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Lipid content and essential fatty acid (EFA) composition of mature Congolese breast milk are influenced by mothers' nutritional status: Impact on infants' EFA supply

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Objective: To measure the lipid content and the fatty acid (FA) composition of breast milk as part of a nutritional survey of the essential fatty acid (EFA) status of 5 months old Congolese infants.
Design: Cross sectional nutrition survey.
Setting: A suburban district of Brazzaville (capital of the Congo).
Subjects: A random sample of nursing mothers and their 5 months old infants ($n = 102$).
Data collection procedures: The mothers were questionned on their socio-economic status, dietary habits, and their body mass index (BMI) was measured. Breast milk samples were collected from each mother. Milk lipid content and fatty acid composition were determined.
Results: Compared with milk from various countries, Congolese women's mature breast milk was low in lipid (28.70 ± 11.33 g/L) but rich in 8:0–14:0 FAs (25.97 ± 8.17% of total FAs) and in polyunsaturated FAs (PUFAs), particularly n-3 PUFAs (2.39 ± 0.68% of total FAs, mainly 18:3 and 22:6). This was associated with the frequent consumption of high-carbohydrate foods (processed cassava roots, wheat bread, doughnuts) known to enhance 8:0–14:0 FA biosynthesis, and with that of foods providing n-6 and n-3 EFAs such as freshwater and saltwater fish, vegetable oil, green leafy vegetables, and high-fat fruit (peanuts, avocado, bushbutter). These foods were traditionally and locally produced. Milk lipid content was negatively related with mothers' BMI ($P < 0.01$) and varied with the frequency of consumption of certain foods corresponding to distinct dietary patterns.
Conclusions: Lipid content and FA composition of Congolese breast milk were dependent on mother's nutritional status. However, despite an adequate EFA composition of breast milk, partially breast-fed 5 months old Congolese infants probably did not get enough n-6 and n-3 EFAs from breast milk to meet their EFA requirements.
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Descriptors: breast milk; lipid content; essential fatty acids; infant nutrition; body mass index; developing countries.

Introduction

During late fetal and early postnatal life, sufficient n-6 and n-3 EFA supplies either from mothers' placenta or breast milk are of the highest importance to ensure the fetus' and infant's optimum growth and development, particularly neurodevelopment (Birch *et al*, 1992; Guesnet & Alessandri 1995) and visual acuity (Crawford, 1993). For that matter, arachidonic (AA) and docosahexaenoic (DHA) acids, which are highly concentrated in the newborn's brain and retinal cell membranes, are crucial in ensuring these functions (Makrides *et al*, 1996; Carlson *et al*, 1996).

Under mothers' optimal nutritional and living conditions, breast milk alone provides enough energy from fat (that is 50–60% of total energy) and all n-6, n-3 parent essential fatty acids (EFAs) as well as derived long-chain polyunsaturated fatty acids (LCPUFAs) to meet the infant's requirements for normal development, at least until 4–6 months of age (FAO/WHO, 1994).

EFA supply in exclusively breast-fed infants depends not only on milk PUFA composition (that is % of total FAs) but also on milk lipid content and intake. In developing countries these amounts can be lower than in affluent countries (WHO, 1985) and infants' EFA intakes may therefore be insufficient. In developing countries, partially breast-fed infants are often given high-carbohydrate, low-fat complementary foods (CF) before 4–6 months of age (WHO, 1993). This causes breast-milk intakes to decrease, thereby aggravating EFA deficiency. To date, very few field studies have focused on the EFA intake and status of young infants in developing countries.

Our study aimed at evaluating the EFA status of population of 5 months old infants in which inappropriate feeding practices are prevalent. It took place in Brazzaville, capital of the Congo, where CF are introduced very early (30% of infants aged less 3 months were receiving gruel) (Tchibindat, 1995). In the Congo, as in other Central African

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countries, traditional CF are mainly starch-based gruels of poor energy density and nutritional value (Trèche, 1995a). Moreover, recent studies (Dop, 1994 and unpublished results) in Brazzaville showed that breast-milk intakes of 4–6 months infants were lower than those usually reported (WHO, 1985). In such poor feeding conditions, the EFA supplies to infants are probably inadequate to meet their requirements (FAO/WHO, 1994).

This paper reports the lipid content and FA composition of breast milk in relation to the nutritional status of mothers nursing 5 months old infants, and their impact on infants' EFA supplies.

Subjects and methods

Characteristics and dietary habits of subjects

Subjects were 102 mothers of 5 months old infants selected by cluster sampling in the population of Talangaï, a suburban district of Brazzaville (capital of the Congo). All measurements and interviews were conducted at the mothers' homes in April 1995. The mothers' weight and height were measured. They were questioned on their socio-economic status and dietary habits using food frequency questionnaires (FFQ). For each of the main foods known to be commonly consumed in urban Congo, each mother was asked to report her frequency of consumption during the week preceding the interview. She had to choose between four frequencies: every day, $\geq$ twice a week, once a week, and seldom or never. Portion sizes were not recorded. Finally, each mother reported foods she avoided eating either during pregnancy or lactation, and reasons why. The questions were asked either in French or in the local language by two Congolese members of our team. It took each mother 30–45 min to answer the FFQ, which was well accepted by all subjects.

