Fatty Acid Composition of Breast Milk from Nigerian and Japanese Women

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Summary The fatty acid composition of samples of breast milk obtained from well-nourished Nigerian and Japanese women was determined by gas chromatography. The cultural differences in dietary intake was reflected in the fatty acid composition of breast milk samples. The milk of Nigerian women contained a significantly higher percentage of saturated fatty acids (48.75%) than that of Japanese women (46.65%). Nigerian milks were also richer in arachidonic (20:4 n-6), eicosatrienoic (20:3 n-6), and docosatetraenoic (22:4 n-6) acids. Conversely, the milk of Japanese woman contained significantly higher percentages of monounsaturates as palmitoleic, heptadecenoic, oleic, and polyunsaturates of n-3 series as α-linolenic, eicosapentaenoic, and docosahexaenoic acid.

Key Words breast milk, fatty acid composition, Nigerian milk, Japanese milk, lipid

Fat is the major energy source and the most variable of the proximate nutrients of breast milk. Examination of fatty acid composition in human milk is of interest because fatty acid have been shown to be of vital importance for normal growth and development (1, 2) of infants. Recent studies suggest that these polyunsaturated fatty acids play a critical role in fluidity of membrane lipids, prostaglandin synthesis, and in the functions of brain and visual cells (3–6). In addition, the role of dietary factors in the pre- and postnatal development of the human brain has

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received increasing attention in recent years. One of the most abundant components of structural lipids in the brain and retina is docosahexaenoic acid (22:6 \(n\)-3) (3), which is mainly derived from dietary eicosapentaenoic acid (20:5 \(n\)-3) or docosahexaenoic acid (27). Thus, an adequate amount of these \(n\)-3 fatty acids in the diet would be vital during both pregnancy and lactation. Although the fatty acid pattern in human milk samples remained constant and was found to be more remarkable for their similarity than dissimilarity in cross-cultural studies (2, 7, 8), it has been reported that the fatty acid composition of human milk generally responded rapidly and markedly to dietary changes (9, 10). The Japanese diets are known to be richer in fish and marine products than Western diets and particularly Nigerian diets (11). Whether supplementation of the diet with fish containing these \(n\)-3 fatty acids during pregnancy and lactation would influence brain development and learning ability in the young has not been experimentally clarified. Nevertheless, the contents of these \(n\)-3 fatty acids in breast milk must be considered. It was of interest, therefore, to compare the fatty acid composition of the lipids in samples of breast milk obtained from well-nourished Nigerian women with those of Japanese women.

MATERIALS AND METHODS

**Description of subject.** Nigerian subjects included 20 well-nourished nonvegetarian women (27–35 years of age) from a university community with from two to four living children. A total of 53 lactating Japanese women of middle socioeconomic status (28–36 years of age) with one or two living children were selected for the study. All mothers were volunteers and information on the health of women and infants, age, parity and food patterns, and history of lactation were obtained through personal interview. All subjects were in good health and delivered a healthy full-term infant.

**Dietary intake of Nigerians.** The dietary habit survey of Nigerian mothers by the methods of food frequency and 24 h-recall conducted by Stoff et al. (11) showed that intake of protein, fat, and carbohydrate was 14%, 29%, and 52% of total energy (7,200 kJ/day), respectively. Although small variations in scores obtained by the two methods on the same individual were observed, there was a good concurrence between the two methods (Pearson’s correlation coefficient = 0.96, \(p < 0.05\)). However, we could not conduct the dietary habit survey of Nigerian volunteer mothers by ourselves.

**Milk sample.** All samples were collected from healthy women volunteers fed on \textit{ad libitum} diets at random times during nursing because these sampling conditions had previously been shown to have no effect on fatty acid composition of human milk (12). The stages of lactation were comparable between the Nigerian (60–90 days postpartum) and Japanese (70–100 days postpartum). An aliquot (5 ml) of samples obtained from Nigerian women were collected at the early morning nursing in the maternity wing of Teaching Hospital Shagamu of the Ogun State...
University in Nigeria by manual expression into plastic containers. One ml 0.01% butylated hydroxytoluene (BHT) was added as an antioxidant before storage at $-30^\circ C$. All samples were later lyophilized and transported to Tokyo for analysis. The Japanese milk samples (5–10 ml) were expressed at an early morning nursing directly into plastic containers at Ina Health Center, Ina City in Nagano and placed on ice for transfer to Tokyo where samples were lyophilized for analysis. All procedures were approved by the National Institute of Nutrition Committee on the use of Human Subjects in Research.

