**Original Article**

# Macronutrient Composition of Breast Milk for Full Term and Premature Infants: Correlation to Nutritional and Socioeconomic Factors

**Panagiota Koutsiafti**
**Faculty of Medicine School of Health Sciences, Department of Neonatology, NICU, University of Thessaly, Larissa, Greece**

**Georgia Soultani**
**PS Food Technology, University of Thessaly, Karditsa, Greece**

**Stergios Kechagias**
**Faculty of Medicine School of Health Sciences, Department of Orthopaedic Surgery & Musculoskeletal Trauma, Larissa, Greece**

**Ioanna Grivea**
**Faculty of Medicine School of Health Sciences, Department of Neonatology-NICU, University of Thessaly, Larissa, Greece**

**Eleni Malissiova**
**PS Food Technology, University of Thessaly, Karditsa, Greece**

**Correspondence:** Koutsiafti Panagiota, 1 Kondylaki st. Larissa,41221, Greece e-mail: pkoutsiafti@gmail.com

**Abstract**

**Background:** Breast milk is recognized as the best food available for optimal baby development. The purpose of this study was to compare the concentrations of macronutrients of human milk from mothers of full term and premature infants and to evaluate changes of breast milk according to maternal social and demographic parameters.
**Methods:** Breast milk samples were obtained from 50 healthy nursing women with an average age of 32.0 ± 3.3 years and the lipid, protein and lactose content was analyzed. A self report questionnaire relating to socio-demographic and nutritional information was completed by each participating mother.
**Results:** The mean maternal milk macro- nutrient concentrations were: fat 3.16 ± 0.97 g / dL, protein 1.90 ± 0.43 g/dL, lactose 6.8 ± 0.83 g / dL. Educational level, occupation and certain foods were found to significantly affect the composition of breast milk. The fat and protein content of the private sector or self-employed mothers was significantly higher in comparison with public sector or un-employed mothers ($p < 0.01$). There were no significant correlations between the concentration of lactose and the parameters analyzed.
**Conclusion:** Socioeconomic factors may contribute to changes in the macronutrients of breast milk. Future studies on cultural and environmental factors' effect on breast milk synthesis could be assessed.

**Key words:** Breast milk, nutrition, socioeconomic factors, premature infants

## Introduction

Breast milk is often described as the golden standard of infant nutrition, since it provides all the macronutrients and vitamins needed for optimal baby growth (Hennet & Borsig, 2016). Human breast milk, with the unique combination of essential nutrients and many bioactive agents, is an ideal food with a variety of benefits for the survival of infants but also for their development, including brain development and cognitive functions. Cytokines, antimicrobial proteins, hormones, and oligosaccharides enhance the intestinal mucosal immunity and promote the infant's physical development (Hennet & Borsig, 2016). Breast milk is a perfect multifunctional fluid blend that has

evolved over the centuries to nourish infants and protect them from various diseases while maturing their own immune system (Andreas, Kampmann & Mehring Le-Doare, 2015).

The lactation stage affects breast milk macronutrients to a greater extent than the nationality and age of mothers or other variables. In general, colostrum has high concentrations of bioactive proteins and oligosaccharides , whereas mature milk has proportionally higher levels of lipids and caseins. Maternal nutrition has little effect on most macronutrients, although lipids taken up by the diet affect the fatty acid composition of breast milk (Hennet & Borsig, 2016). However, socioeconomic factors seem to exert an (indirect or direct) effect on breastfeeding, mainly through maternal support or protein intake adequacy (Daglas et al., 2020; Lin et al., 2020). These two factors, along with duration of pregnancy and past experience of breastfeeding have been proven significant for breastfeeding at home (Daglas et al., 2020).

Lipids are the largest source of calories, yielding 40-50% of the total diet of breast milk. Apart from triglycerides and cholesterol, the lipid fraction of the colostrum comprises a variety of lipids, such as anti-inflammatory lipoxins and resolvins. Milk proteins are often classified as insoluble casein and soluble proteins, which comprise bioactive proteins, such as secretory IgA (sIgA), lactoferrin , lysozyme, and alpha- lactalbumin. The carbohydrate fraction includes lactose (50-70 g / l) and oligosaccharide complex (7-12 g / l). The Antonakou et al. study (2013) in Greek mother milk samples at the start of the 1st, 3rd and 6th months postpartum showed that the intake of monounsaturated and polyunsaturated lipids of the mother significantly affected the lipid profile of the milk. Also, maternal weight gain during pregnancy was associated with a higher proportion of saturated lipids in milk, and it was also found that multiparous mothers' milk had a higher total lipid concentration compared with milk from uniparous mothers (Antonakou et al. 2013).