Milk sampling and lipid analysis

At the time of sampling, mothers had been nursing for 5 ± 0.5 months. They expressed a small volume of breast milk ($\approx$ 1–5 mL) from each breast into a sterile plastic container. Samples were collected at two consistent times in the day, in the mid morning when lipid content is supposed to be the highest (Ferris & Jensen 1984), and in the mid-afternoon, with a minimum interval of 4 h between collections. The two samples were thoroughly mixed together, and, in the same evening, a 1 mL aliquot of milk was pipetted into a 10 mL screw-capped glass vial containing 2 mL of dichloromethane-methanol (2:1). This procedure limited the lipolytic and oxidative degradation of milk lipids before extraction (Bitman *et al*, 1983). Samples were stored at $+4°C$ until air transportation to France where they were analyzed.

Total lipids were solvent-extracted from breast milk according to Folch *et al* (1957). Extracted lipids were quantified gravimetrically after complete solvent evaporation under nitrogen stream at 40°C. Fatty acid methyl esters (FAME) were obtained by trans-methylation of total lipids with 1 mL of 7% boron-trifluoride in methanol, in a water bath heated at 90°C for 10 min. FAME were extracted twice with 2×1 mL of hexane. Solvent was removed under gentle nitrogen stream. Care was taken not to reach complete dryness when FAME were concentrated, in order to limit losses of volatile short-chain FAs which are normally present in breast milk. FAME were separated and identified by gas-liquid chromatography with a 200 Delsi-Nermag gas chromatograph equipped with a split (50:1) injector, a flame ionization detector, and a DB-23 bonded fused-silica capillary column (30 m $\times$ 0.25 mm I.D. with a 0.25 μm film thickness) (Alltech, USA). This column provided a good separation of *trans* 16:1, 18:1, and 18:2 FAME isomers as well as of all the polyunsaturated FAME in human milk. Helium at a 1–2 mL/min flow rate was the carrier gas. Column temperature was programmed from 140–200°C at 5°C/min to correctly elute all of the short-chain ($\leq$ 10 C) saturated and long-chain ($\geq$ 20 C) polyunsaturated FAME. The injection port was maintained at 230°C, and the detector at 250°C. FAME with 8–24 carbons were identified by comparison of their relative retention times with appropriate commercial standards (Alltech, USA and Spi-Bio, France) chromatographed in the same conditions. These FAME were quantified with an Enica integrator-calculator. FA composition of milk lipids was expressed as a percentage of total FAs (wt/wt). Peaks lower than 0.01% of the total area were omitted.

Data processing and statistical analysis

Data are presented as means $\pm$ s.d. The anthropometric, dietary, and socio-economic data as well as some of the statistical analyses were processed using Epi-Info version 5 (Center for Disease Control, Atlanta, GA, 1990). The SAS System, release 6.09 for Unix (SAS Institute, Cary, NC, 1995) was used for further data management and statistical analyses.

Pearson correlation coefficients were computed to describe relationships between FA variables. A principal component analysis (PCA) was done on this correlation matrix (Lebart *et al*, 1984). The purpose of PCA is to derive a small number of linear combinations (principal components) of a set of variables that retain as much of the original variables as possible. For some of the FA variables simple linear regression analysis was done. Analysis of variance was used to compare milk lipid content and FA composition of mothers grouped according to their frequency of consumption of different foods. Finally, multivariate dietary profiles, including simultaneously the frequency of consumption of various foods, were assessed with two methods: correspondence analysis on the frequencies of consumption, and hierarchical clustering on the principal coordinates. The purpose of cluster analysis is to form groups of subjects (clusters) with similar dietary profiles. The type I error risk was set at 0.05.

Permissions

Permission to conduct the study was obtained from the Congolese Ministry of Research but no specific ethical approval could be obtained because no ethical review committees existed in the Congo. Parents were informed in local languages about the study and gave their oral consent.

Results

Mothers' BMI and living conditions

Table 1 summarizes the anthropometric data of the mothers. The mean body mass index (BMI) was 22.3 ± 4.5. There were 14% of energy deficient women (BMI $<$ 18.5) and 22% were overweight (BMI $>$ 25.0). Compared to mothers' BMI distribution in Brazzaville in 1991 (Cornu *et al*, 1995) there was an increase in the prevalence of low BMIs (11% in 1991, 14% in 1995, $P < 0.01$) but the mean BMI was not significantly affected

Table 1 Anthropometric status of the Congolese mothers

	Mean ± SD (n = 102)	Range Min	Range Max
Age (y)	27.0 ± 6.8	17	46
Weight (kg)	57.8 ± 13.3	40.4	108.0
Height (cm)	160.7 ± 6.0	145	175
BMI[a]	22.3 ± 4.5	15.9	38.7
% BMI < 18.5	13.7		
18.5 ≤ %BMI < 24.9	64.7		
% BMI ≥ 25	21.6		

[a]BMI, body mass index = weight in kg/(height in m)^2.

