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LONG CHAIN POLYUNSATURATED FATTY ACIDS IN BREAST MILK IN LA PLATA, ARGENTINA: RELATIONSHIP WITH MATERNAL NUTRITIONAL STATUS.

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SUMMARY

Milk fat is the major source of energy for breastfed infants; it also supplies polyunsaturated fatty acids (PUFAs) essential for the development of brain, retina, and other organs. Maternal nutritional status is critical for the newborn, and little information exists regarding the PUFA status of vulnerable populations living in Southern regions. We studied the relationship between maternal nourishment and milk fatty acid composition. Mother nutritional status (normal, overweight or obese) was estimated on the body mass index. Milk protein, total lipid, and fatty acid composition were determined. Milk protein was not affected by mother's nutritional status. In obese mothers an increase in lipid content, linoleic acid, total n-6 and total polyunsaturated fatty acids was observed comparing to the other groups.

Disregarding the nutritional status, the ratio n-6/n-3 fatty acids was very high and the 22:6n-3 content was very low, when compared with those of mothers from other countries. This finding led us to urge Public Health officers to promote changes in the dietary habits of nursing women.

INTRODUCTION

Breast milk from healthy and well-nourished women is the best method of feeding for healthy infants during the first 6 months of life [1,2]. It is an exceptionally complex fluid, containing carbohydrates and salts in true solutions, casein in colloidal dispersion, cells and cellular debris, and lipids contained mostly in emulsified globules. These globules contain a lot of triacylglycerols in the core, and the main lipid constituents of the amphypathic fat globule surface membrane are phospholipids and cholesterol [3,4,5]. Human milk fat is the major source of energy for the breastfed infant, providing 40-55% of the total energy intake. Milk fat also supplies essential nutrients such as fat-soluble vitamins and n-6 and n-3 polyunsaturated fatty acids (PUFAs) necessary for the full development of brain, retina, and other organs including the skin [6,7]. Fatty acids of human milk are derived from three sources: mobilization of endogenous stores of fatty acids, *de novo* synthesis of fatty acids by the liver or breast tissue, and the maternal diet [8-11] The n-3 fatty acids, 22:6 n-3 (docosahexaenoic acid or DHA) and the n-6 fatty acids, 20:4 n-6 (arachidonic acid or AA) are stored in adipose tissue and can be secreted into breast milk after mobilization. Dietary sources can supply DHA and AA directly, or they can be synthesized from their precursors (α linolenic, ALA 18:3 n-3, and linoleic, LA 18:2 n-6, acids, respectively), through desaturation and elongation pathways [12]. The fatty acid composition of human milk reflects the type of dietary fat consumed , in the short term and the long term by the mother [13-15]. The n-3 fatty acids are of particular interest because of their role in the development of the infant brain and retina [16-18], and it was demonstrated that a dietary deficiency in n-3 fatty acids is associated with visual abnormalities in rats [19], in preterm infants [20-23],and in formula-fed infants [24]

Since maternal nutritional status during pregnancy is critical for the newborn, and little information exists regarding the PUFA status of populations living in South America, we designed the present protocol to study the relationship between maternal nourishment and fatty acid composition of human milk.

SUBJECTS AND METHODS

Subject characteristics and anthropometry

Forty-six mothers were recruited to participate in the present study. Breast milk samples were obtained from these mothers, who had given birth to healthy, full-term infants (38-42 week gestation). They were between 16-39 years old, and their nutritional state was estimated on the body mass index (BMI). According to BMI, 21 women were considered as normal, (BMI lower than 25), 16 were considered overweight (BMI between 25 and 30), and 9 mothers were obese (BMI up to 30). Mother diet was evaluated according to food frequency questionnaires. Consent for this study was obtained from volunteers according to a protocol dealing with scientific and ethical aspects approved by the Board of Directors of Comisión de Investigaciones Científicas, Buenos Aires Province, Argentina.

Breast milk samples

Samples corresponded to mature milk, and were obtained 1-3 months after delivery, they were collected at 3rd min into a nursing, by hand expression. The milk was transported on ice and immediately frozen at -70 ^{0} C until analysis.