Previous studies of maternal milk macronutrients from mothers of different nationalities showed little variation with regard to diet as the change in milk lipid concentration seems to be independent of maternal diet. However, certain fatty acids that form the lipid fraction depend on the mother's diet. These fatty acids are either intrinsically synthesized by the mammary gland, or are taken up by the maternal plasma. These two sources of fatty acids are affected by the mother's diet (Ballard & Morrow, 2013). The fatty acid profile in breast milk can be modified by controlling the diet of the mother, especially with regard to monounsaturated omega-6 fatty acids and omega-3 fatty acids. As dietary fatty acids are rapidly transferred to breast milk within 2-3 days, the breast milk composition changes. The mammary gland is able to synthesize the medium chain fatty acids (MCFAs). In women receiving a diet high in carbohydrates and low in fat, an increase of MCFA synthesis is observed, in order to maintain the amount of triglycerides in human breast milk (Andreas, Kampmann & Mehring Le-Doare, 2015).

The purpose of this study was to assess the concentrations of macronutrients in milk available for mature and premature infants and to evaluate their changes according to nutritional, social and demographic parameters (such as age, infant immaturity, residential area, educational level, occupation and socio-economic condition).

**Methodology**

The study sample comprised mothers of newborns in the Larissa region, Thessaly, central Greece. The Intensive Nursing Unit of the University Hospital of Larissa was selected. Of the 50 mothers included in the study, 27 were full term infants' mothers and 23 premature infants' mothers. Mothers were administered a self report questionnaire with closed type questions for the collection of socio-demographic and nutritional information. The survey took part with the permission of the hospital and the mothers. Mothers were informed about the aim of study and the way they should complete the questionnaire. The milk collection was done in a special area of the hospital during the morning and two hours at least following breastfeeding. A total of 30 mL milk was collected from one breast by an electric breast pump. Milk was placed in three dark sterile plastic tubes without preservatives and was immediately transferred in a cool-box, in order to be stored at -80 _C until analysed. The samples analysis was performed with the spectroscopy, Milkoscan 4000 device which identifies

the contents fractions mass of fat, protein, lactose of milk.

Regarding mothers' diet, beef, pork chicken, milk and feta cheese consumption was questioned among other variables. However, only these foods were included in univariate analysis for breast milk content in fat, protein, and lactose, due to limited number of participants, for statistical reliability reasons

Values are expressed as Mean (standard deviation). Statistical analysis was performed with Statistical Package for Social Sciences 22.0 (SPSS 22.0). ANOVA, t-test were and linear regression models were applied. Statistical significance was set at p=0.05.

**Results**

Regarding mothers' age, 20.0% were under 26 years old, 30.0% were aged 26-31, 36.0% aged 32-37 and 14.0% older than 37 years of age. The 74.0% of mothers were urban residents, while 42% were university-educated (65.8%). The 4.0% of mothers surveyed were unemployed, 32.0% employed in the private sector or freelancers and 24.0% employed in the public sector; 42.0% had premature babies. In the 55.2% of the sample, the annual family income ranged from 8000 € to 15000 €, in 32.0%, the annual income exceeded 15000. (Table 1)

The following data concerning fat, protein and lactose content were obtained from the laboratory analysis of breast milk samples: fat 3.16 ± 0.97 g / dL, protein 1.90 ± 0.43 g/dL, lactose 6.8 ± 0.83 g / dL.

Table 2 shows the associations between the amount of fat in breast milk and socio-demographic variables. There was a statistically significant difference regarding the mean values "fat content in breast milk of mothers" depending on the variable "mother profession" : the milk fat of mothers working in the public sector is lower than the other two categories. p =0.009.

