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Fatty acid composition of mature human milk in Nigeria

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Summary: The fatty acid composition of mature human milk from 10 rural Nigerian women was analyzed by high-resolution capillary gas-liquid chromatography and compared to previously determined results on mature human milk from 15 German mothers. Human milk of the Nigerian group contains significantly higher proportions of saturated fatty acids (median 54.07 vs. 42.76 % wt/wt). The difference is primarily caused by high values for lauric (C12:0, 8.34 %) and myristic acids (C14:0, 9.57 %), but not of medium chain fatty acids (C8:0, C10:0), presumably due to increased de novo fatty acid synthesis in the African women consuming a high carbohydrate and low-fat diet. Markedly lower values of oleic and total cis-monounsaturated (22.82 vs. 37.98 %) as well as trans-isomeric fatty acids (1.20 vs. 4.40 %) in Nigerian milk appear to result from low dietary intakes of animal and partially hydrogenated fats, respectively. Although percentage contribution of linoleic acid (18:2n-6) is similar, arachidonic acid (C20:4n-6) and total n-6 long-chain polyunsaturates with 20 and 22 carbons (n-6 LCP) are higher in the African samples. N-6 LCP secretion with human milk lipids is not correlated to the precursor linoleic acid and seems not to depend on maternal dietary intake of preformed dietary LCP with animal fats. N-3 LCP are very high in milk of the Nigerian women who obtain a large portion of dietary lipids from sea fish, but even then docosahexaenoic (C22:6n-3) and not eicosapentaenoic (C20:5n-3) is the predominant n-3 LCP in milk. We conclude that, in addition to dietary effects, metabolic processes regulate the milk content of n-6 and n-3 LCP. We speculate that such metabolic regulation may protect the breastfed infant by providing a relatively constant supply of the physiologically important LCP.

Zusammenfassung: Die Fettsäuren in reifer Muttermilch von 10 Frauen aus einer ländlichen Region Nigerias wurden mit hochauflösender Kapillar-Gaschromatographie untersucht und mit früher erhobenen Ergebnissen aus der Milch von 15 deutschen Frauen verglichen. Die Frauenmilch in Nigeria enthält signifikant höhere Anteile an gesättigten Fettsäuren (Median 54,07 vs. 42,76 Gew.-%). Dieser Unterschied entsteht vorwiegend durch hohe Anteile an Laurin- (C 12:0, 8,34 %) und Myristinsäure (C14:0, 9,57 %), aber nicht an mittelkettigen Fettsäuren (C8:0, C10:0), wahrscheinlich als Folge einer vermehrten De-novo-Fettsäuresynthese bei den afrikanischen Frauen mit einer kohlenhydratreichen und fettarmen Ernährung. Wesentlich niedrigere Anteile der Ölsäure und der Summe an Monoenfettsäuren (22,82 vs. 37,98 %) sowie der trans-isomeren Fettsäuren (1,20 vs. 4,40 %) in nigerianischer Frauenmilch dürften aus der niedrigen Nahrungszufuhr an tierischen bzw. partiell gehärteten Fetten resultieren. Obwohl sich in beiden Gruppen

Abkürzungen/Abbreviation index: LCP = Long-chain polyunsaturated fatty acids with 20 and 22 carbon atoms and 2–6 double bonds

ähnliche Gehalte an Linolsäure finden, zeigen die afrikanischen Milchproben höhere Werte für Arachidonsäure und die Summe der n-6-langkettigen Polyenfettsäuren mit 20 und 22 Kohlenstoffatomen (LCP). Der n-6-LCP-Gehalt der Frauenmilch korreliert nicht mit dem Präkursor Linolsäure und scheint nicht von der mütterlichen Nahrungsaufnahme an präformierten LCP aus tierischen Fetten abhängig zu sein. Sehr hohe Werte ergeben sich für n-3-LCP in der Milch der nigerianischen Frauen, bei denen ein relativ großer Anteil der Nahrungsfette durch Seefisch beigetragen wird. Dabei bleibt aber Docosahexaensäure die quantitativ wichtigste n-3-LCP-Fettsäure in der Milch und wird nicht von Eicosapentaensäure verdrängt. Wir folgern, daß der LCP-Gehalt der Frauenmilch nicht allein von der Zusammensetzung der mütterlichen Ernährung abhängt, sondern zusätzlich durch metabolische Prozesse reguliert wird. Wir spekulieren, daß eine solche metabolische Regulation einen Schutzmechanismus für das gestillte Kind darstellen könnte, durch den die kindliche Nahrungszufuhr der physiologisch wichtigen LCP relativ konstant gehalten wird.