(23.1 in 1991, 22.3 in 1995). Table 2 shows the general socio-economic status and living conditions of the mothers and their families. Family size was large. Most homes were shared by two families or more. Most mothers had gone to high school but the households' living conditions were precarious due to a chronically deprived economic environment (Cornu *et al*, 1995). The 50% currency devaluation in the Congo in February 1994, and the irregular payment of salaries to most public servants have worsened the situation. Among the heads of households, 35% were public servants and 11% were unemployed; the others worked as vendors or employees in informal small businesses of low income. Among mothers, one out of two was unemployed.

Mothers' dietary habits

Table 3 lists foods currently consumed by the mothers. Diets usually included a limited variety of foods. Processed cassava roots (foufou and chikwangue), vegetable oil, and wheat bread were consumed every day by a majority of mothers. Some fatty foods (bushbutter, dried peanuts, doughnuts, and avocado) and green leafy vegetables were also frequently eaten. Frozen meat (beef, chicken) were consumed several times a week by half of the women. Mothers ate numerous saltwater or freshwater fish species. Only those most frequently consumed were listed in Table 3 (namely horse mackerel, sheat, cod, and sole).

Table 2 Socio-economic status of the mothers and their families (n = 102)

Size of family (= number of household members, mean ± SD)	7.3 ± 4.3
	%
Families sharing home with others (one or more):	63.1
Mother's education	
No education	3.9
Primary school level	14.5
Secondary school level	77.7
Higher level	3.9
Proportion of homes with:	
No electricity	63.1
No water supply inside the house	91.3
No gas burner for cooking	66.0
No refrigerator or freezer	86.4
No private toilets	58.3
Head of household's occupation:	
Public servant	35.1
Informal business (merchant or employee)	47.9
Unemployed	10.6
Other	6.4
Mother's occupation:	
Student	9.7
Public servant	4.9
Merchant	33.0
Unemployed	50.5
Other	1.9

Most of these foods were traditionally and locally produced. Only meat, garlic sausage, salted cod, vegetable oil, margarine, and mayonnaise were imported. In fact, the Talangaï district is not fully urbanized, and imported foods and dietary habits from Western societies have not reached all the population. Moreover, although imported and local foods were available on the markets, prices had increased since the devaluation, and money was scarce. Mothers said they spent less on expensive imported foods and went back to cheaper local and traditional foods. A majority of mothers (57%) avoided consuming fish and/or meat during pregnancy whereas only 16% refrained from eating fish or green leafy vegetables during lactation. The reasons for refusal were mainly physiological during pregnancy (nausea) and taboos or nutrition misknowledge during lactation (fears concerning the infant's health or development) but the latter were few (10% of mothers).

Breast-milk lipid content and FA composition

The total lipid content, and the fatty acid profile of the breast-milk samples are presented in Tables 4 and 5.

The mean lipid content was low (28.70 g/L) but there was a wide range of values (7.90–74.80 g/L). Thirty-six FAs were identified, representing more than 98% of the total FAs of breast-milk lipids. Only 1.25% of total FAs remained unidentified. All FAs showed high coefficients of variation (CV); among the main FAs, the largest CVs were observed for myristic (14:0), α-linolenic (18:3, n-3), and DHA (22:6, n-3) acids. SFAs were the major FAs of breast milk (more than 50%); half of them were medium-chain (MC = 8:0 and 10:0) and intermediary-chain (IC = 12:0 and 14:0) SFAs. Shorter-chain FAs (< 8:0) were barely detected in these samples. There were no 18:1 *trans* isomers. Only small amounts of 16:1 *trans* (0.30%) and 18:2 *trans* (0.05%) isomers were identified. Linoleic (18:2, n-6) and α-linolenic (18:3, n-3) acids were the main PUFAs (13.65% and 1.19% of total FAs respectively). Their respective main metabolites (AA and DHA) were also found and to a lesser extent all the other LCPUFAs ($\geq$ 20 C) of the n-6 and n-3 families. Amounts of LC n-6, and n-3 PUFAs were equivalent (respectively 1.18% and 1.20% of total FAs) although their precursor contents differed widely (18:2, n-6 = 13.65% and 18:3, n-3 = 1.19%). The 18:2, n-6/18:3, n-3 was not high (mean value = 12.24, range 8.00–24.62). The pattern of relationships between FA variables on the first two principal components is shown in Figure 1. This correlation circle gives a projection of the variables on the first two principal components. It is an accurate representation of their respective position in the initial high dimensional space. Groups of well correlated variables (for example 10:0–14:0) can thus be visualized.