Characterization of the newborn as premature or mature had no significant effect (although a difference did emerge ) on the fat content of breast milk, that is, both groups of mothers do not produce milk with the same amount of fat (premature: 3.34 ± 0,95g dL vs mature: 2,99± 0.93 g / dL), p = 0,203

Table 3 shows the correlations between the amount of protein in breast milk and socio-demographic variables. The maternal educational level (Elementary/Junior high school/High School graduates and University graduates) considerably affects the factor "amount of protein in human milk", i.e. the two maternal groups do not produce milk with about the same amount of protein (mean values for elementary and secondary educational group:2.01 ± 0.41 g/dL vs University Group:1.74 ± 0.42 g/dL) , p = 0.029 Also, mothers working in the private sector or self-employed exhibited significantly higher protein content than those who were unemployed or working in the public sector (p <0.001). Milk protein content differed marginally between preterm and full-term group: 2.03±0.43 vs 1.79±0.42 No statistically significant associations were observed between the amount of lactose and the socio-demographic variables (Table 4).

According to univariate analysis, milk and chicken were significantly related to protein content (Table 5). In the multivariate analysis which comprised the sociodemographic variables, educational level and profession were related to protein content: milk consumption augmented and being civil servant decreased the probability of protein presence in breast milk.(Table 6)

**Table 1. Demographic characteristics of the sample.**

| **Age** | **N** | **%** |
|---|---|---|
| <26 years | 10 | 20.00 |
| 26-31 years | 15 | 30.0 |
| 32-37 years | 18 | 36.0 |
| >37 years | 7 | 14.0 |
| Total | 50 | 100.0 |
| **Place of residence** | | |
| Urban area | 37 | 74.0 |
| Semi-urban/rural | 13 | 26.0 |

| | | |
|---|---|---|
| Total | 50 | 100.0 |
| **Educational level** | | |
| Elementary | 1 | 2.0 |
| Junior High School | 1 | 2.0 |
| High School | 11 | 22.0 |
| College | 2 | 4.0 |
| Tertiary Technical Institute | 14 | 28.0 |
| University | 15 | 30.0 |
| Postgraduate | 5 | 10.0 |
| Doctoral | 1 | 2.0 |
| Total | 50 | 100.0 |
| **Profession** | | |
| Unemployed | 2 | 4.0 |
| Public Sector | 12 | 24.0 |
| Private Sector | 16 | 32.0 |
| Free Lancer | 2 | 4.0 |
| Total | 50 | 100.0 |
| **Financial Status (income/year)** | | |
| Low (<8000€) | 8 | 16.0 |
| Moderate (8000-15000€) | 26 | 52.0 |
| High (>15000€) | 16 | 32.0 |
| Total | 50 | 100.0 |
| **Infant maturity** | | |
| Preterm | 21 | 42.0 |
| Fullterm | 29 | 58.0 |
| Total | 50 | 100.0 |

**Table 2. Breast milk fat content association with sociodemographic variables.**

| Fat content (g/dl) | N | Mean | SD | p |
|---|---|---|---|---|
| **Age** | | | | |
| <32 years | 25 | 3.11 | 0.99 | 0.745 |
| ≥32 years | 25 | 3.20 | 0.97 | |
| **Place of residence** | | | | |
| Urban area | 37 | 3.07 | 0.85 | 0.266 |
| Semi-urban/Rural | 13 | 3.42 | 1.28 | |
| **Educational level** | | | | |
| Elementary and Junior High School graduates | 29 | 3.08 | 0.78 | 0.172 |
| Tertiary(Technical & University) | 21 | 3.25 | 1.20 | |
| **Profession** | | | | |
| Unemployed | 20 | 3.00 | 0.94 | **0.009** |
| Public Sector | 12 | 2.57 | 0.68 | |
| Private Sector/Freelancer. | 18 | 3.72 | 0.92 | |
| **Financial Status (income/year)** | | | | |
| Low (<8000€) | 8 | 2.97 | 1.02 | 0.233 |
| Moderate (8000-15000€) | 26 | 3.00 | 0.84 | |
| High (>15000€) | 16 | 3.50 | 0.99 | |
| **Infant maturity** | | | | |
| Preterm | 23 | 3.34 | 0.95 | 0.203 |
| Fullterm | 27 | 2.99 | 0.93 | |
| SD: Standard Deviation | | | | |

