Human Milk Fatty Acids Profile Changes during Prolonged Lactation: A Cross-Sectional Study

Ronit Lubetzky MD[1,2,5], Galit Zaidenberg-Israeli MD[1,2], Francis B. Mimouni MD[3,5], Shaul Dollberg MD[1,5], Eyal Shimoni PhD[4], Yael Ungar PhD[4] and Dror Mandel MD[1,5]

[1]Department of Neonatology, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel
[2]Department of Pediatrics, Dana Children's Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel
[3]Department of Pediatrics, Shaare Zedek Medical Center, Jerusalem, Israel
[4]Faculty of Biotechnology & Food Engineering, Technion-Israel Institute of Technology, Haifa, Israel
[5]Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

ABSTRACT: **Background:** Human milk produced during prolonged lactation (> 1 year) is extraordinarily rich in fat and has a higher energy content than human milk produced during short lactation.

Objectives: To estimate the fatty acid (FA) profile of human milk and to test the hypothesis that the proportion of C12 and C14 (two dietary saturated FA known to most promote hypercholesterolemia) in human milk during prolonged lactation is similar to that in short lactation.

Methods: We conducted a cross-sectional study of 30 mothers of term infants lactating for more than 1 year as compared with 25 mothers of full-term infants who lactated for 2–6 months. Milk was collected by manual expression in mid-breastfeeding.

Results: The two groups did not differ in maternal height, weight, body mass index, diet, infant birth weight and gestational age, but mothers in the prolonged lactation group were significantly older. There was a significant correlation between lactation duration and C12 or C14. The percentage of all FA combined (except for C12 and C14) decreased significantly over time. In contrast, C12:0 and C14:0 combined increased significantly during lactation ($R^2 = 10.0\%$, $P < 0.03$).

Conclusions: Women who lactated for more than 1 year had higher C12 and C14 FA percentages in their milk than women who lactated for 2–6 months.

IMAJ 2012; 14: 7–10

KEY WORDS: human milk, fatty acids, cholesterol, breastfeeding, lactation

The optimal duration of breastfeeding is unknown. Since 1979, the World Health Organization [1], the American Academy of Pediatrics [2] and the American Academy of Family Physicians [3] recommend that children throughout the world be breastfed for a minimum of 1–2 years, with no defined upper limit on duration of breastfeeding. In 2007, the American College of Obstetricians and Gynecologists stated that its members' "professional objectives are to encourage and enable as many women as possible to breastfeed and to help them continue as long as possible" [4].

One of the important long-term benefits of breastfeeding is reduced cardiovascular risk in adulthood [2,5]. However, two studies have suggested that the beneficial effect of breastfeeding on cardiovascular risk exists only if weaning is performed prior to 1 year of age; moreover, some researchers have suggested that beyond 1 year, prolonged breastfeeding might actually increase the cardiovascular risk [5,6]. In a previous study we demonstrated that human milk produced during prolonged lactation (> 1 year) is extraordinarily rich in fat and has a higher energy content than human milk produced during the first half-year of lactation [7].

Dietary fat has differential effects on hypercholesterolemia. Some dietary saturated fatty acids, in particular C12:0 and C14:0, are known to most promote hypercholesterolemia in humans [8]. In order to estimate the FA profile of human milk we conducted a cross-sectional study of 55 women lactating for periods ranging from 2 to 6 months (short lactation duration) and for more than 1 year (prolonged lactation duration). We tested the null hypothesis that the proportion of "cholesterol-promoting" fatty acids, namely C12 and C14, in human milk during prolonged lactation is similar to that in short lactation.

SUBJECTS AND METHODS

The milk of 30 mothers of full-term, healthy, growing children lactating for more than 1 year was compared to that of 25 mothers of full-term infants who lactated for 2–6 months. All infants were healthy, free of congenital malformations, and had been born after a normal pregnancy, labor and delivery. In order to control for possible diurnal variations [9,10], for the analyses we used one sample collected between 9.00 p.m. and midnight from every subject. Milk was collected by manual expression

This study was presented in part at the Pediatric Academic Societies annual meeting 2010, Vancouver, BC, Canada

FA = fatty acids

in mid-breast feeding. Maternal type of diet (omnivorous vs. lacto-ovo-vegetarianism) was recorded.