Many highly significant ($P < 0.01$) correlations were found within or between FA families. The highest positive correlations were between 12:0 and 14:0 ($r = +0.85$), 18:2, n-6 and 18:3, n-3 ($r = +0.84$), 22:5, n-3 and DHA ($r = +0.84$), 10:0 and 12:0 ($r = +0.80$), and 18:2, n-6 and 20:2, n-6 ($r = +0.63$). The highest negative correlations were between 18:1, n-9 and 14:0 ($r = -0.89$), and 12:0 ($r = -0.85$). On the other hand, there was no correlation between parent EFAs (18:2, n-6, and 18:3, n-3) and their respective main metabolites (AA and DHA), or between ICSFAs and total LC n-6, n-3 PUFAs.

Table 3 Frequency of food consumption by the mothers (% of subjects, $n = 102$)

	Frequency of consumption			
Food	*Every day*	*≥ Twice a week*	*Once a week*	*Seldom or never*
Vegetables				
Foufou and chikwangue[a]	90.2	9.8	—	—
Leafy green vegetables	14.7	80.4	2.9	2.0
Dried peanuts	21.6	46.1	16.7	15.7
Peanut paste	3.9	71.6	13.7	10.8
Fried plantain	3.9	15.7	11.8	68.6
Squash paste	2.0	14.7	17.6	65.7
Cereal products				
Wheat bread	59.8	26.5	3.9	9.8
Doughnuts	11.8	52.9	9.8	25.5
Rice	1.0	33.7	20.8	44.6
Biscuits	8.8	15.7	5.9	69.6
Meat and meat products:				
Frozen beef	3.1	52.5	11.3	33.0
Frozen chicken	1.0	55.7	14.4	28.9
Giblets	—	24.5	32.4	43.1
Garlic sausage	2.0	19.8	11.9	66.3
Fresh meat	—	20.6	33.3	46.1
Game	—	11.8	21.6	66.7
Palmtree worms	2.2	1.1	—	96.7
Fish				
Frozen horse mackerel	—	53.5	22.8	23.7
Smoked sheat	1.0	48.5	11.9	38.6
Fresh sheat	2.9	34.3	14.7	48.0
Salted cod	—	24.5	15.7	56.9
Salted sole	—	18.2	9.1	72.7
Canned sardines in oil	1.0	2.9	9.8	86.3
Fruits				
Bushbutter[b]	37.3	54.9	3.9	3.9
Avocado	7.8	46.1	18.6	27.5
Coconut	—	6.9	19.6	73.5
Palmnut	—	4.9	5.9	89.2
Eggs:	2.9	27.5	18.6	51.0
Fats and oils				
Vegetable oil[c]	66.7	27.5	2.0	3.9
Margarine	23.5	25.5	7.8	43.1
Palm oil	—	23.5	60.8	15.7
Mayonnaise	2.9	14.7	4.9	77.5
Dairy products				
Ice cream	2.9	37.3	11.8	48.0
Yoghurt	1.0	16.7	7.8	74.5
Melted cheese	—	12.7	4.9	82.4
Beverages				
Tea + condensed milk	31.4	39.2	5.9	23.5
Sauces				
Palmnut juice	—	54.9	32.4	12.7

[a]Processed cassava roots.
[b]*Dacryodes edulis*, also named safou or african pear.
[c]Vegetable oil was sold mostly in small unlabeled glass bottles. Mothers said it was peanut oil but FA analysis showed that refined soybean oil was commonly used. This oil came from the United States and appeared in the Congo on the local city markets at the beginning of 1994.

Relationship between mothers' BMI, milk lipid content, and diet

There was a significant negative correlation between BMI and milk lipid content ($r = 0.28$, $P < 0.01$). Underweight mothers (BMI < 18.5) had a higher milk lipid content ($P < 0.01$) than average and overweight mothers (Table 6). Mothers with low BMIs ate saltwater fish, giblet, bushbutter, yoghurt, and cheese less frequently, whereas they ate ice cream, doughnuts, and fried plantain more often than mothers with average or high BMIs. The lipid content of breast milk was not related with mothers' age or socio-economic status.

Influence of diets on breast-milk lipid content and fatty acid composition

Whenever possible, we compared the breast-milk lipid content or FA composition according to mothers' frequency of consumption of each food. Influence of processed cassava roots (foufou and chikwangue), leafy green vegetables, wheat bread, bushbutter, and peanut oil on milk lipid content could not be determined because they were frequently consumed by a large majority of mothers (Table 3). Among other foods whose frequency of weekly consumption differed widely between subjects, frequent consumption (≥ twice a week) of frozen horse-mackerel by 53% of the mothers was related to a lower lipid content of breast milk (25.24 g/L *vs* 32.0 g/L among mothers consuming horse-mackerel once a week or less, $P < 0.01$). On the other hand, consumption of ice cream at least once a week (51% of the mothers) had the opposite effect on milk lipid content (31.64 g/L *vs* 24.88 g/L among mothers eating no ice cream, $P < 0.01$). The multivariate analysis of dietary profiles showed that consumption of horse-mackerel and of ice cream corresponded to distinct dietary