Key words: Infant nutrition; breastfeeding; lactation; lipids; fatty acids

Schlüsselwörter: Säuglingsernährung, Stillen, Laktation, Lipide, Fettsäuren

Introduction

The quality of dietary lipids is of great importance for growth and development of the human infant. Fat is the major source of energy for the infant, providing 45–55 % of calories in human milk (12). Moreover, the composition of dietary lipids modulates the structure of biological membranes in growing tissues and, thereby, membrane functions such as fluidity, permeability, and activity of membrane-bound enzymes and receptors. Essential fatty acids of both the n-6 and n-3 series are also required as precursors for the synthesis of various prostaglandins and other eicosanoids with important regulatory functions. Results of recent studies in premature infants demonstrate that the quality of perinatal lipid supply is correlated to the functional development of membrane-rich tissues such as the brain and retina (1, 19, 33) and to early human growth (22).

The optimal amounts and relative ratios of different fatty acids and fatty acid families in infantile diets which are associated with the best outcome cannot be determined with certainty at present. However, human milk is recommended as the preferred diet for healthy infants born at term, and full breastfeeding is considered to meet human nutrient requirements during the first 6 postnatal months. Hence, the composition of human milk as the biological model of postnatal nutrient supply has been used to develop recommendations on lipid content and composition of infant formulas (4), and to evaluate enteral and parenteral feeding regimens (17, 20). The question has been raised whether the fatty acid composition of human milk may serve as a model for artificial infant nutrition, because it is quite variable (3). Dietary changes in lactating women may alter the fatty acid pattern of human-milk lipids, which was first demonstrated by Thiemich in 1899 (31), and later confirmed in many other studies (reviewed in (12, 12a)). Other factors associated with dietary habits such as geographic and ethnic background of lactating women and their cultural

traditions and socioeconomic status may also affect milk lipid composition (12, 16). Since the variation of human milk fatty acids in different parts of the world and its limitations by possible regulatory mechanisms are of interest, we studied the fatty acid composition of mature human milk of rural Nigerian women consuming a largely traditional diet.

Material and methods

Samples of human milk were donated by 10 apparently healthy and well-nourished women from the village of Udo, Bendel State, Nigeria. All subjects consumed an ad libitum diet and were fully breastfeeding one infant for a duration of 3 to 5 months. In this part of Nigeria, the traditional diet in rural populations is low in animal and total fat, high in carbohydrates and fiber. Fresh and dried sea fish is readily available due to the proximity of the ocean and contribute a relatively large portion of dietary fat.

The participating mothers manually expressed a small amount of milk into a clean plastic container after breastfeeding their baby. One ml of milk was then aspirated with a syringe and injected through a teflon coated rubber septum into a dark glass vial prefilled with 5 ml chloroform/methanol (1:1, vol/vol) containig 1 mg/L butylated hydroxytoluene as an antioxidant, 1 ml of heptadecanoic acid in chloroform as an internal standard, and nitrogen gas to replace oxygen, as we have previously described for collection of blood plasma under tropical conditions (14, 21). The vials were transported within a few hours without cooling to the University of Benin, where they were kept frozen until shipped to Düsseldorf by air for analysis. Completion of lipid extraction, preparation of fatty acid methyl esters and high-resolution gas-liquid chromatography were performed as reported before (16), but using a Hewlett-Packard 5890 A gaschromatograph with automated cool on column injection (Hewlett-Packard, Böblingen, FRG).

Results are expressed as weight percentage (% wt/wt) of all fatty acids measured, because in contrast to absolute concentrations the percentage values of major and minor human milk fatty acids are unaffected by changing milk lipid contents (6, 13) and can be reliably determined from random samples, as well as from expressions obtained with milk pumps. Since results for some fatty acids appeared to have a skewed distribution, we choose to present the results as median values and ranges. The results of the Nigerian milk samples were compared with the composition of mature human milk in Germany previously studied (16) by a Mann-Whitney test with Minitab release 7.2 for DOS microcomputers (28), which was also used for linear regression analysis of selected variables.