LABORATORY METHODS

All milk samples were stored at -20°C until analyzed. Lipids were extracted with chloroform-methanol (2:1) with butylatedehydroxyanisole used as an antioxidant, and fatty acids were identified as published previously [11,12].

STATISTICAL ANALYSES

Results are expressed as mean ± SD. Student's *t*-tests were used to determine the differences between the two groups (2–6 months and > 1 year) for continuous variables, and chi-square tests for categorical variables. Linear regression was used to determine the correlation between lactation duration and the percentage of each individual fatty acid. Stepwise backward multiple regression was used to determine the effect of variables found to be significant in univariate analysis on fatty acid profile.

RESULTS

The demographic and maternal characteristics of the study participants are presented in Table 1. There were 55 infants breastfed for either 2–6 months (short duration group, n=25) or 12–39 months (long duration group, n=30). The two groups (short versus prolonged lactation) did not differ in terms of maternal height, weight, body weight index, diet (most were omnivorous and had a Mediterranean-type diet, except for four mothers who were lacto-ovo-vegetarians – three in the short duration and one in the long duration lactation groups), infant birth weight and gestational age. They, however, differed significantly in terms of maternal age, with mothers in the prolonged lactation group being older ($P = 0.02$) [Table 1].

Table 2 depicts the individual fatty acid measured, expressed as a percentage of total FA in milk. There were no significant differences between the two groups in terms of FA percentages for all FA considered except for C14 ($P = 0.046$). Four fatty acids from the long chain polyunsaturated group were undetectable in a large percentage of samples and fell below the limit of detection of the method we used in this study.

Table 1. Demographic and clinical characteristics

	Short lactation (2–6 mos) (n=25)	Prolonged lactation (> 1 yr) (n=30)	*P*
Maternal age (yrs)	31.4 ± 3.3	34.8 ± 4.2	0.02
Maternal weight (kg)	59.6 ± 8.8	62.7 ± 14.0	NS
Maternal height (m)	1.65 ± 0.05	1.64 ± 0.06	NS
Maternal BMI (kg/m²)	22.3 ± 2.7	23.1 ± 4.3	NS
Duration of lactation (mos)	3.65 ± 1.1	18.6 ± 6.2	< 0.001
Infant gestational age (wks)	39.0 ± 1.7	39.6 ± 1.5	NS
Infant birth weight (kg)	3.25 ± 0.4	3.20 ± 0.45	NS

Data are expressed as mean ± SD
BMI = body mass index, NS = not significant

Table 2. Laboratory characteristics

	Short lactation (2–6 mos) (n=25)	Prolonged lactation (> 1 yr) (n=30)
C4:0	3.81 ± 6.75	2.793 ± 2.48
C6:0	0.79 ± 1.73	1.73 ± 4.99
C8:0	3.84 ± 6.2	3.94 ± 6.9
C10:0	2.88 ± 4.2	5.14 ± 6.0
C12:0	3.1 ± 2.0	3.8 ± 2.1
C14:0*	3.9 ± 2	5.4 ± 3.4
C14:1	1.2 ± 1.2	1.8 ± 1.8
C16:0	15.0 ± 9.5	14.6 ± 9.4
C16:1	5.2 ± 7.6	6.8 ± 7.9
C18:0	15.9 ± 13.4	14.9 ± 12.1
C18:1 *n*-9	8.3 ± 7.3	6.6 ± 7.7
C18:1 (total)	24.2 ± 8.6	21.5 ± 10.4
C18:2 *n*-6	27.5 ± 17.4	21.2 ± 14.7
C18:2 *n*-7	0.45 ± 0.9	0.18 ± 0.6
C18:3 *n*-3	0.46 ± 0.72	0.9 ± 1.2
C18:3 *n*-6	0.43 ± 0.6	0.64 ± 1.3
C20:0	0.58 ± 0.6	1.07 ± 1.4
C20:3 *n*-6	Undetectable	Undetectable
C20:4 *n*-6	0.85 ± 1.24	1.82 ± 2.33
C20:5 *n*-3	Undetectable	Undetectable
C22:5 *n*-3	Undetectable	Undetectable
C22:6 *n*-3	Undetectable	Undetectable