Table 4 Total lipid content (g/L) and fatty acid (FA) composition (wt% of total FAs) of mature breast milk from Congolese women ($n = 102$)

	Mean ± s.d.	*Range* *Min*	*Range* *Max*	*CV*
Total lipid content	28.70 ± 11.33	7.90	74.80	
SFAs[a]				
8:0	0.28 ± 0.07	0.14	0.58	26.7
10:0	1.91 ± 0.44	1.08	3.42	23.0
12:0	10.53 ± 2.91	4.14	18.90	27.6
13:0	0.02 ± 0.03	0.00	0.13	131.0
14:0	13.24 ± 5.26	4.14	29.57	39.7
15:0	0.18 ± 0.06	0.09	0.40	33.7
16:0	21.22 ± 2.56	13.89	30.89	12.1
17:0	0.27 ± 0.08	0.08	0.58	29.9
18:0	4.89 ± 1.08	1.78	8.95	22.2
20:0	0.15 ± 0.07	0.00	0.36	42.2
22:0	0.09 ± 0.06	0.00	0.29	63.0
24:0	0.07 ± 0.06	0.00	0.38	81.0
MUFAs				
14:1, n-5	0.14 ± 0.08	0.00	0.37	54.6
16:1, n-7 t[b]	0.30 ± 0.09	0.00	0.56	31.3
16:1, n-7	1.90 ± 0.74	0.38	4.60	39.0
18:1, n-9	24.76 ± 5.17	12.09	37.76	20.9
18:1, n-7	1.19 ± 0.26	0.65	1.93	21.6
20:1, n-9	0.26 ± 0.09	0.05	0.49	33.6
22:1, n-9	0.07 ± 0.07	0.00	0.49	90.3
24:1, n-9	0.04 ± 0.04	0.00	0.16	80.0
n-6 PUFAs				
18:2, n-6 t	0.05 ± 0.04	0.00	0.18	86.7
18:2, n-6	13.65 ± 3.63	6.84	22.99	26.7
18:3, n-6	0.08 ± 0.05	0.00	0.23	64.7
20:2, n-6	0.26 ± 0.08	0.06	0.44	30.2
20:3, n-6	0.31 ± 0.10	0.06	0.66	33.2
20:4, n-6	0.44 ± 0.09	0.23	0.68	20.8
22:4, n-6	0.10 ± 0.04	0.00	0.25	41.0
22:5, n-6	tr	0.00	tr	—
n-3 PUFAs				
18:3, n-3	1.19 ± 0.46	0.42	2.56	38.8
18:4, n-3	0.05 ± 0.07	0.00	0.33	129.4
20:3, n-3	0.06 ± 0.07	0.00	0.68	120.7
20:4, n-3	0.10 ± 0.08	0.00	0.78	82.0
20:5, n-3	0.17 ± 0.10	0.02	0.51	62.1
22:4, n-3	0.05 ± 0.04	0.00	0.24	95.1
22:5, n-3	0.24 ± 0.11	0.08	0.68	46.4
22:6, n-3	0.55 ± 0.22	0.21	1.28	39.4

[a]SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; CV, coefficient of variation; tr, traces
[b]t, trans double bond.

Table 5 Composition (wt% of total FAs) of FA families and FA ratios ($n = 102$)

	Mean ± s.d.
FA families[a]	
Total SFAs	52.86 ± 7.09
Total MC and ICSFAs	25.97 ± 8.17
Total MUFAs	28.66 ± 5.64
Total n-6 PUFAs	14.88 ± 3.73
Total n-3 PUFAs	2.39 ± 0.68
Total (n-6 + n-3) PUFAs	17.27 ± 4.18
Total LC n-6 PUFAs	1.18 ± 0.25
Total LC n-3 PUFAs	1.20 ± 0.43
Total (n-6 + n-3) PUFAs/SFAs	0.34 ± 0.11
Total unidentified FAs	1.25 ± 0.68
FA ratios	
18:2, n-6/18:3, n-3	12.24 ± 3.25
18:2, n-6/(12:0 + 14:0)	0.66 ± 0.33
20:4, n-6/22:6, n-3	0.88 ± 0.30
Total LC n-6 PUFAs/Total LC n-3 PUFAs	1.08 ± 0.37

[a]SFAs, saturated fatty acids; MC and ICSFAs, medium-chain (8:0–10:0) and intermediary-chain (12:0–14:0) SFAs; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; LCPUFAs, long-chain PUFAs (≥ 20 C).