Breast milk protein content was measured according to Lowry et al procedure [25]. Total lipids were extracted according to Folch [26] with chloroform and methanol (2:1 v/v), and the content in aliquots of the extract was determined gravimetrically after solvent evaporation. Fatty acid methyl esters were prepared by transesterification with boron trifluoride/methanol [27] and analyzed by gas liquid chromatography (GLC), on an Omegawax 250 (30 m, 0.25 mm i.d., 0.25 μm film thickness) capillary column in a Hewlett Packard HP – 6890, equipped with a Flame Ionization Detector. The fatty acid methyl esters were identified by comparison off their relative retention times with authentic standards, and the mass distribution was calculated electronically by quantification of the peak areas. Authentic standards of fatty acids were purchased from Sigma Chemical Co.

Statistical analysis:

Statistical analysis was performed by one-way ANOVA. When differences were detected (P < 0.05), means were tested with Tukey´s test (GB- STAT Professional Statistics and Graphics 4.0; Dynamic Microsystems Inc., Silver Spring; MD).

RESULTS

Table 1 shows the total fat and protein content in mature milk from Argentine mothers grouped according to their BMI. Milk protein was not affected by mother's nutritional status. The total lipid content of milk samples from obese mothers showed a significant increase relative to the normal or overweight groups.

The percentage fatty acid composition of human milk was also analyzed. Saturated and monounsaturated fatty acids are listed in **Table 2.** There were no differences in the individual saturated fatty acids between different groups, but a lower proportion of the total saturated fatty acids of chain length from 10 to 14 carbons was observed in obese mothers relative to the overweight group. In monounsaturated fatty acids (**Table 2)**, a significant increase in the proportion of 20:1 n-9 in obese mothers was demonstrated in respect to the control ones. Total monounsaturated fatty acids were significantly lower in overweight and obese mothers relative to the normal ones.

In **Table 3** milk polyunsaturated fatty acid composition is listed. In milk from obese mothers there were significant increases in the relative proportion of 18:2 n-6, in the total n-6 fatty acids, and in total polyunsaturated fatty acids, compared with controls. In obese mothers a higher value in the ratio 18:2 n-6/total n-6, was also detected relative to the control. There were no differences in the proportion of fatty acids from the n-3 series. The ratio between n-6 and n-3 fatty acids showed an increase in obese mothers compared with normal and overweight mothers.

Such a ratio was then compared with maternal milk data from different countries, concerning different degree of development as well as different nutritional dietary habits (**Figure 1)**. Irrespective of the maternal nutritional status, breast milk of Argentine mothers showed the highest n-6/ n-3 ratio followed by that of USA mothers, while milk of Japanese and Chinese mothers contained the lowest values **(Figure1).**

These data were well-correlated with the breast milk 22:6n-3 level **(Figure 2)**, being Japanese and Chinese mothers who evidenced the major content of this acid in breast milk.

DISCUSSION

In the current study, we described the composition of fatty acid in breast milk from women living in the city of La Plata, Argentina. The kind of diet consumed by women from a relatively middle socioeconomic status, can be generalized to others urban populations of our country.

Considering protein and fat content, mature milk from Argentine mothers in normal or overweight nutritional status was in average within the range reported in the literature for human bank milk from other countries [28]. However, the fat content increases in obese mothers towards the higher end of the range.

The fatty acid composition pattern was also different in the milk from obese mothers, where a decrease in the amount of saturated fatty acids ≤ 14 C was observed (Table 2). Neville and Picciano [29] discussed the fact that FFA inhibit acetyl-CoA carboxylase in a feedback regulatory loop. This hypothesis explains why synthesis of 8:0-14:0 C fatty acids in the mammary gland is decreased on high-fat and fasting diets. Conversely, it is well known that a high-carbohydrate low-fat diet stimulates synthesis of 8:0-14:0 in the mammary gland. The total 8:0-14:0 rises, in this case, from about 10 to 20% or more. Thus, the results obtained in our work with obese mothers could probably be due to a higher intake of food rich in saturated fat rather than carbohydrates, which are indeed, the favorite food of an urban population in Argentine.