Results

Human milk of the Nigerian women contained a large portion of saturated fatty acids with significantly higher values than in the German samples (Table 1). The difference in total saturates was almost entirely due to increased proportions of intermediate chain fatty acids (C12:0, C14:0). In contrast, medium chain fatty acids (C8:0, C10:0) were not different. The proportions of lauric (C12:0) and myristic (C14:0) acids in Nigerian milk lipids were significantly correlated ($R = 0.81$, $p = 0.004$), but there were no correlations between any of the intermediate and medium-chain fatty acids. Cis-monounsaturated fatty acids contributed lower proportions in Nigerian than in German samples, primarily due to small proportions of oleic acids, whereas larger values were found in Nigerian samples for the

Table. Comparison of fatty acid composition of mature human milk from 10 Nigerian and 15 German* women. Data represent median values and ranges, % wt/wt. n.d. = not detected. (*Data on German milk samples taken from Koletzko et al. 1988.)

	Nigeria	Germany	p-value
Saturated fatty acids:			
C6:0	0.01 (0.00–0.05)	n.d.	
C8:0	n.d.	n.d.	
C10:0	0.54 (0.00–1.14)	0.71 (0.23–1.14)	
C11:0	0.06 (0.03–0.23)	0.00 (0.00–0.04)	< 0.0001
C12:0	8.34 (1.05–11.87)	4.41 (2.29–7.78)	= 0.006
C13:0	0.15 (0.03–0.94)	0.05 (0.04–0.08)	= 0.01
C14:0	9.57 (4.38–21.90)	6.73 (4.40–10.50)	= 0.0099
C14:0iso	0.00 (0.00–0.25)	n.d.	
C15:0	0.54 (0.16–2.34)	0.46 (0.32–0.74)	
C15:0aiso	0.04 (0.00–1.18)	n.d.	
C16:0	23.35 (16.09–30.42)	21.83 (15.74–24.68)	
C16:0iso	0.00 (0.00–5.08)	n.d.	
C17:0aiso	0.44 (0.20–1.41)	n.d.	
C18:0	10.15 (6.86–14.76)	8.15 (5.29–10.33)	= 0.038
C19:0aiso	0.09 (0.06–0.44)	n.d.	
C20:0	0.42 (0.26–0.58)	0.22 (0.17–0.32)	= 0.0001
C20:0iso	0.00 (0.00–0.23)	n.d.	
C22:0	0.41 (0.19–0.50)	0.09 (0.06–0.21)	= 0.0001
C24:0	0.39 (0.17–0.58)	0.00 (0.00–0.06)	< 0.0001
Total saturated	54.07 (38.42–71.74)	42.76 (34.69–53.46)	= 0.001
Cis-monounsaturated fatty acids:			
C14:1n–5	0.08 (0.05–2.40)	0.29 (0.20–0.49)	= 0.001
C15:1n–5	0.05 (0.00–2.28)	n.d.	
C16:1n–7	0.91 (0.64–2.19)	2.68 (2.38–3.90)	< 0.0001
C17:1n–7	0.13 (0.00–1.58)	0.32 (0.26–0.45)	= 0.0009
C18:1n–9	18.52 (9.44–25.30)	34.31 (29.82–39.69)	< 0.0001
C18:1n–7	0.95 (0.77–2.32)	(above value includes n–9+n–7)	
C20:1n–9	0.34 (0.12–0.69)	0.52 (0.34–0.99)	= 0.0099
C22:1n–9	0.75 (0.12–2.06	0.08 (0.04–0.11)	< 0.0001
C24:1n–9	0.59 (0.31–1.25)	0.00 (0.00–0.04)	< 0.0001
Total cis-monounsat.	22.82 (14.76–29.30)	37.98 (34.69–53.46)	< 0.0001
Trans-isomeric fatty acids:			
C14:1t	0.04 (0.03–1.04)	0.19 (0.12–0.33)	= 0.0009
C16:1t	0.27 (0.08–3.91)	0.46 (0.32–1.15)	= 0.001
C18:1t	0.86 (0.52–4.94)	3.12 (1.47–4.38)	= 0.0017
C18:2tt	0.12 (0.06–0.39)	0.14 (0.09–0.25)	
Total trans	1.20 (0.79–10.29)	4.40 (2.17–6.04)	= 0.0012
N–6 PUFA:			
C18:2n–6	11.06 (5.40–13.78)	10.76 (5.58–21.65)	
C18:3n–6	0.12 (0.01–0.35)	0.16 (0.00–0.27)	
C20:2n–6	0.26 (0.19–2.04)	0.34 (0.28–0.48)	= 0.018
C20:3n–6	0.49 (0.39–0.98)	0.26 (0.19–0.38)	< 0.0001

Table (contd.)