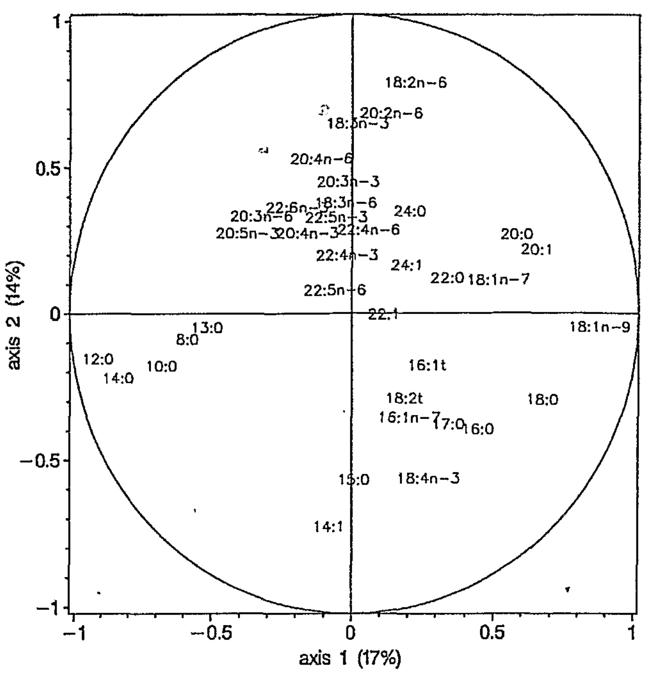

Figure 1 Projections of correlations between FAs on the first two axes of PCA.

Table 6 Breast-milk lipid content (g/L) by category of BMI

BMI category	*Total lipid content*[a]
BMI < 18.5 ($n = 14$)	35.53 ± 15.56
18.5 ≤ BMI < 24.9 ($n = 64$)	28.94 ± 10.45
BMI ≥ 25 ($n = 21$)	21.68 ± 6.27

[a]Mean ± s.d.
Differences between BMI groups were statistically significant ($P < 0.01$).

patterns. Frequent consumption of frozen horse-mackerel was linked with infrequent intakes of peanuts (dried or paste), butter, biscuits, mayonnaise, margarine, eggs, and smoked freshwater fish, whereas that of ice cream was associated with more butter, biscuits, mayonnaise, eggs, frozen chicken, and deep-fried plantain. The former dietary profile would be lower in fat and carbohydrate than the latter.

We also compared % of n-3 PUFAs in breast milk of mothers eating fish (namely frozen horse mackerel, smoked or fresh sheat, salted cod, and salted sole) at least twice a week to that of mothers eating fish less frequently. Only the frequent consumption of frozen horse mackerel was associated with a significant increase in breast-milk DHA (Figure 2).

Discussion

Breast-milk lipid content

The mean lipid content of breast milk of Congolese mothers living in Talangaï, a suburban district of Brazzaville, was notably lower (28.70 g/L) than that usually reported for human milk (35–40 g/L) but the range of values was wide. More precisely 75% of milk samples had a lipid content below reference values. Our observation concurs with that of the WHO collaborative study (WHO, 1985), namely that the lipid content of human milk in developing countries is generally lower than that in affluent populations. However, comparisons from one study to

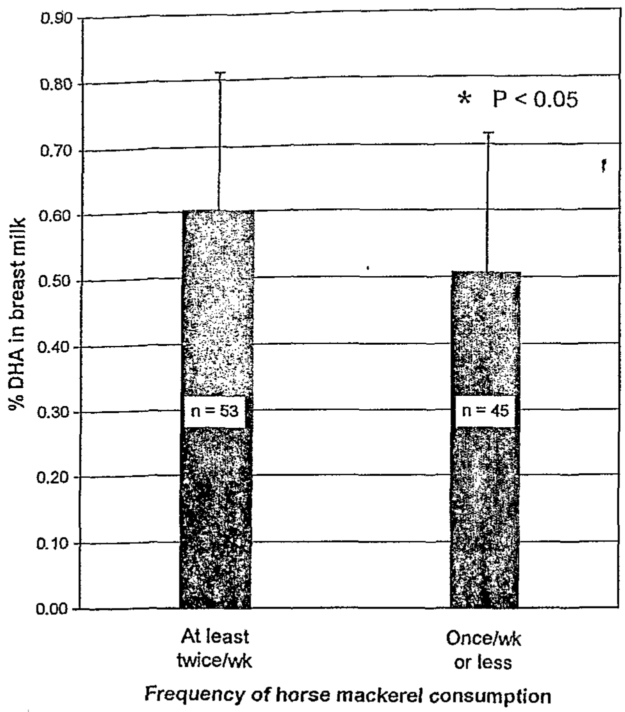

Figure 2 Level of breast-milk DHA by frequency of consumption of horse mackerel.

another are questionable because methods for collecting milk and extracting lipid may affect the lipid content of breast-milk samples. The sampling procedure we used minimized the bias due to known variations in milk lipid content during a single feed, between breasts, between feeds and within the day (Jensen *et al*, 1995; WHO, 1985). Therefore, the wide range in milk lipid content between subjects was probably not due to the sampling procedure but to other factors such as the mothers' diet or health (Jensen *et al*, 1995).