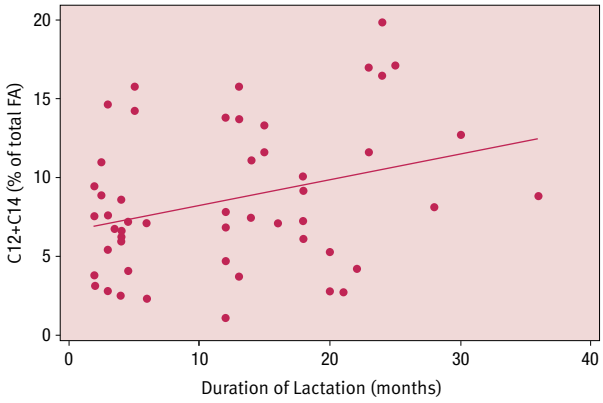
Data are expressed as mean ± SD (% of total FA)
*The groups did not significantly differ in all fatty acids measured except for C14 ($P = 0.046$)

When the duration of lactation was used as a continuous variable and not as a categorical variable, there was a significant correlation between lactation duration and C12 ($R^2 = 7.4\%$, $P = 0.05$) and lactation duration and C14 ($R^2 = 9.2\%$, $P = 0.029$). No other fatty acid correlated significantly with lactation duration. The percentage of all fatty acids combined (except for C12 and C14) decreased significantly over time in contrast to C12:0 and C14:0 combined which significantly increased during lactation ($R^2 = 10.0\%$, $P < 0.03$) [Figure 1]. In univariate and multiple regression, maternal age was not found to significantly influence the FA profile.

DISCUSSION

We found that the fatty acid profile of human milk during prolonged lactation (> 1 year) was not identical to that of human milk during short lactation (2–6 months). The dif-

Figure 1. Correlation between C12+C14 (percent of total fatty acids) and lactation duration (months) ($R^2 = 10.0\%$, $P < 0.03$)



ferences found were not striking and became apparent only when we used duration of lactation as a continuous variable. Doing so, there was a significantly higher percentage of C12 and C14 FA and a lower percentage of all other combined fatty acids in human milk during prolonged lactation.

It must be emphasized that a limitation of our study was its cross-sectional design. Thus, we cannot conclude with certainty that C12 and C14 increase over time during lactation, and we can only state that women who lactated for more than 1 year had higher C12 and C14 percentages in their milk than women who lactated for 2–6 months. It is indeed possible that the differences between the two groups were not related to lactation duration but to differences between the two groups for some important but unrecognized other variable(s). For example, a systematic difference in dietary intake of fat between the two groups cannot be excluded. However, most women in the study ate a Mediterranean-type diet, and only four women were lacto-ovo-vegetarian. More subtle dietary differences (fish, vegetable oil, fruit/vegetable consumption) might have had a substantial influence on fatty acid intake and breast milk composition. The two groups were also similar in terms of maternal height, weight, body mass index, infant birth weight and gestational age. They were significantly different in terms of maternal age (the mothers were older in the prolonged duration group). The presence of older mothers in the prolonged duration group may indicate significant socioeconomic differences between the two groups, which theoretically may have influenced the results. However, in multiple regression analysis, maternal age was not found to affect the fatty acid profile. Thus, we emphasize that the results of our study, a pilot study, should be confirmed in a longitudinal approach.

We are aware of another study that also examined the fatty acid profile of human milk during prolonged (14 months) and short (3 months) lactation [13]. In that study, there were no differences in FA profile between groups, but the study was limited by a similar cross-sectional approach in addition to a very small sample size (fewer than 14 patients in each group).