Fatty acids analysis. Fatty acids of lyophilized milk samples were methylated according to the procedure of Stoffel et al. (13), and fatty acid methylesters were stored at $-20^\circ C$ in a nitrogen atmosphere until analysis.

Fatty acid methyl esters were separated and estimated using a Varian 3500 Gas Chromatograph attached to a Shimadzu C-R 4A Chromatopac Data System. The fatty acid analysis of the methyl esters was performed on a FFS capillary column (Shinwakako Co., Ltd., 25 m × 0.25 mm id, ULBON HR Thermon 3000B), with injector temperature of 250$^\circ C$, detector flame ionization at 250$^\circ C$, and He carrier. Temperature was programmed with column initial temperature at 140$^\circ C$ and raised 3$^\circ C$/min to a final temperature of 240$^\circ C$. The split ratio was 50 : 1. Fatty acid peaks were identified by comparing their retention times to known standards (Spelco, Barcelona), and by mass spectrographic analysis. Mass spectrography (Incos 50, Finnigan MAT Inst. Inc., U.S.A.) was used as a monitor of gas-liquid chromatograph in order to identify fatty acids. The method used allowed a well separation of at least 30 identified fatty acid peaks.

Statistics analysis. Statistics analysis of variance (ANOVA) were carried out to determine statistical significance of results (14).

RESULTS

The mean values of saturated fatty acids in the lipid fraction of milks from Nigerian and Japanese women are listed in Table 1. Nigerian women produced a milk richer in saturated fatty acids (48.75%) when compared to Japanese women milk (46.65%). The lipids from the milks of Nigerian women contained significantly ($p<0.001$) higher laureate (12:0), 10.20%, and lower palmitate (16:0), 22.14%, than the milk from Japanese women (12:0; 7.82%, 16:0; 23.19%). Percentages of saturated fatty acids as myristic (14:0), heptadecanoic (17:0), stearic (18:0), arachidic (20:0), behenic (22:0), and lignoceric (24:0) acids were similar for both groups.

The unsaturated fatty acids found in the lipids of milk obtained from Nigerian and Japanese lactating women showed important differences. The largest difference was found in the level of palmitoleic acid (16:1 n-7), which accounted for 2.58% of the fatty acids in the Japanese and just 0.74% in the Nigerian milks. Japanese milk contained more monounsaturated fatty acids (34.29%) than Nigerian milk (32.37%) (Table 2). There were no significant differences in the level of total

Vol. 37, No. 4, 1991
Table 1. Saturated fatty acids in the lipid fraction of breast milk from Nigerian and Japanese women.

| Fatty acid | Nigerian (n = 20) | Japanese (n = 53) |
|------------|--------------------|-------------------|
| 12:0       | 10.20±0.26***      | 7.82±1.99         |
| 14:0       | 9.05±0.11          | 8.69±3.67         |
| 16:0       | 22.14±0.10*        | 23.19±3.06        |
| 17:0       | 0.31±0.01          | 0.30±0.12         |
| 18:0       | 6.77±0.04          | 6.34±1.83         |
| 20:0       | 0.17±0.01          | 0.19±0.09         |
| 22:0       | 0.06±0.02          | 0.07±0.09         |
| 24:0       | 0.05±0.01          | 0.05±0.01         |
| Total      | 48.75±0.20         | 46.65±2.79        |

Mean values expressed in mol% of milk fat±SD. *Saturated fatty acids are designated by:0 notation. **Significantly different from Japanese values at p<0.05; ***significantly different from Japanese values at p<0.01; ****significantly different from Japanese values at p<0.001.

polyunsaturated fatty acids (PUFA) of n-6 series in both groups. However, the significantly (p<0.05) higher mean values of arachidonic (0.56%), eicosatrienoic (0.40%), and docosatetraenoic (0.22%) acids were recorded for Nigerian milk. Milk of Japanese women contained higher percentages of polyunsaturated fatty acids of n-3 series as α-linolenic (18:3 n-3), eicosapentaenoic (20:5 n-3), and docosahexaenoic (22:6 n-3) acids compared to milk of Nigerian women. The differences were statistically significant, as shown in Table 2.

