobilization of fat during breastfeeding may contribute to the human milk fat level. A study from Iran and Argentina showed that the BMI of the mother has an association with the human milk fat level [33, 34]. Many investigations have attempted to correlate poor maternal nutrition with the total concentration of milk fat. Low fat levels were also discovered in the milk of malnourished women. A study of Filipino mothers found that milk fat increased with breastfeeding duration but not with body mass index [35]. Rather than having a high BMI without adequate nursing, mothers should feed continuously to mobilize their stored body fat. This may indicate that human milk fat adequacy depends on the duration of breastfeeding by mobilizing the existing fat.

5. Limitation

The association between human milk fat level and micronutrient consumption of lactating mothers was not assessed. The last meal of the mother at the time of human milk sample collection was not assessed, and the immediate effect on human milk fat level was not addressed. In addition, the leptin level in human milk, which regulates infant appetite to increase the demand for feeding, was also not investigated.

6. Conclusions

The fat content of human milk was within the normal recommended range. It was unaffected by maternal nutrition in terms of energy, fat, carbohydrate intake, and the mother's BMI. The fat content of human milk has a significantly positive association with the frequency of eating meat and feeding frequency. The mean human milk fat level is lower than that reported in other countries; however, it fulfills most infant energy requirements. Therefore, breastfeeding mothers should regularly increase their meat or high-protein diet group during breastfeeding. Mothers should also have adequate time for breastfeeding by maintaining the emptiness of one breast before shifting to the other side. Policymakers and formula milk producers should consider that human milk fat levels should not be as minimum as 3.2 g/100 kcal if formula milk is mandatory to be prepared. Healthcare providers are also recommended to counsel mothers to eat foods rich in high-quality proteins during service delivery and lactation. Longitudinal studies are recommended to analyze human milk fat components and last meal intake before human milk sample collection among lactating mothers to check changes within the feeding frequency.

Abbreviations/Acronyms

AA	Archoidonic Acid
ADH	Adhanine dehexosnicacid
BMI	Body Mass Index
CHO	Carbohydrate
FA	Fatty acid
FAO	Food and Agriculture Organization of the United Nations
H-F	High fat
H-CHO	High carbohydrate
H_2SO_4	Sulphuric acid
IRB	Institutional Review Board
kg	Kilogram
LCPUFA	long-chain polyunsaturated fatty acid
LCPUFA	National Health and Nutrition Examination Study
ml	Mililitre
NHANES	National Health and Nutrition Examination Study
PUFA	Polyunsaturated Fatty acid
rda	Recommended dietary allowance
RPM	Rate per minute
SD	Standard Deviation

SRS Simple Random Sampling
USAD United Sates Academic Decathlon
WHO World Health Organization

Author Contributions

HB: Conceptualization, writing – original draft, formal analysis, methodology, writing – review and editing, software. SS: Methodology, writing – review and editing. TTB: Review, writing, editing and editing, writing – review and editing. Each author contributed significantly to the conception and design of the study without reservation. All authors have read and approved the published version of the manuscript.

Competing Interests

The authors have declared that no competing interests exist.

References

1. Prentic A, Prentice AM, Whitehead RG. Breast-milk fat concentrations of rural African women: 1. Short-term variations within individuals. Br J Nutr. 1981; 45: 483-494.
2. Trahms CM, McKean KN. Nutrition during infancy. Krause's food and nutrition therapy. 12th ed. Amsterdam, Netherlands: Elsevier; 2008.
3. Segura SA, Ansótegui JA, Díaz-Gómez NM. The importance of maternal nutrition during breastfeeding: Do breastfeeding mothers need nutritional supplements? An Pediatr. 2016; 84: 347.e1-347.e7.
4. Prentice A, Prentice AM, Whitehead RG. Breast-milk fat concentrations of rural African women. 2. Long-term variations within a community. Br J Nutr. 1981; 45: 495-503.
5. Lauritzen LA, Hansen HS, Jørgensen MH, Michaelsen KF. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res. 2001; 40: 1-94.
6. Koletzko B, Rodriguez-Palmero M, Demmelmair H, Fidler N, Jensen R, Sauerwald T. Physiological aspects of human milk lipids. Early Hum Dev. 2001; 65: S3-S18.
7. Picciano MF. Nutrient composition of human milk. Pediatr Clin North Am. 2001; 48: 53-67.
8. Perez-Escamilla R, Cohen RJ, Brown KH, Rivera LL, Canahuati J, Dewey KG. Maternal anthropometric status and lactation performance in a low-income Honduran population: Evidence for the role of infants. Am J Clin Nutr. 1995; 61: 528-534.
9. WHO. Fourth WHO-coordinated survey on human milk for persistent organic pollutants in cooperation with UNEP: Guidelines for developing a national protocol [Internet]. Geneva, Switzerland: World Health Organization; 2007. Available from: https://www.tuttosteopatia.it/wp-content/uploads/popprotocol.pdf.
10. Scientific Advisory Committee on Nutrition. Dietary reference values for energy. London, UK: The Stationery Office; 2011.
11. Jelliffe DB, Jelliffe EFP. Human Milk in the Modern World. Br Med J. 1978; 2: 1573. PMCID: PMC1608823.