If we postulate that indeed C12 and C14 increase during prolonged lactation over time, this has to be considered together with the striking finding that human milk produced during prolonged lactation (> 1 year) is extraordinarily rich in fat and has a higher energy content than human milk produced during the first half-year of lactation [7]. Indeed, C12 and C14 are the saturated fatty acids that have been shown to have the greater hypercholesterolemic effect in humans [8]. Moreover, human milk has considerably higher concentrations of cholesterol than most available formulas, and infants fed human milk have higher plasma cholesterol concentrations than formula-fed infants [14]. The long-term impact of prolonged human milk feeding on cholesterol metabolism and on cardiovascular risk is unclear and controversial. Relative hypercholesterolemia in infancy might be beneficial in that it may "program" endogenous cholesterol synthesis rates by lowering synthesis [14], but whether lower cholesterol synthesis rates persist into adulthood is unknown, although it has been demonstrated in rats [15].

The importance of a high percentage of C12 (lauric acid) and C14 (myristic acid) in the diet of human milk-fed infants may not be restricted to cholesterol metabolism and cardiovascular protection. Indeed, lauric acid, metabolized into monolaurin, has been shown to play multiple roles as an antibacterial and antiviral agent [16-18]. Infants fed with human milk are notably protected against a multitude of infectious diseases, and it is possible that one of the many agents involved is indeed lauric acid. Whether or not prolonged lactation protects infants against infections has not been studied systematically.

The fact that the concentrations of docosahexaenoic acid were low or undetectable in the samples studied was surprising to us. Indeed, generally reported concentrations of DHA in human milk range between 0.27 and 0.48 and are reported to be little influenced by maternal diet [19]. We must point out that Shehadeh et al. [13] in a study of human milk during prolonged lactation also found undetectable values of DHA in the majority of samples. The latter study was also performed in Israel, thus raising the question whether the maternal Israeli diet or prolonged lactation can be responsible for this finding. Kosher food restrictions forbid the use of seafood other than scaly fish, which might impose a restriction on LC-PUFA intake among religiously observant Israeli women. Moreover, research from Israel reveals that the Israeli population consumes a high level of omega-6 fatty acids while the intake of omega-3 fatty acids is relatively low [20,21]. Essentially all cold water fish, kosher fish (salmon, herring, cod, sole, etc.) or non-kosher seafood (shrimp, lobster, etc.) produce and contain omega-3 and omega-6 fatty acids. Thus, as long as patients eat seafood,

DHA = docosahexaenoic acid
LC-PUFA = long chain polyunsaturated fatty acids

kosher or non-kosher, they probably obtain an adequate supply of omega-3 or 6 fatty acids, assuming they consume two portions a week as suggested by the American Heart Association [22]. The price of fish in Israel is usually higher than that of poultry, meat and dairy products, which might be a deterrent to adequate fish intake in the Israeli diet. Indeed, an international comparison shows that the average percent of total food expenditure in Israel is 14.11% for meat products, 12.97% for dairy products, and only 2.51% for fish products [23].

We conclude that women who lactated for more than 1 year had higher C12 and C14 fatty acid percentages in their milk than women who lactated for 2–6 months. This finding must be confirmed in a longitudinal prospective approach. Whether or not clinical outcomes – both short term (such as visual and early motor and cognitive outcomes, etc.) and long term (better IQ scores, improved immunological health, reduced atherosclerosis risk, etc.) – in an individual are affected by the composition of human milk fat remains to be studied.

Corresponding author:
Dr. D. Mandel
Dept. of Neonatology, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, 6 Weizmann Street, Tel Aviv 64239, Israel
Phone: (972-3) 692-5690
Fax: (972-3) 692-5681
email: drorm@tasmc.health.gov.il