**Table 3. Breast milk protein content association with sociodemographic variables.**

| Protein content (g/dl) | N | Mean | SD | p |
|---|---|---|---|---|
| **Age** | | | | |
| <32 years | 25 | 1.96 | 0.34 | 0.283 |
| ≥32 years | 25 | 1.83 | 0.50 | |
| t (150) = 2.355 | | | | |
| **Place of residence** | | | | |
| Urban area | 37 | 1.81 | 0.43 | 0.022 |
| Semi-urban/Rural | 13 | 2.13 | 0.36 | |
| **Educational level** | | | | |
| Elementary and Junior High School graduates | 29 | 2.01 | 0.41 | **0.029** |
| Tertiary(Technical & University) | 21 | 1.74 | 0.42 | |
| **Profession** | | | | |
| Unemployed | 20 | 1.98 | 0.42 | **<0.001** |
| Public Sector | 12 | 1.61 | 0.39 | |
| Private Sector/Freelancer. | 18 | 2.00 | 0.41 | |
| **Financial Status (income/year)** | | | | |
| Low (<8000€) | 8 | 1.90 | 0.48 | 0.938 |
| Moderate (8000-15000€) | 26 | 1.96 | 0.43 | |
| High (>15000€) | 16 | 1.80 | 0.41 | |
| **Infant maturity** | | | | |
| Preterm | 23 | 2.03 | 0.43 | 0.060 |
| Fullterm | 27 | 1.79 | 0.42 | |
| SD: Standard Deviation | | | | |

**Table 4. Breast milk lactose content association with sociodemographic variables.**

| Lactose content (g/dl) | N | Mean | SD | p |
|---|---|---|---|---|
| **Age** | | | | |
| <32 years | 25 | 6.80 | 0.71 | 0.968 |
| ≥32 years | 25 | 6.79 | 0.96 | |
| t (150) = 0.843 | | | | |
| **Place of residence** | | | | |
| Urban area | 37 | 6.90 | 0.49 | 0.126 |
| Semi-urban/Rural | 13 | 6.49 | 1.41 | |
| t (150) = 1.423 | | | | |
| **Educational level** | | | | |
| Elementary and Junior High School graduates | 29 | 6.74 | 0.94 | 0.609 |
| Tertiary(Technical & University) | 21 | 6.87 | 0.67 | |
| t (150) = 1.162 | | | | |
| **Profession** | | | | |
| Unemployed | 20 | 6.64 | 1.09 | 0.168 |
| Public Sector | 12 | 6.75 | 0.79 | |
| Private Sector/Freelancer. | 18 | 6.99 | 0.46 | |
| t (150) = 1.803 | | | | |
| **Financial Status (income/year)** | | | | |
| Bad (<8000€) | 8 | 6.54 | 1.52 | 0.242 |
| Moderate (8000-15000€) | 26 | 6.84 | 0.77 | |
| Good (>15000€) | 16 | 6.83 | 0.78 | |
| t (150) = 1.431 | | | | |

| **Infant maturity** | | | | |
|---|---|---|---|---|
| Preterm | 23 | 6.89 | 1.05 | 0.429 |
| Fullterm | 27 | 6.69 | 0.67 | |
| t (150) = 0.124 | | | | |
| SD: Standard Deviation | | | | |

**Table 5. Univariate analysis for certain foods according to breast content in fat, protein and lactose.**

| | **Lipids** | | **Proteins** | | **Lactose** | |
|---|---|---|---|---|---|---|
| **Foods*** | B | p | B | p | B | p |
| Milk | 0.133 | 0.705 | 0.443 | **0.003** | -0.481 | 0.104 |
| Feta cheese | 0.479 | 0.096 | 0.099 | 0.440 | -0.366 | 0.139 |
| Beef | 0.642 | 0.074 | -0.126 | 0.431 | 0.427 | 0.128 |
| Chicken | -0,202 | 0.563 | 0.297 | **0.050** | -0.254 | 0.397 |
| Pork | -0.043 | 0.893 | 0.218 | 0.117 | -0.431 | 0.111 |

**Table 6. Multivariate analysis for breast milk proteins.**

| $R^2$=0,40 | | Unstandardized Coefficients | | Standardized Coefficients | t | p |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | 1,642 | 0,178 | | 9,203 | <0,001 |
| | Place of residence | -0,225 | 0,117 | -0,232 | -1,935 | 0,059 |
| | **Employment (civil servant)** | **-0,329** | **0,119** | **-0,329** | **-2,754** | **0,009** |
| | Educational level | 0,094 | 0,103 | 0,109 | 0,907 | 0,369 |
| | **Milk** | **0,384** | **0,132** | **0,360** | **2,911** | **0,006** |
| | Chicken | 0,178 | 0,130 | 0,167 | 1,377 | 0,176 |
| | | | | | | |
| a. Dependent Variable: Proteins | | | | | | |