Regarding polyunsaturated fatty acids, it is known that they are required in an adequate amount in the early infancy in order to achieve an optimal neurologic development [30,31]. Linoleic [32] and 18:3n-3 or α–linolenic acids [33] are essential, that is, they are required because they cannot be produced in humans. Innis [34] reviewed the role of these acids in growth and development. Linoleic acid is the precursor of arachidonic acid (20:4n-6), and 18:3n-3 of 20:5n-3 and 22:6n-3. These acids are formed from their precursors by elongation-desaturation, though 22:6n-3 is also formed by an oxidation reaction converting 24:6n-3 to 22:6n-3 by retroconversion [35,36]. Docosahexaenoic (22:6n-3) acid is particularly important in neural development during the perinatal period [34]. Retinal membrane phospholipids, particularly the phosphatidylethanolamine fraction contain large amounts of DHA [31,37]. It was demonstrated that a dietary deficiency in n-3 fatty acids is associated with visual abnormalities in rats [19], in preterm infants [20-23],and in formula-fed infants [24].

Moreover, a close correlation was found between erythrocyte DHA content and visual-evoked potential acuity [24], and we have also demonstrated the relationship between the proportion of DHA in erythrocyte phospholipid and visual function which was assessed using full-field flash electroretinography in malnourished nursing infants [38].

The amount of 18:2n-6, 18:3n-3 and LCPUFA derivatives in milk respond to their quantities in the maternal diet. In Argentine mothers the relative percentage of linoleic acid was similar to that of mothers from other countries such us Sudan [39], China [39] and Cuba [40] (Fig. 1). It increased when mother nutritional status was overweight or obese, reaching the highest values. The sum of total n-6 fatty acids supports this fact. However, the most interesting finding was the fact that this increase was not accompanied by a simultaneous increase in α-linolenic acid or its desaturation-elongation products. In consequence, the relationship between total n-6/ n-3 fatty acids is very high, only similar to data obtained from American mothers (Figure 1). This ratio between 24 to 30 depends on the maternal nutritional status being the highest values found in obese women. It is evident that this value is by far much more elevated as the ratio LA/ ALA between 5 and 15 has been recommended [41]. The amount of 22:6n-3 derived from α-linolenic acid in milk which is within a range from 0 to 2.78 wt% with means of 0.45 for Western women and 0.88 for non-Western women [38] whereas the present study shows values between 0.13- 0.15. The quantities of this acid in milk respond to the amounts of DHA in foods, primarily fish, in the maternal diet. Notwithstanding the infants may be able to convert 18:2n-6 and 18:3n-3 to sufficient 20:4n-6 and 22:6n-3 for normal growth and visual acuity if the precursor acid is available, the low level of 22:6n-3 observed in Argentine mothers as well as that reported in the literature in respect with USA [3] mothers is worrying.

In conclusion, these results demonstrate that the milk fatty acid composition in Argentine mothers, at least those which are representative of an urban population is far from being the ideal one. We were unable to find any data in the literature with reference to composition of milk in relation with the nutritional status of mothers from other countries. However, we can assess that overweight and obesity are factors that contribute to impair the quality of the milk considering that it contains the highest levels of n-6/n-3 EFAs ratio, factor that was detrimental for development, specially in reference to central nervous system and visual acuity. In summary, the n-3 polyunsaturated fatty acid content in breast milk of women living in Argentina appears to be deficient; fact that was also assessed in others countries. Thus, infants development may be affected; so this finding allowed us to urge Public Health officers to actively support a campaign, promoting changes in the dietary habits of nursing women.

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REFERENCES

[1] ESPGAN Committee on Nutrition. Guidelines on infant nutrition: I. Recommendations for the composition of an adapted formula. Acta Paediatr. Scand. 262 (1977) 1-20 (Suppl)

[2] ESPGAN Committee on Nutrition. Guidelines on infant nutrition: III. Recommendations for infant feeding. Acta Paediatr. Scand. 302 (1982) 1-27 (Suppl)

[3] R.G. Jensen. The lipids in human milk. Prog. Lipid Res. 35 (1996) 53-92.

[4] J. Bitman, D.L. Wood, M. Hamosh, P. Hamosh, N.R. Mehta. Comparison of the lipid composition of breast milk from mothers of term and preterm infants. Am. J. Clin Nutr, 38 (1983) 300-312.

[5] J.Bitman, D.L. Wood, N.R. Mehta, P. Hamosh, M. Hamosh. Comparison of the phospholipid composition of breast milk from mothers of term and preterm infants during lactation. Am. J. Clin. Nutr. 40 (1984) 1103-1119.