DISCUSSION

It is well established that the fatty acid composition of breast milk lipids are affected by diet. The human mammary gland is capable of synthesizing fatty acids of 10 to 14 carbons in length. Longer chain lengths (16>) of fatty acids are not synthesized in the mammary gland and can be obtained only from diet or mobilized from fat deposits (2,15,16).

The selected well-nourished Nigerian women in this study are non-Islamic and tended to eat at least weekly all meats including chicken, beef, and pork, as well as fish, mainly cooked with vegetable oil, whereas the Japanese diets are known to be richer in fish and marine products, and complex carbohydrates, and lower in animal protein and fat (17). The mean level of saturated fatty acids in Nigerian milk was significantly higher than the mean value recorded for the Japanese but similar to the level reported in Egyptian women (18). Lauric acid (12:0) accounted for 10.2% of the fatty acids in the milk of Nigerian women. Elevated levels of lauric acid was reported in the lipids of milk from women on high-carbohydrate diets living in Ivory Coast (19). The content of palmitic acid (16:0) in milk of Nigerian women is in agreement with the levels recorded for Egyptian, American, and German

J. Nutr. Sci. Vitaminol.
FATTY ACID OF HUMAN MILK SAMPLES

Table 2. Unsaturated fatty acids in the lipid fraction of breast milk from Nigerian and Japanese women.

| Fatty acid \(n\) | Nigerian | Japanese |
|-----------------|----------|----------|
|                 | Monounsaturated fatty acids | Polyunsaturated fatty acids -n-6 series | Polyunsaturated fatty acids -n-3 series | Total | Total |
| 16:1 n-7        | 0.74±0.22*** | 15.57±0.17 | 0.93±0.02* | 1.53±0.03*** | 0.092 |
| 17:1            | 0.09±0.00**  | 0.07±0.01  | 0.09±0.01***| 1.82±0.06    | 0.157 |
| 18:1 n-9        | 28.38±0.17   | ND        | 0.17±0.10   | 0.13±0.09    |
| 18:1 n-7        | 2.61±0.13**  | ND        | 0.34±0.01** | 0.53±0.10    |
| 20:1 n-11       | 0.45±0.02    | 0.40±0.01* | 0.22±0.00*  | 0.20±0.09    |
| 20:1 n-9        | 0.04±0.01    | 0.56±0.02* | ND         | 0.36±0.01    |
| 24:1 n-9        | 0.06±0.03    | 0.22±0.00* | ND         | 0.07±0.01    |
| Total           | 32.37±0.14***| 17.32±0.16| 1.53±0.03***| 16.30±1.04   |
| Japanese        | 2.58±0.76    | 15.38±1.11| 1.82±0.06    | 2.71±0.06    |
| Mean values expressed in mol% of milk fat±SD. ND: not detectable. * Specific unsaturated fatty acids are designated by \(n\) notation, indicating the position of the first double bond relative to the terminal methyl end group. ** Significantly different from Japanese values at \(p<0.05\); *** significantly different from Japanese values at \(p<0.01\); **** significantly different from Japanese values at \(p<0.001\). 

women (18,20) but lower than that of Japanese women. Such monounsaturated fatty acids as palmitoleic (16:1), heptadecenoic (17:1), and oleic (18:1) acids were significantly higher in Japanese women’s milk than in the Nigerian (Table 2).

It is of interest that palmitoleic acid has been implicated in the prevention of death from cerebrovascular disorders in SHRSP rats (21).