	Nigeria	Germany	p-value
N-6 PUFA:			
C20:4n–6	0.82 (0.38–1.48)	0.36 (0.30–0.54)	= 0.0001
C22:2n–6	0.14 (0.05–0.91)	0.11 (0.00–0.28)	
C22:4n–6	0.09 (0.05–0.16)	0.08 (0.00–0.16)	
C22:5n–6	0.09 (0.05–0.59)	0.00 (0.00–0.07)	< 0.0001
Total n–6 LCP	2.01 (1.37–5.08)	1.14 (0.84–1.73)	= 0.0001
Total n–6 PUFA	12.52 (7.47–16.64)	12.26 (6.45–22.76)	
N–3 PUFA			
C18:3n–3	1.41 (0.64–5.45)	0.82 (0.51–1.12)	= 0.006
C18:4n–3	0.09 (0.04–0.32)	n.d.	
C20:3n–3	0.27 (0.05–1.09)	0.06 (0.00–0.10)	
C20:4n–3	0.14 (0.00–0.28)	n.d.	
C20:5n–3	0.48 (0.17–1.57)	0.04 (0.00–0.16)	< 0.0001
C22:3n–3	0.21 (0.03–2.02)	n.d.	
C22:5n–3	0.39 (0.12–0.72)	0.17 (0.11–0.26)	= 0.0017
C22:6n–3	0.93 (0.70–2.16)	0.22 (0.15–0.60)	= 0.003
Total n–3 LCP	2.88 (1.45–4.50)	0.51 (0.31–1.14)	< 0.0001
Total n–3 PUFA	4.63 (2.16–7.97)	1.38 (0.84–1.73)	< 0.0001
Total n–6+n3-LCP	5.26 (2.82–8.22)	1.66 (1.18–2.69)	< 0.0001
Total n–6+n–3 PUFA	17.88 (11.02–21.53)	13.82 (7.33–14.84)	= 0.018
Other:			
C20:3n–9	0.43 (0.22–0.72)	0.04 (0.03–0.05)	< 0.0001
Ratios:			
Ratio 18:2n–6/18:3n–3	9.33 (1.16–17.18)	11.48 (9.36–29.66)	= 0.025
Ratio 20:4n–6/22:6n–3	0.72 (0.44–3.90)	1.64 (0.65–2.22)	= 0.0087
Ratio n–6 LCP/n–3 LCP	0.80 (0.44–1.62)	2.30 (1.36–3.17)	= 0.0001
Ratio Polyunsat./Satur.	0.34 (0.15–0.45)	0.31 (0.14–0.65)	
Ratio Monounsat./Satur.	0.44 (0.21–0.59)	0.90 (0.64–1.30)	< 0.0001

long-chain monounsaturates erucic (C22:1n-9) and nervonic (C24:1n-9) acids which are required for postnatal myelination of the central nervous system. Trans isomeric fatty acids were extremely low in the milk of all Nigerian women, except for one outlying sample with very high results. Median total trans content in Nigeria was only about one-quarter of the one found in Germany.

Linoleic acid (C18:2n-6) values did not differ between the two groups, but the Nigerian milk contained significantly higher proportions of its long-chain polyunsaturated metabolites (n-6 LCP) such as arachidonic acid (C20:4n-6). In the n-3 series, alpha-linolenic acid (C18:3n-3) was only slightly higher in Nigeria, but n-3 LCP such as docosahexaenoic acid (C22:6n-3) reached very high values and total n-3 LCP percentage was almost six times larger than in Germany. Similar to our previous findings in human milk of German women, there was no correlation between milk content of the parent essential fatty acids' linoleic or alpha-linolenic acids and their respective LCP products.

Discussion

Traditional diets in many parts of Africa comprise high intakes of complex carbohydrates and fiber, but they are low in animal protein and in total and animal fat. Thus, there are some similarities to the composition of diets advocated in industrialized countries for prevention of heart disease and for other health reasons, and also to vegetarian diets. The information available on the effect of such diets in lactating women on the lipid composition of human milk is limited. Some studies on fatty acid composition in Africa reported only data on a restricted number of major fatty acids which were generated by gas-liquid chromatography with packed analytical columns (2, 24, 26). Packed columns tend to separate fewer fatty acids and be less accurate in quantitation than capillary columns (8, 15, 21). Analysis with capillary columns, today the method of choice, was used in only two previous studies on African milks (25, 34).