[6] B. Koletzko, M. Rodriguez-Palmero. Saturated fatty acids in human milk and their role in early infant development. J. Mammary Gland. Biol. Lactation 4 (1999) 269-284..

[7] M. Rodriguez-Palmero, B. Koletzko, C. Kunz, R. Jensen. Nutritional and biochemical properties of human milk:II. Lipids, micronutrients and bioactive factors. Clin. Perinatol. 26 (1999) 335-359.

[8] N. Ortiz-Olaya, M.E. Flores, E.E. Deschner. Significance of lipid consumption during lactation. Rev. Invest. Clin 48 (1996) 473-478.

[9] O. Hernell. The requirements and utilization of dietary fatty acids in the newborn infant. Acta Paediatr. Scand. (Suppl) 365 (1990) 20-27.

[10] J.E. Chappell, M.T. Clandinin, C. Kearney-Volpe. *Trans* fatty acids in human milk lipids: Influence of maternal diet and weight loss. Am. J. Clin. Nutr. 42 (1985) 49-56.

[11] R.G. Jensen, M.M. Hagerty, K.E. McMahon. Lipids of human milk and infant formulas: a review. AmJ.Clin.Nutr. 31 (1978) 990-1016.

[12] R.R. Brenner. Factors influencing fatty acid chain elongation and desaturation. In: A.J. Vergrosen & M. Crawford (eds.), The role of fats in human nutrition. Academic Press, London, England, 1989, pp 45-79.

[13] R.A. Henderson, R.G. Jensen, C.F. Lammi-Keefe, A.M. Ferris, K.R. Dardick K.R. Effect of fish oil on the fatty acid composition of human milk and maternal and infant erythrocytes. Lipids 27 (1992) 863-869.

[14] W.S. Harris, W.E. Connor, S. Lindsey. Will dietary omega-3 fatty acids change the composition of human milk? Am. J. Clin. Nutr. 40 (1984) 780-785.

[15] C.A. Francois, S.L. Connor, R.C. Wander, W.E. Connor. Acute effects of dietary fatty acids on the fatty acids of human milk. Am. J. Clin. Nutr. 67 (1998) 301-308.

[16] M. Martinez. Tissue levels of polyunsaturated fatty acids during early human development. J. Pediatr. 120 (1992) 129-138.

[17] S.M. Innis, C.M. Nelson, M.F. Rioux, D.J. King. Development of visual acuity in relation to plasma and erythrocyte omega-6 and omega-3 fatty acids in healthy term gestation infants. Am. J. Clin. Nutr. 60 (1994) 347-352.

[18] E.E. Birch, D.R. Hoffman, R. Uauy, D.G. Birch, C. Prestidge. Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. Pediatr.Res. 44 (1998) 201-209.

[19] B.M. Benolken, R.E. Anderson, T.G. Wheeler. Membrane fatty acid associated ith the electrical response in visual excitation. Science 182 (1973) 1253-1254.

[20] E.E. Birch, D.G. Birch, D.R. Hoffman, R.D. Uauy. Dietary essential fatty acid supply and visual acuity development. Invest. Ophthalmol. Vis. Sci. 32 (1992) 3245-3253.

[21] D.G. Birch, E.E. Birch, D.R. Hoffman, R.D. Uauy. Retinal development in very low-birth-weight infants fed diets differing in omega-3 fatty acids. Invest. Ophthalmol. Vis. Sci. 33 (1992) 2365-2376.

[22] S.E. Carlson, R.G. Cooke, J.M. Peeples. Docosahexaenoate (DHA) and eicosaenoate (EPA) status of preterm infants: relationship to visual acuity in n-3 supplemented and unsupplemented infants. Pediatr. Res. 25 (1989) 285-287.

[23] R.D. Uauy, D.G. Birch, E.E. Birch, J. Tyson, D.R. Hoffman. Effect of dietary omega-3 fatty acids on retinal function of very-low-birth-weight neonates. Pediar.Res. 28 (1990) 485-492.

[24] M. Makrides, K. Simmer, M. Goggin, R.A. Gibson. Erythrocyte docosahexaenoic acid correlates with the visual response of healthy term infants. Pediatr. Res. 33 (1993) 425-427.