12. Nikniaz L, Mahdavi R, Arefhoesseini S, Khiabani MS. Association between fat content of breast milk and maternal nutritional status and infants' weight in Tabriz, Iran. Malays J Nutr. 2009; 15: 37-44.
13. Villalpando SF, Butte NF, Wong WW, Flores-Huerta S, Hernandez-Beltran MD, Smith EO, et al. Lactation performance of rural Mesoamerindians. Eur J Clin Nutr. 1992; 46: 337-348.
14. Rocquelin G, Tapsoba S, Dop MC, Mbemba F, Traissac P, Martin-Prével Y. Lipid content and essential fatty acid (EFA) composition of mature Congolese breast milk are influenced by mothers' nutritional status: Impact on infants' EFA supply. Eur J Clin Nutr. 1998; 52: 164-171.
15. da Cunha J, Macedo da Costa TH, Ito MK. Influences of maternal dietary intake and suckling on breast milk lipid and fatty acid composition in low-income women from Brasilia, Brazil. Early Hum Dev. 2005; 81: 303-311.
16. WHO. The quantity and quality of breast milk: Report on the WHO collaborative study on breast-feeding. Geneva, Switzerland: World Health Organization; 1985.
17. Brown KH, Akhtar NA, Robertson AD, Ahmed MG. Lactational capacity of marginally nourished mothers: Relationships between maternal nutritional status and quantity and proximate composition of milk. Pediatrics. 1986; 78: 909-919.
18. Finley DA, Lönnerdal B, Dewey KG, Grivetti LE. Breast milk composition: Fat content and fatty acid composition in vegetarians and non-vegetarians. Am J Clin Nutr. 1985; 41: 787-800.
19. Kent JC, Mitoulas LR, Cregan MD, Ramsay DT, Doherty DA, Hartmann PE. Volume and frequency of breast feedings and fat content of breast milk throughout the day. Pediatrics. 2006; 117: e387-e395.
20. Andres A, Cleves MA, Bellando JB, Pivik RT, Casey PH, Badger TM. Developmental status of 1-year-old infants fed breast milk, cow’s milk formula, or soy formula. Pediatrics. 2012; 129: 1134-1140.
21. Del Prado M, Villalpando S, Elizondo A, Rodríguez M, Demmelmair H, Koletzko B. Contribution of dietary and newly formed arachidonic acid to human milk lipids in women eating a low-fat diet. Am J Clin Nutr. 2001; 74: 242-247.
22. Mohammad MA, Sunehag AL, Haymond MW. Effect of dietary macronutrient composition under moderate hypocaloric intake on maternal adaptation during lactation. Am J Clin Nutr. 2009; 89: 1821-1827.
23. Paul VK, Singh M, Srivastava LM, Arora NK, Deorari AK. Macronutrient and energy content of breast milk of mothers delivering prematurely. Indian J Pediatr. 1997; 64: 379-382.
24. Atwood CS, Hartmann PE. Collection of fore and hind milk from the sow and the changes in milk composition during suckling. J Dairy Res. 1992; 59: 287-298.
25. Neville MC, Allen JC, Watters C. The mechanisms of milk secretion. In: Lactation. Boston, MA: Springer; 1983. pp. 49-102.
26. Whittlestone WG. Variations in the fat content of milk throughout the milking process. J Dairy Res. 1953; 20: 146-153.
27. Section on Breastfeeding, Eidelman AI, Schanler RJ, Johnston M, Landers S, Noble L, et al. Breastfeeding and the use of human milk. Pediatrics. 2012; 129: e827-e841.
28. Weigle DS, Breen PA, Matthys CC, Callahan HS, Meeuws KE, Burden VR, et al. A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. Am J Clin Nutr. 2005; 82: 41-48.

29. Palou A, Picó C. Leptin intake during lactation prevents obesity and affects food intake and food preferences in later life. Appetite. 2009; 52: 249-252.
30. Dewey KG, Heinig MJ, Nommsen LA, Lonnerdal B. Maternal versus infant factors related to breast milk intake and residual milk volume: The DARLING study. Pediatrics. 1991; 87: 829-837.
31. Karatas Z, Durmus Aydogdu S, Dinleyici EC, Colak O, Dogruel N. Breastmilk ghrelin, leptin, and fat levels changing foremilk to hindmilk: Is that important for self-control of feeding? Eur J Pediatr. 2011; 170: 1273-1280.
32. Hassiotou F, Geddes DT. Programming of appetite control during breastfeeding as a preventative strategy against the obesity epidemic. J Hum Lact. 2014; 30: 136-142.
33. Brenna JT, Varamini B, Jensen RG, Diersen-Schade DA, Boettcher JA, Arterburn LM. Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. Am J Clin Nutr. 2007; 85: 1457-1464.
34. Marín MC, Sanjurjo A, Rodrigo MA, de Alaniz MJ. Long-chain polyunsaturated fatty acids in breast milk in La Plata, Argentina: Relationship with maternal nutritional status. Prostaglandins Leukot Essent Fatty Acids. 2005; 73: 355-360.
35. Quinn EA, Largado FE, Power M, Kuzawa CW. Predictors of breast milk macronutrient composition in Filipino mothers. Am J Hum Biol. 2012; 24: 533-540.