The mean total levels of polyunsaturated fatty acids of -n-6 series in both Japanese and Nigerian breast milk were similar and agree with recorded values for American women (17.3%) but were higher than levels reported for German

Vol. 37, No. 4, 1991
However, dietary intakes of linoleic acid (18:2 n-6) have been reported to influence the content of this fatty acid in milk (7, 9, 10, 23, 24). Higher levels of eicosapentaenoic (0.20%) and docosahexaenoic (0.53%) acids were recorded for Japanese milk compared to Nigerian milk (eicosapentaenoic acid, 0.09%; docosahexaenoic acid, 0.34%), and furthermore the total levels of long chain polyunsaturates of n-3 series were higher than levels reported in groups of Australian women (25), and well-nourished Western women (26). Fish oil produced significant increases in the eicosapentaenoic and docosahexaenoic acids of milk and plasma (27), and docosahexaenoic acid has been implicated in brain and retinal development (27–31), and the Japanese diets are known to be rich in fish and marine products. Therefore, the lower levels of n-6 metabolites as 20:3 n-6, 20:4 n-6, and 22:4 n-6 in Japanese milk can be adduced to the possible fact that higher proportions of n-3 fatty acids inhibit the synthesis of these fatty acids (32).

The fatty acid compositions of the milk of Nigerian women were, with some exceptions, markedly different from those of the Japanese women in this study. This was expected since Nigerian diets differed considerably from those of Japanese. In addition, the statistical deviations of fatty acid compositions were generally higher in Japanese milks than in Nigerian milks, suggesting that the foods in the daily diets of Japanese women were more variable than those of Nigerian women. It is interesting to note that, in this study and others reported herein, the quality of human breast milk with regard to fatty acids is highly dependent on the dietary variations of lactating mothers.

Further study on the effect of dietary habit on the fatty acid composition of breast milk in Japanese and Nigerian women is now proceeding in our laboratory.

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REFERENCES

1) Jelliffe, D. B., and Jelliffe, E. F. P. (1978): The volume and composition of human milk in poorly nourished communities. A review. *Am. J. Clin. Nutr.*, 31, 492–515.
2) Crawford, M. A., Hall, B., Laurance, B. M., and Munhambo, A. (1976): Milk lipids and their variability. *Curr. Med. Res. Opin.*, 4, 33–43.
3) Gomperts, B. D. (1977): The plasma membrane, in Models for Structure and Function, Academic Press, New York, pp. 1–50.
4) Van Dorf, D. A., Beerthuis, R. K., Nugteren, D. H., and Vonkerman, H. (1964): The biosynthesis of prostaglandins. *Biochim. Biophys. Acta*, 90, 204–207.
5) Sinclair, A. J., and Crawford, M. A. (1972): The incorporation of linolenic acid and docosahexaenoic acid into liver and brain lipids of developing rats. *FEBS Lett.*, 26, 127–129.

*J. Nutr. Sci. Vitaminol.*
6) Wheeler, T. G., and Anderson, R. (1975): Visual membranes specificity of fatty acid precursors of the electrical response to illumination. *Science*, 188, 1312–1314.

7) Hall, B. (1979): Uniformity of human milk. *Am. J. Clin. Nutr.*, 32, 304–312.

8) Clark, R. M., Ferris, A. M., Fey, M., Brown, P. B., Hundrieser, K. E., and Jensen, R. G. (1982): Changes in the lipids of human milk from 2 to 16 weeks postpartum. *J. Pediatr. Gastroenterol. Nutr.*, 1, 311–315.

9) Jensen, R. G., Hagerty, M. M., and McMahon, E. E. (1978): Lipids of human milk and infant formulas, a review. *Am. J. Clin. Nutr.*, 31, 990–1016.

10) Lammi-Keefe, C. L., and Jensen, R. G. (1984): Lipids in human milk: A review 2: Composition and fat soluble vitamins. *J. Pediatr. Gastroenterol. Nutr.*, 3, 172–198.

11) Stoff, J., Garza, C., Smith, E., Nichols, B., and Montandon, C. (1983): A comparison of dietary methods in nutritional studies. *Am. J. Clin. Nutr.*, 37, 300–306.

12) Gibson, R. A., and Kneebone, G. M. (1980): Effect of sampling on fatty acid composition of human colostrum. *J. Nutr.*, 110, 1671–1675.

13) Stoffel, W., Chu, F., and Ahrens, E. H. (1959): Hydrogen chloride and methanol methylation of extracts from animal tissues. *Anal. Chem.*, 31, 307.

14) Steel, R. G. D., and Torrie, J. H. (1960): *in* Principles and Procedures of Statistics with Special Reference to the Biological Sciences. McGraw-Hill, New York, Toronto, and London.