References

1. http://www.who.int/topics/breastfeeding/en. Accessed: January 20, 2010.
2. Gartner LM, Morton J, Lawrence RA, et al.; American Academy of Pediatrics Section on Breastfeeding. Breastfeeding and the use of human milk. *Pediatrics* 2005; 115: 496-506.
3. American Academy of Family Physicians. Breastfeeding (position paper). http://www.aafp.org/online/en/home/policy/policies/b/breastfeedingpositionpaper.html. Accessed January 20, 2010.
4. American College of Obstetricians and Gynecologists. Breastfeeding: maternal and infant aspects. Special report from ACOG. *ACOG Clin Rev* 2007; 12 (Suppl): 1-16S.
5. Fall CH, Barker DJ, Osmond C, Winter PD, Clark PM, Hales CN. Relation of infant feeding to adult serum cholesterol concentration and death from ischaemic heart disease. *BMJ* 1992; 304: 801-5.
6. Leeson CP, Kattenhorn M, Deanfield JE, Lucas A. Duration of breast feeding and arterial distensibility in early adult life: population based study. *BMJ* 2001; 322: 643-7.
7. Mandel D, Lubetzky R, Dollberg S, Barak S, Mimouni FB. Fat and energy contents of expressed human breast milk in prolonged lactation. *Pediatrics* 2005; 116: e432-5.
8. Hayes KC. Saturated fats and blood lipids: new slant on an old story. *Can J Cardiol* 1995; 11 (Suppl G): 39-46G.
9. Clark RM, Ferris AM, Fey M, Brown PB, Hundrieser KE, Jensen RG. Changes in the lipids of human milk from 2 to 16 weeks postpartum. *J Pediatr Gastroenterol Nutr* 1982; 1: 311-15.
10. Ruel MT, Dewey KG, Martinez C, Flores R, Brown KH. Validation of single daytime samples of human milk to estimate the 24-h concentration of lipids in urban Guatemalan mothers. *Am J Clin Nutr* 1997; 65: 439-44.
11. Mitoulas LR, Gurrin LC, Doherty DA, Sherriff JL, Hartmann PE. Infant intake of fatty acids from human milk over the first year of lactation. *Br J Nutr* 2003; 90: 979-86.
12. Chouinard PY, Corneau L, Barbano DM, Metzger LE, Bauman DE. Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. *J Nutr* 1999; 129: 1579-84.
13. Shehadeh N, Aslih N, Shihab S, Werman MJ, Sheinman R, Shamir R. Human milk beyond one year post-partum: lower content of protein, calcium, and saturated very long-chain fatty acids. *J Pediatr* 2006; 148: 122-4.
14. Cruz ML, Wong WW, Mimouni F, et al. Effects of infant nutrition on cholesterol synthesis rates. *Pediatr Res* 1994; 35: 135-40.
15. Reiser R, Sidelman Z. Control of serum cholesterol homeostasis by cholesterol in the milk of the suckling rat. *J Nutr* 1972; 102: 1009-16.
16. Rouse MS, Rotger M, Piper KE, et al. In vitro and in vivo evaluations of the activities of lauric acid monoester formulations against Staphylococcus aureus. *Antimicrob Agents Chemother* 2005; 49: 3187-91.
17. Carpo BG, Verallo-Rowell VM, Kabara J. Novel antibacterial activity of monolaurin compared with conventional antibiotics against organisms from skin infections: an in vitro study. *J Drugs Dermatol* 2007; 6: 991-8.
18. Hornung B, Amtmann E, Sauer G. Lauric acid inhibits the maturation of vesicular stomatitis virus. *J Gen Virol* 1994; 75: 353-61.
19. Koletzko B, Rodriguez-Palmero M. Polyunsaturated fatty acids in human milk and their role in early infant development. *J Mammary Gland Biol Neoplasia* 1999; 4: 269-84.
20. Endevelt R, Shahar DR. Omega-3: the vanishing nutrient beyond cardiovascular prevention and treatment. *IMAJ Isr Med Assoc J* 2004; 6: 235-9.
21. Dubnov G, Berry EM. Omega-6/omega-3 fatty acid ratio: the Israeli paradox. *World Rev Nutr Diet* 2003; 92: 81-91.
22. Krauss RM, Deckelbaum RJ, Ernst N, et al. Dietary guidelines for healthy American adults: a statement for health professionals from the Nutrition Committee, American Heart Association. *Circulation* 1996; 94: 1795-800.
23. United States Department of Agriculture, technical bulletin No 1904, Oct 2003. http://www.ers.usda.gov/publications/tb1904/tb1904.pdf (Accessed November 13, 2011).