Mothers' diet and breast-milk lipid content

We showed that breast-milk lipid content varied according to dietary profiles. Our data are in contradiction with those of the WHO study (1985) reporting that the lipid content of mature breast milk is little influenced by variations in the mothers' nutritional status or dietary intake. In contrast, our data confirmed those of an experimental trial, not mentioned in the WHO report, showing that high-carbohydrate, low-fat diets lead to higher breast-milk lipid content than do low-carbohydrate, high-fat diets (Harzer *et al*, 1984). Although we did not quantify fat and carbohydrate intakes, we can assume they differed from one dietary profile to another and consequently affected milk lipid content. This issue needs to be further investigated.

Mothers' BMI and breast-milk lipid content

The negative relationship shown between mothers' BMI and milk lipid content was reported for Kenyan women (van Steenbergen *et al*, 1983). These authors and others (Rasmussen, 1992) also found low milk volumes in mothers with a very poor nutritional status. In these women, an increase in milk lipid content appears to have compensated the reduction in their milk volume, but this observation was not confirmed by other investigators (Brown & Dewey, 1992). The negative correlation between the BMI and the milk lipid content could be mediated by the effect of the mothers' intakes of carbohydrates and lipids on milk lipid content. Finally, more information is needed about how external factors (nutritional, seasonal, and socio-economic) and metabolic mechanisms regulate maternal lactational performance in developing countries.

Mothers' diets and breast-milk fatty acids

The mean fatty acid composition of breast milk from Congolese mothers showed similar as well as different features in comparison with those reported for lactating mothers from developing and developed countries. MC and ICSFA represented 25.97% of total FAs, about twice as much as in most affluent countries (Jensen *et al*, 1995; Laryea *et al*, 1995; Guesnet & Alessandri, 1993; de la Presa-Owens *et al*, 1996). High-carbohydrate, low-fat diets commonly consumed in many developing countries are known to enhance endogenous MC and ICSFA biosynthesis in the mammary gland during lactation. This was established as early as 1958 (Insull *et al*, 1958) and largely confirmed since. In the Congo, the mean daily consumption of processed cassava roots (foufou and chickwangue) is ~800 g, that is 35% of mean total energy intake, the second highest value in the world (Trèche, 1995b). Hence it is not surprising that frequent consumption of these high-carbohydrate, low-fat foods led to high amounts of MC and ICSFAs in breast milk. The high concentration of ICSFAs in Congolese breast milk was mainly correlated with a reduction of 18:1, n-9, as illustrated by a highly significant negative correlation between ICSFAs and 18:1, n-9. Our data confirm recent works (Jensen *et al*, 1995; Guesnet *et al*, 1993).

A substantial amount of MC and ICSFAs in breast milk is regarded by many nutritionists as beneficial for the infant because these FAs represent a readily absorbable and rich source of energy (Jensen & Jensen, 1992a). However, some expert committees (Tomarelli, 1988) favor limiting lauric and myristic acids in infant formulas (no more than 15% of total FAs for each of these fatty acids) since these FAs are hypercholesterolemic. In our study, nearly 1 milk sample out of 4 had ICSFA contents higher than these values; their hypercholesterolemic effect on these infants, however, are unknown. ICSFAs are added to infant formulas as medium-chain triacylglycerols mainly from lauric acid oils (namely coconut oil) whose properties (triacylglycerol structure, fatty acid absorption, and metabolic effects, etc.) are perhaps different from those naturally present in human milk (Jensen & Jensen, 1992b). Thus, similar hypercholesterolemic effects might not occur with high ICSFA breast-milk intakes, but further research is needed on this subject.

No 18:1 *trans* was detected in Congolese breast milk, and it must be regarded as beneficial to the infant's nutrition (FAO/WHO, 1994). Presence of *trans* FA isomers in breast milk generally reflects mothers' intakes of hydrogenated fats and oils (mainly margarines) or of ruminant fats. *Trans* isomers are often detected in milk of women consuming Western-type diets which comprise more processed foods or butter fat (Boatella *et al*, 1993; Jensen *et al*, 1995; Chardigny *et al*, 1995; Chen *et al*, 1995). In Brazzaville, margarine was the main if not the only source of *trans* FA isomers but mothers consumed little of it. It was costly and most mothers bought it in nearby shops in small quantities for spreading on bread for breakfast. Consequently, the very low FA isomer intakes did not show up in breast-milk fatty acids. The detection of small amounts of 16:1 *trans* and 18:2 *trans* in some milks cannot be explained.

Congolese breast milk was particularly rich in 18:3, n-3 (1.19% of total FAs). This probably reflects the frequent consumption of soybean oil, green leafy vegetables, and to some extent, of processed cassava roots. The last two, although low in fat, are a significant source of 18:3, n-3 (unpublished results). Other commonly consumed foods (avocado, bushbutter, peanut oil, or dried peanuts) were high in fat and 18:2, n-6, thus contributing to the enrichment of breast milk in this EFA (13.65% of total FAs). Also because of a noticeable quantity of 18:3, n-3 in breast milk, the mean 18:2, n-6/18:3, n-3 ratio ($\approx$12/1) was low. Indeed, it was within, or very close to values presently recommended for optimum infant nutrition (FAO/WHO, 1994).