## Discussion

In the present study, the chemical composition of breast milk and its association with socio-demographic characteristics of the mother and infant maturity was studied. The results of the fat content, protein and lactose are consistent with other studies performed (Gidrewicz & Fenton, 2014; Yang et al., 2014; Chang et al., 2015). The maternal milk lipid concentration was found to be higher in mothers of preterm infants, a finding consistent with previous studies (Anderson, Atkinson & Bryan, 1981; Hausman Keedem et al., 2013; Lubetzky et al., 2015). In the study by Anderson conducted on women who delivered prematurely between 26 $^{th}$ and 33 $^{th}$ week of pregnancy has been found that the percentage of fat contained in milk was 20 30% higher than this, women who gave birth to full-

term newborns (Anderson, Atkinson & Bryan, 1981). This increase in lipid content may be due to increased lipid synthesis and excretion in milk, reduced milk water content, or a combination of both (Hausman Keedem et al., 2013). Breast milk fat is necessary not only because it provides the energy required to achieve the rapid growth rate of the newborn but also because it provides important nutrients that are vital for the development of the neonatal central nervous system and which cannot be synthesized by the newborn (Lauritzen & Carlson, 2011).

Few studies have investigated the association between the area of residence of breast-feeding mothers with the mother's milk content in various ingredients such as lipids and proteins. In the present study, there was no correlation of the residence area (rural or urban areas) with the maternal milk content of macronutrients. In the study of (Qian et al., 2010), which compared maternal milk in lactating mothers living in different regions of Shanghai, found that mothers living in rural areas had lower lipid and protein concentrations than mother milk living in urban areas. It should be noted that women from rural areas of Shanghai were of a lower socio-economic status as indicated educational level, occupation and income. The Thiombiano-Coulibaly et al., (2003) examined a milk sample of 70 mothers living in rural and 52 urban areas during a time of poor production of local products. Respectively, they examined samples from 100 mothers living in rural and 98 urban areas during post-harvest period. They found that regardless of the time and place of residence, the milk had a low fat content compared to milk samples from other countries. Breast milk fat content may vary depending on income, especially in developing countries.

Another study (Al-Tamer & Mahmood, 2006) showed that the socio-economic status of breastfeeding Iranian mothers negatively affected the content of breast milk fat compared to that of mothers from developed countries. Additionally, older studies have found that maternal milk fat content decreased significantly only during the second month of lactation, (Fornes & Dorea, 1995) in the case of low income mothers. Concerning the effect of the socio-economic level on protein levels, it is reported that low- income mothers carry significantly lower protein concentrations in their breast milk (Sanchez-Pozo et al., 1987; Donangelo et al., 1989). A crossover study from Sweden reported higher breast-milk total protein content for a maternal diet high in protein than with a low-protein diet (8.83 compared with 7.31 g/d; $p< 0.05$) (Forsum & Lonnerdal, 1980). Moreover, the total daily protein intake has been with body weight growth, a fact indicating the significance of protein intake and indirectly underlining the crucial role of socioeconomic factors (Lin et al., 2020).

There is some evidence regarding the correlation between maternal nutrition and the fatty acids content of breast milk. A study in South Korea has shown that dietary intakes of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), omega 3 ( n -3) fatty acids, omega 6 (n- 6) SFAsfatty acids and polyunsaturated fatty acids (PUFAs ) were positively correlated with the corresponding fatty acids in breast, whereas a Chinese study concluded that dietary intake and breast milk content of ω-3 PUFA were also positively correlated (Urwin et al., 2012). In addition, it has been shown that women consuming fish and other foods containing high levels of PUFA have relatively higher n- 3 maternal fatty acids than milk from women consuming diets low in these ingredients (Wang et al., 2000). It is also likely that the economic prosperity of households also indirectly affects the composition, because it affects the mother's diet. In a survey in Bangladesh, the welfare of households was independent predictor of (increased) ratio PUFA compared to SFA and an increased ω6- PUFA ratio compared to ω3- P UFA (Nayak et al., 2017).