[25] O.H. Lowry, N.J. Rosembrough, R.J. Randall. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193 (1951) 265-275.

[26] J. Folch, M. Lees, G.H. Sloane Stanley. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226 (1957) 497-509.

[27] W.R. Morrison, L.M. Smith, L.M. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5 (1964) 600-608.

[28] K.F. Michaelsen, L. Skafte, J.H. Badsberg, M. Jorgensen. Variation in micronutrients in human bank milk: Influencing factors and implications for human milk banking.. J. Pediatr. Gastroenterol. Nutr. 11 (1990) 229-239

[29] M.C. Neville, M.D. Picciano. Regulation of milk lipid secretion and composition. Annu. Rev. Nutr. 17 (1997) 159-184.

[30] C.D. Stubbs, A.D. Smith. The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and functions. Biochim. Biophys. Acta 779 (1984) 89-137.

[31] P.S. Sastry. Lipids of nervous tissue: composition and metabolism. Prog. Lipid. Res. 24 (1985) 69-176

[32] R.T. Holman, W.O. Caster, H.G. Wiese. The essential fatty acid requirment of infants and assessment of their dietary intake of linoleate by serum fatty acid analysis.. Am. J. Clin. Nutr. 14 (1964) 70-75.

[33] R.T. Holman, S.B. Johnson, T.F. Hatch. A case of human linolenic acid deficiency involving neurological abnormalities. Am J. Clin. Nutr 35 (1982) 617-623.

[34] S.M. Innis. Essential fatty acids in growth and development.. Prog. Lipid Res. 30 (1991) 39-103.

[35] H. Sprecher H., D.L. Luthria, B.S. Mohammend, S.P. Baykouskera. Re-evaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. J. Lipid Res. 36 (1995) 2471-2477.

[36] R.C.S. Greiner, Q. Zhang, K.L. Goodman, D.A. Giusanni, P.M. Nathanielsz, T.W. Brenna. Linoleate, linolenate and docosahexaenoate recycling into saturated and monounsaturated acids is a major pathway in pregnant or lactating adults and fetal or infant Rhesus monkeys. J. Lipid. Res. 37 (1996) 2675-2686.

[37] S.J. Fiesler, R.E. Anderson. Chemistry and metabolism of lipids in the vertebrate retina. Prog. Lipid. Res. 22 (1983) 79-131

[38] M.C. Marín, G.E. Rey, L.C. Pedersoli, M.A. Rodrigo, M.J.T. de Alaniz, Dietary long-chain fatty acids and visual response in malnourished nursing infants. Prost. Leukot. Essent. Fatty Acids 63 (2000) 385-390

[39] R.G. Jensen. Lipids in Human Milk. Lipids 34 (1999) 1243-1271.

[40] J.M. Krasevec, P.J. Jones, A. Cabrera-Hernández, D.L. Mayer, W.E. Connor. Maternal and infant essential fatty acid status in Havana, Cuba. Am. J. Clin. Nutr. 76 (2002) 834-844

[41] ESPGAN Committee on Nutrition. Comment on the content and composition of lipid in infant formulas. Acta Paediatr. Scand. 80 (1991) 887-896.

Table 1 Protein and fat content in mature milk from Argentine mothers according their BMI

	Normal n= 21	Overweight n = 16	Obese n = 9
Protein (g/L)	9.7 ± 0.8	9.1 ± 1.0	9.1 ± 1.0
Fat (g/L)	69.2 ± 4.3	71.5 ± 7.7 [a]	98.1 ± 9.4 [*,b]

Values are mean ± SEM; n = number of samples

Statistically significant differences ($P < 0.05$) among groups are indicated by different roman superscripts (a,b)
* $P < 0.01$ probability value relative to normal mothers

Table 2 Saturated and monounsaturated fatty acids (wt%) in human milk from Argentine mothers according their BMI