The total amounts of LC n-6 and n-3 PUFAs in the breast milk of Congolese mothers were similar to or even higher than that of most milk samples from either developed or developing countries (Guesnet *et al*, 1993; Jensen *et al*, 1995; Laryea *et al*, 1995; Makrides *et al*, 1995; de la Presa-Owens *et al*, 1996). Total LC n-3 PUFAs averaged 1.20% of total FAs and reached values observed in Asian populations eating large amounts of sea food (Kneebone *et al*, 1985; Chulei *et al*, 1995) or in nursing mothers experimentally fed n-3 PUFA-enriched diets (Henderson *et al*, 1992; Cherian & Sim, 1996). Congolese mothers frequently ate freshwater or saltwater fish, which are good sources of the LC n-3 PUFAs incorporated in elevated amounts in their breast milk. Consumption of horse mackerel in particular (7.5% of 20:5, n-3, and 13.7% of DHA, unpublished result), was shown to be associated with higher breast-milk DHA when it was eaten by mothers at least twice per week. Our data are in agreement with others from developing countries where usual diets contain fish (Innis, 1992; Jensen *et al*, 1995; Chulei *et al*, 1995). Moreover, the noticeable amount of 18:3, n-3 in the mothers' diets is likely to compete favourably with 18:2, n-6 for the same enzyme system involved in LCPUFAs biosynthesis and, consequently, to further increase the LC n-3 PUFA concentration in breast milk. Finally, the ratio LC n-6 PUFAs/LC n-3 PUFAs was near 1 instead of 2 in most other studies. Because the newborn's ability to biosynthesize LCPUFAs (AA and DHA in particular) from parent precursors (18:2, n-6 and 18:3, n-3) is limited, it is probably beneficial for the Congolese infant to get enough preformed LCPUFAs from breast milk (Koletzko *et al*, 1996).

Five months old infants' EFA requirements and breast-milk supplies

Will such a milk meet all the EFA requirements of a 5 months old Congolese infant? The minimum daily 18:2, n-6 supply needed to prevent clinical symptoms for example skin lesions) in young infants is estimated (Tomarelli, 1988) at 72 mg/100 kJ/d (namely 300 mg/100 kcal or $\approx$3% of total energy), that is $\approx$1.7 g/d in 4–5 months old infants whose energy requirements in kJ/d/kg are 364 KJ (87 kcal) for boys and 368 kJ (88 kcal) for girls (Butte, 1996). Given the 18:2, n-6 concentration (mg/100 mL) found in the milk samples, intakes of breast milk needed to meet the requirement vary between 138 mL–1848 mL. Two recent studies (Dop, 1994 and unpublished results) conducted by our research unit among 4–6 months old infants living in two districts of Brazzaville, one of them socio-economically comparable and bordering Talangaï, showed average breast-milk intakes to be between 429 g/d and 475 g/d. Assuming the infants' milk intakes in Talangaï are near these values (for example $\approx$450 g or mL/d), 55% of infants cannot fulfill their minimum 18:2, n-6 need. If we consider the 18:3, n-3 minimum requirement (that is 0.5% of total energy, that is a dietary intake of $\approx$320 mg/d) and make the same assumption we find that 94% of infants did not receive enough 18:3, n-3 from breast milk to meet the requirement. FAO/WHO recommendations for term infants (FAO/WHO, 1994) are much higher than minimum requirements and the same calculations as above show that only 5% of infants received enough EFA to meet the recommendations. The other infants must obtain these EFAs from complementary foods (CF).

Unfortunately, CF in Brazzaville were mainly high-carbohydrate, low-fat gruels (that is made from fermented corn paste) in which sugar and sometimes small amounts of condensed milk and/or peanut paste were added. Our analyses of the lipid content and FA composition (unpublished results) showed these CF were either poor in 18:2, n-6 and LCPUFAs (condensed milk), or poor in 18:3, n-3 and completely devoid of LCPUFAs (peanut paste, fermented corn paste). Current intakes of CF in the Congo (Tchinbindat, 1995) could probably compensate for the 18:2, n-6 deficit in milk but not for the 18:3, n-3 and LCPUFA deficit.

Conclusions

The mean FA composition of breast milk of Congolese mothers, particularly the EFA composition, was noticeable because of traditional dietary habits (for example frequent consumption of high-carbohydrate foods, leafy green vegetables, fish, and high-fat fruit) which lead to breast-milk enrichment in ICFAs and PUFAs (particularly n-3 PUFAs). From this point of view the current Congolese diet was more adequate than most Western diets. Since the EFA content of traditional Congolese CF is lower than that of breast milk, mothers should be advised to delay the introduction of CF until their infant reaches 4–6 months. They should be encouraged to use local foods that are good sources of EFAs to complement the infant's diet from the age of 6 months.

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