15) Garton, G. A. (1963): The composition and biosynthesis of milk lipids. *J. Lipid. Res.*, 4, 137–154.

16) Vuori, E., Kuru, K., Makinen, S. M., Vavrynen, P., Kara, R., and Kutunen, P. (1982): Maternal diet and fatty acid pattern of breast milk. *Acta Pediatr. Scand.*, 71, 959–963.

17) Kagawa, Y., Nishizawa, M., Suzuki, M., Miyatake, T., Hamamoto, T., Goto, K., Motonaga, E., Izumikawa, H., Hirata, H., and Ebihara, A. (1982): Eicosapentaenoic acid of serum lipids of Japanese islanders with low incidence of cardiovascular diseases. *J. Nutr. Sci. Vitaminol.*, 28, 441–453.

18) Borschel, M. W., Elkin, R. G., Kirksey, A., Story, J. A., Gala, O., Harrison, G., and Jerome, N. W. (1986): Fatty acid composition of mature human milk of Egyptian and American women. *Am. J. Clin. Nutr.*, 44, 330–335.

19) Lauber, E., and Reinhardt, M. (1979): Studies on the quality of breast milk during 23 months of lactation in a rural community of the Ivory Coast. *Am. J. Clin. Nutr.*, 32, 1159–1173.

20) Koletzko, B., Mróz, M., and Bremer, H. J. (1988): Fatty acid composition of mature human milk in Germany. *Am. J. Clin. Nutr.*, 47, 945–949.

21) Yamori, Y., Nara, Y., Tsubouchi, T., Sogara, Y., Ikeda, K., and Horie, R. (1986): Dietary prevention of stroke and its mechanisms in stroke-prone SHR (SHRSP): Preventive effect of dietary fiber and palmitoleic acid. *J. Hypertension*, 4 (Suppl. 3), 449–452.

22) Kneebone, G. M., Kneebone, R., and Gibson, R. A. (1985): Fatty acid composition of breast milk from three racial groups from Penang, Malaysia. *Am. J. Clin. Nutr.*, 41, 765–769.

23) Insull, W., Hirsch, F., James, T., and Ahrens, E. H. (1959): The fatty acids of human milk. II. Alterations produced by manipulation of caloric balance and exchange of dietary fats. *J. Clin. Invest.*, 38, 443–450.

24) Read, W. W. C., Lutz, P. G., and Tashjian, A. (1965): Human milk lipids. II. The
influence of dietary carbohydrates and fat of the fatty acids of mature milk. A study in four ethnic groups. *Am. J. Clin. Nutr.*, 17, 180–183.

25) Gibson, R. A., and Kneebone, G. M. (1981): Fatty acid composition of human colostrum and mature breast milk. *Am. J. Clin. Nutr.*, 34, 252–257.

26) Vuori, E., Kiuru, K., Makinen, S. M., Väyrynen, P., Kara, R., and Kuitunen, P. (1972): Maternal diet and fatty acid pattern of breast milk. *Acta Paediatr. Scand.*, 71, 959–963.

27) Harris, W. S., Connor, W. E., and Lindsey, S. (1984): Will dietary n-3 fatty acids change the composition of human milk? *Am. J. Clin. Nutr.*, 40, 780–785.

28) Walker, B. L. (1967): Maternal diet and brain fatty acids in young rats. *Lipids*, 2, 497–500.

29) Benolken, R. M., Anderson, R. E., and Wheeler, T. G. (1973): Membrane fatty acids associated with the electrical response in visual excitation. *Science*, 182, 1253–1254.

30) Lamptey, M. S., and Walker, B. L. (1976): A possible essential role for dietary linolenic acid in the development of the young rat. *J. Nutr.*, 106, 86–93.

31) Neuringer, M., Connor, W. E., Petten, C. V., and Barstad, L. (1984): Dietary omega-3 fatty acid deficiency and visual loss in infant rhesus monkeys. *J. Clin. Invest.*, 73, 272–276.

32) Holman, R. T. (1964): Nutritional and metabolic interrelationships between fatty acids. *Fed. Proc.*, 23, 1062–1067.