In agreement with our results, most other publications on human milk of rural African mothers (2, 24, 25, 34) also reported large contents of saturated fatty acids exceeding 50 % of total fatty acids, except for one study in The Gambia (26). These high values are primarily caused by large amounts of intermediate chain fatty acids (C12:0, C14:0) that usually contribute more than 20 % of total milk fat in Africa (2, 24, 25, 34). A high milk content of lauric acid may be advantageous, especially under tropical conditions, because it is not only well-absorbed, but it also has antiinfective properties (32a). Dietary composition rather than hereditary factors appear responsible for the high proportions of lauric and myristic acids found. Van der Westhuizen et al. (34) found significantly greater human milk contents of intermediate chain and total saturated fatty acids in rural South African women, who consumed a traditional diet, than in an urban population with a partly westernized diet. In vitro studies on lactating human breast epithelial cells demonstrated de novo synthesis of fatty acids, primarily lauric and myristic acids, from radiolabeled acetate (32), and in vivo investigation of lactating women given experimental diets high in carbohydrates showed a resulting increase of lauric and myristic acids, but not of medium- (C8:0, C10:0) and long- ($\geq$C16:0) chain fatty acids (11, 12).

In contrast to saturates, cis-monounsaturated fatty acids tend to be lower in African than in the European milk samples and usually do not exceed 30 % (2, 24, 25, 34), which may be explained by a lower maternal dietary intake of monounsaturates from animal fats in Africa. A large supply of trans-isomers of unsaturated fatty acids to infants during the perinatal period may have untoward effects, such as disturbance of essential fatty acid metabolism and inhibition of growth (4, 21, 23). Human milk content of trans-isomers of unsaturated fatty acids appears to reflect primarily the maternal dietary intake from partially hydrogenated fats and ruminant fats, which tends to be small in rural African populations (15, 23); this explains the rather low values found in Nigerian human milk. It appears that trans fatty acid content of human milk in industrialized countries may be reduced by respective dietary changes of lactating women.

Median values for the classical essential fatty acids' linoleic (C18:2n-6) and alpha-linolenic (C18:3n-3) acids are rather similar in Nigerian and German milk samples. The extremely low linoleic acid content of 1 % reported in human milk in Tanzania by an older paper (27) has not been confirmed in the present or any other study on African human milk, and it might have resulted from limitations of the methodology used at that time. In agreement with our previous findings in human milk of German women (16), we did not find any correlation between the parent essential fatty acids and their LCP metabolites in the Nigerian samples. Obviously, human milk contents of precursor and product essential fatty acids are differentially regulated. The metabolites of linoleic acid, arachidonic acid and other n-6 LCP, tend to be high in Nigerian milks, similar to relatively high contents reported for the milk of rural South African women (34). These findings are in accordance with observations in vegetarian women consuming diets low in total and animal fat, whose breastmilk has similar or slightly higher n-6 LCP contents than that of omnivorous women (5, 29, 30). It seems that n-6 LCP secretion with human milk does not depend on maternal dietary intake of preformed LCP with animal fats, but that n-6 LCP in milk can be derived from endogenous stores or from metabolically regulated desaturation and chain elongation of the precursors in the maternal organism.

The relatively large proportions of n-3 LCP that we found in the milk of Nigerian women correspond to high n-3 LCP values in plasma lipids of Nigerian infants from the same geographical region (14) and may be explained by the high habitual intake of saltwater fish in the Bendel state. Intervention studies in lactating women with oral administration of encapsulated fish oil in very large doses documented a consecutive increase of milk n-3 LCP (7). The major LCP in marine lipids is eicosapentaenoic acid (C20:5n-3), whereas docosahexaenoic acid (C22:6n-3) usually contributes only smaller amounts. Nonetheless, the predominant n-3 LCP in human milk, both in the Nigerian women and in Canadian Inuits with a high fish consumption (10) is still docosahexaenoic acid, which is not replaced by eicosapentaenoic acid. It appears that, in addition to dietary effects, there is metabolic regulation of both n-6 and n-3 LCP content in human milk. It is tempting to speculate that such regulatory processes may represent a protective mechanism for the infant, providing a relatively constant dietary supply of LCP which is not directly dependent on dietary composition of the lactating mother, because LCP may have a greater physiological importance for babies than the precursor fatty acids (9, 18).

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