Fatty acid	**Normal** n=21	**Overweight** n=16	**Obese** n=9
10:0	0.91 ± 0.10	1.18 ± 0.13	0.89 ± 0.11
12:0	4.67 ± 0.41	5.55 ± 0.52	4.15 ± 0.57
14:0	6.02 ± 0.40	6.18 ± 0.46	4.93 ± 0.56
15:0	0.43 ± 0.03	0.40 ± 0.03	0.41 ± 0.03
16:0	20.58 ± 0.43	20.61 ± 0.57	21.19 ± 0.58
18:0	9.78 ± 0.99	9.63 ± 0.89	9.21 ± 1.16
20:0	0.26 ± 0.03	0.25 ± 0.02	0.25 ± 0.02
22:0	0.05 ± 0.01	0.03 ± 0.01	0.03 ± 0.02
≤14-carbon saturated	11.60 ± 0.80	12.91 ± 0.95 [a]	9.97 ± 0.85 [b]
Total saturated	42.70 ± 1.21	44.30 ± 1.32	41.57 ± 1.54
16:1 n-7	3.22 ± 0.24	3.54 ± 0.22	3.08 ± 0.33
18:1 n-9	33.36 ± 1.73	29.05 ± 1.17	30.05 ± 1.50
18:1 n-7	0.28 ± 0.03	0.44 ± 0.19	0.38 ± 0.18
20:1 n-9	0.08 ± 0.02	0.16 ± 0.03	0.19 ± 0.04 **
Total monounsaturated	36.94 ± 0.81	33.90 ± 1.30 *	33.70 ± 1.25 *

Values are mean ± SEM; n = number of samples. Some minor components omitted
Statistically significant differences ($P < 0.05$) among groups are indicated by different roman superscripts (a,b)
* $P < 0.05$ ** $P < 0.01$ probability value relative to normal mothers

Table 3 Polyunsaturated fatty acids (wt%) in human milk from Argentine mothers according their BMI

Fatty acid	**Normal** n=21	**Overweigh** n=16	**Obese** n=9
18:2 n-6	16.61 ± 1.06	19.12 ± 1.06	22.71 ± 0.91 **
18:3 n-6	0.59 ± 0.06	0.51 ± 0.06	0.41 ± 0.12
20:2 n-6	0.36 ± 0.02	0.38 ± 0.03	0.37 ± 0.04
20:3 n-6	0.40 ± 0.03	0.49 ± 0.06	0.46 ± 0.05
20:4 n-6	0.45 ± 0.03	0.51 ± 0.06	0.43 ± 0.02
22:4 n-6	0.09 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
22:5 n-6	0.11 ± 0.01	0.13 ± 0.01	0.13 ± 0.01
Total n-6	18.61 ± 1.11	21.25 ± 1.09	23.58 ± 1.38 *
18:2 n-6/total n-6	0.89 ± 0.16	0.90 ± 0.18	0.96 ± 0.11 *
18:3 n-3	0.47 ± 0.04	0.49 ± 0.06	0.47 ± 0.02
20:3 n-3	0.04 ± 0.01	0.05 ± 0.02	0.02 ± 0.01
20:5 n-3	0.09 ± 0.03	0.14 ± 0.05	0.07 ± 0.01
22:4 n-3	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
22:5 n-3	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
22:6 n-3	0.13 ± 0.01	0.15 ± 0.02	0.14 ± 0.01
Total n-3	0.78 ± 0.03	0.88 ± 0.07	0.76 ± 0.03
Total n-6/total n-3	23.86 ± 2.01	24.14 ± 2.64 [a]	31.03 ± 2.53 **, [b]
Total polyunsaturated	19.37 ± 1.01	22.12 ± 1.02	24.34 ± 1.16 *

Values are mean ± SEM; n = number of samples
Statistically significant differences ($P < 0.05$) among groups are indicated by different roman superscripts (a,b)
* $P < 0.05$ ** $P < 0.01$ Probability value relative to normal mothers

CAPTIONS TO ILLUSTRATIONS

Figure 1. Breast-milk n-6 and n-3 fatty acids content. Comparison of values from Argentine mothers according to their BMI with values reported in the literature for other countries: (1) Adapted from Jensen [3]. (2) Adapted from Jensen [39]. (3) Adapted from Krasevec et al. [40].

r = total n-6/ total n-3 fatty acids ratio.

Figure 2. Mean (± SEM) breast-milk 22:6 n-3 fatty acid content. Comparison of values from Argentine mothers according to their BMI with values reported in the literature for other countries: (1) Adapted from Jensen [3]. (2) Adapted from Jensen [39]. (3) Adapted from Krasevec et al. [40].