Further epidemiological and nutritional studies are required to clarify the underlying mechanisms of these effects. In the earlier study by Pita et al., (1985) it has been found that oleic acid is reduced in milk by mothers with medium and low socioeconomic status who consumed almost exclusively vegetable seed oils. The mother's weight did not affect the fatty acid composition of the milk. However, a study in New Zealand found no strong correlations between dietary nutrients and breast milk components were found (Butts et al., 2018).

***Limitations:*** The origin of the study sample from a single region of Greece and the size of the sample limits the generalization of our results. Also, some diurnal variation in protein and fat in human milk, present especially in the preterm infants (Paulavicience et al., 2020) may have

affected substances concentration in the breast milk.

***Conclusions:*** The present study concluded that fats and proteins in maternal milk working in the private sector or self-employed are significantly more than in the milk of non-working mothers and those working in the public sector. This is likely explained by the economic situation of the above categories, as our data suggests that civil servants and the unemployed are in a significantly worse economic situation than private employees and freelancers. Moreover, milk consumption was related to increased protein content. The latter differed marginally between preterm and full-term infants groups The association of certain foods with breast macronutrients content needs further investigation. Future studies on larger and more representative samples could provide more insight into the relationship between breast milk composition and nutritional and socio-demographic factors in our country.

## References


Al-Tamer, Y.Y. & Mahmood, A.A. 2006. The influence of Iraqi mothers' socioeconomic status on their milk-lipid content. *European Journal of Clinical Nutrition*, 60(12): 1400-1405

Anderson, G.H., Atkinson, S.A. & Bryan, M.H. (1981). Energy and macronutrient content of human milk during early lactation from mothers giving birth prematurely and at term. *American Journal of Clinical Nutrition*, 34(2): 258-265

Andreas, N.J., Kampmann, B. & Mehring Le-Doare, K. (2015). Human breast milk: A review on its composition and bioactivity. *Early Human Development*, 91(11): 629-635

Antonakou, A., Skenderi, K.P., Chiou, A., Anastasiou, C.A., Bakoula, C. & Matalas, A.L. (2013). Breast milk fat concentration and fatty acid pattern during the first six months in exclusively breastfeeding Greek women. *European Journal of Nutrition*, 52(3): 963-973,

Ballard, O. & Morrow, A.L. (2013). Human milk composition: nutrients and bioactive factors. *Pediatric Clinics of North America*, 60(1): 49-74

Butts, C.A., Hedderley, D.I., Herath, T.D., Paturi, G., Glyn-Jones, S., Wiens, F., Stahl, B. & Gopal, P. (2018). Human Milk Composition and Dietary Intakes of Breastfeeding Women of Different Ethnicity from the Manawatu-Wanganui Region of New Zealand. *Nutrients*, 10(9): 1231, doi: 10.3390/nu10091231

Chang, N., Jung, J.A., Kim, H., Jo, A., Kang, S., , Lee, S.W., Yi, H. , Jihee Kim, J., Yim, J.G. & Jung, B.M. (2015). Macronutrient composition of human milk from Korean mothers of full term infants born at 37-42 gestational weeks. *Nutrition Research and Practice*, 9(4): 433-438

Daglas, M., Galanis, P., Bilali, A., Antoniou, E., & Iatrakis, G. (2020). Maternal and Neonatal Factors Associated with Successful Breastfeeding in Preterm Infants. *International Journal of Caring Science*, 13(1): 152-162.

Donangelo, C.M., Trugo, N.M., Koury, J.C., Barreto Silva, M.I., Freitas, L.A., Feldheim, W. & Barth, C. (1989). Iron, zinc, folate and vitamin B12 nutritional status and milk composition of low-income Brazilian mothers. *European Journal of Clinical Nutrition*, 43(4): 253-266.

Fornes, N.S. & Dorea, J.G. (1995) Subcutaneous fat changes in low-income lactating mothers and growth of breast-fed infants. *Journal of the American College of Nutrition*, 14(1): 61-65

Forsum, E. & Lonnerdal, B. (1980). Effect of protein intake on protein and nitrogen composition of breast milk. *American Journal of Clinical Nutrition*, 33(8): 1809–13

Gidrewicz, D.A. & Fenton T.R. (2014). A systematic review and meta-analysis of the nutrient content of preterm and term breast milk. *BMC Pediatrics*, 14: 216, doi: 10.1186/1471-2431-14-216

Hausman Kedem, M., Mandel, D., Domani, K.A., Mimouni, F.B., Shay, V., Marom, R., Dollberg, S., Herman, L. & Lubetzky, R. (2013). The effect of advanced maternal age upon human milk fat content. *Breastfeed Medicine*, 8(1): 116-119

Hennet, T. & Borsig, L. (2016). Breastfed at Tiffany's. *Trends in Biochemical Sciences*, 41(6): 508-518

Lauritzen, L. & Carlson, S.E. (2011). Maternal fatty acid status during pregnancy and lactation and relation to newborn and infant status. *Maternal & Child Nutrition*, 7 suppl2: 41-58

Lin, Y.H., Hsu, Y.C., Lin, M.C., Chen, C.H., & Wang, T.M. (2020). The association of macronutrients in human milk with the growth of preterm infants. *PloS one*, *15*(3), e0230800. https://doi.org/10.1371/journal.pone.0230800

Lubetzky, R., Sever, O., Mimouni, F.B. & Mandel, D. (2015). Human Milk Macronutrients Content: Effect of Advanced Maternal Age. *Breastfeed Medicine*, 10(9): 433-436

Nayak, U., Kanungo, S., Zhang, D., Colgate, R.E., Carmolli, M.P., Dey, A., Alam, M., Manna, B. , Nandy, R.K., Kim, D.R., Paul, D.K., Choudhury, S., , Sahoo, S. , Harris, W.S. , Wierzba, T.F., Ahmed, T. , Kirkpatrick, B.D. , Haque, R., Petri Jr, W.A. & Mychaleckyj, J.C. (2017). Influence of maternal and socioeconomic factors on breast milk fatty acid composition in urban, low-income families. *Maternal & Child Nutrition*, 13(4), e12423, doi: 10.1111/mcn.12423

Paulaviciene, I.J., Liubsys, A., Molyte, A., Eidukaite, A., & Usonis, V. (2020). Circadian changes in the composition of human milk macronutrients depending on pregnancy duration: a cross-

sectional study. *International breastfeeding journal*, *15*(1), 49, https://doi.org/10.1186/s13006-020-00291-y

Pita, M.L., Morales, J., Sánchez-Pozo, A., Martínez-Valverde, J.A. & Gil A. (1985). Influence of the mother's weight and socioeconomic status on the fatty acid composition of human milk. *Annals of Nutrition & Metabolism*, 29(3): 366-373

Qian, J., Chen, T., Lu, W., Wu, S. & Zhu J. (2010). Breast milk macro- and micronutrient composition in lactating mothers from suburban and urban Shanghai. *Journal of Paediatrics and Child Health*, 46(3): 115-120

Sanchez-Pozo, A., Lopez Morales, J., Izquierdo, A., Martinez-Valverde, A. & Gil A. (1987). Protein composition of human milk in relation to mothers' weight and socioeconomic status. *Human Nutrition Clinical Nutrition*, 41(2): 115-125.

Thiombiano-Coulibaly, N., Rocquelin, G., Eymard-Duvernay, S., Kiffer-Nunes, J., Tapsoba, S. & Traoré, S.A. (2003). Seasonal and environmental effects on breast milk fatty acids in Burkina Faso and the need to improve the omega 3 PUFA content. *Acta Paediatrica*, 92(12): 1388-1393.

Urwin, H.J., Miles, E.A., Noakes, P.S., Kremmyda, L.S., Vlachava, M., Diaper, N.D., Pérez-Cano, F.J., Godfrey, K.M., Calder, P.C. & Yaqoob P. (2012). Salmon consumption during pregnancy alters fatty acid composition and secretory IgA concentration in human breast milk. *The Journal of Nutrition*, 142(8): 1603-1610

Wang, L.W., Shimizu, Y., Kaneko, S., Hanaka, S., Abe, T., Shimasaki, H., Hisaki, H. & Nakajima, H. (2000). Comparison of the fatty acid composition of total lipids and phospholipids in breast milk from Japanese women. *Pediatrics International*, 42(1): 14–20

Yang, T., Zhang, Y., Ning, Y., You, L., Ma, D., Zheng, Y., Yang, X., Li, W., Wang, Z. & Wang, P. (2014). Breast milk macronutrient composition and the associated factors in urban Chinese mothers. *Chinese Medical Journal* (Engl), 127(9): 1721-1725