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Fatty acids in formulae for term infants: compliance of present recommendations with the actual human milk fatty acid composition of geographically different populations

EN Smit[1], IA Martini[2], RFJ Kemperman[3], A Schaafsma[4], FAJ Muskiet[3] and ER Boersma[1]

Department of Obstetrics/Pediatrics, Perinatal Nutrition and Development Unit[1], Laboratory Center[2], Pathology and Laboratory Medicine[3], Groningen University Hospital, and Research & Development, Friesland Nutrition, Leeuwarden[4], The Netherlands

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Aim: Recommendations for formula fatty acids (FA) are largely based on the mature human milk FA composition. This study aimed to investigate whether current recommendations for formula FA for term infants comply with the actual breast-milk FA composition of geographically distinct populations and to provide more realistic grounds for future recommendations. *Methods:* 455 mature breast-milk samples were collected in different countries over 25 y. Recommendations of different organizations were projected on their FA data. FA interrelationships were calculated with Spearman's rank tests. FA compositions of 30 formulae were compared with those of breast milk. *Results:* Many samples from non-Western communities did not meet the recommendations for formula 12:0, 14:0 and 18:2ω6, since these are mainly based on breast milk of mothers living in Western countries. Recommendations for 18:3ω3, 18:2ω6/18:3ω3, 20:4ω6 and 22:6ω3 were not met by many milk samples, which may point to the poorly developed recommendations for long-chain polyunsaturated FA. Most of the investigated breast-milk FA (12:0, 14:0, 16:0, 18:0, 18:3ω3, 22:6ω3, 18:2ω6, 20:4ω6, 18:1ω9) were either positively or negatively interrelated. Many formulae had FA compositions that were not consistent with the physiological interrelationships of FA in breast milk. *Conclusion:* Future recommendations, if based on human milk, should derive from its FA balance, as indicated by the FA interrelationships. A "humanized" formula FA composition would in this sense be any composition that cannot be distinguished from that of breast milk by techniques such as principal component analysis.

Key words: *Breast milk, fatty acids, infant formula, long-chain polyunsaturated fatty acids, recommendations*

EN Smit, c/o FAJ Muskiet, Pathology and Laboratory Medicine, CMC-V, Y1.147, Groningen University Hospital, PO Box 30.001, NL-9700 RB Groningen, The Netherlands (Tel. +31 50 3612 733/3612 203, fax. +31 50 3612 290, e-mail. ella.smit@undp.org or f.a.j.muskiet@lab.azg.nl)

Human milk is the single best food for babies and young infants (1). Its superiority relates to many factors, including its specific fatty acid (FA) composition. The FA composition of human milk is, however, strongly dependent on maternal diet and to a small extent affected by other factors such as time postpartum, gestational age, parity and certain diseases (2). Together these, and probably other, factors give rise to high inter-individual biological variation (3). Most national and international authorities have based their recommendations for the manufacturing of infant formulae on mature human milk FA composition as the gold standard (4). Possibly because of a lack of additional information, these "standards" are mostly based on milk obtained from mothers with Caucasian ethnicity, consuming typically Western diets. For instance, based on human milk data derived from Sweden, Germany and the UK, the ESPGHAN recommends a formula linoleic acid/α-linolenic acid (18:2ω6/18:3ω3) ratio around 10 g g^{-1}, with a range of 5–15 (5). Yet there are several reasons why not only milk from Western women should be taken into consideration. First, at present there is no scientific evidence that the composition of breast milk obtained from Caucasian women is superior to that of other ethnic populations. On the contrary, motor development of breastfed black children from African descent is often precocious compared with Caucasian breastfed children raised in Western countries (6). Secondly, there is growing concern regarding the tremendous changes in the intakes of total fat, ω6 and ω3 FA and *trans*-FA over the past 100–150 y in Western countries (7), which may each have influenced the present Western human milk FA composition (2). In addition, as a result of increased migration, many non-

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Caucasian ethnic populations (with their typical dietary patterns) have settled in the USA and Europe, and many Europeans and North Americans have included food from Asian, African and South American countries in their diets.

Next to human milk, recommendations may also be based on estimated requirements or proposed potential beneficial effects of supplementation of certain FA. The minimal requirement of 18:2ω6 is estimated at 1% of energy, which together with a safety margin has resulted in a recommendation of a minimum of 11 g% 18:2ω6. A maximum intake to prevent untoward effects was set by ESPGHAN at 20 g% (5). Another expert panel provided a wider margin for 18:2ω6 with a minimum of 8 g% and a maximum of 35 g% of total FA (8). The latter recommendations were based on the large range in human milk samples and the observation that no adverse effects have been reported of formulae with LA beyond 35% as used in the past. There is good evidence from randomized controlled trials that enrichment of formula with long-chain polyunsaturated FA (LCP), notably 22:6ω3, improves early cognitive and visual development, especially in preterm and possibly also in term newborn infants (reviewed in Refs 9–11). Although these effects seem to be transient, they have been the basis for recommendations by several authorities to add 22:6ω3 and 20:4ω6 to infant formula for preterm and also term infants (12–15). Others were more hesitant to include LCP in formula for term children and advised addition of LCP, but not beyond 1 and 2 g% for 22:6ω3 and 20:4ω6, respectively (5, 16, 17). The American Life Sciences Research Office (LSRO) did not recommend addition of 22:6ω3 and 20:4ω6 to infant formulae for term newborns in 1998, but wished to reassess their point of view within 5 y, when the results of more randomized controlled trials will be available (8).

This study investigated whether current recommendations for term infant formula FA composition comply with that of human milk obtained from different populations living in a wide range of industrialized and non-industrialized countries. For this purpose, these recommendations were projected on a human milk FA dataset that has been compiled over the past 25 y. To provide more realistic grounds for future recommendations based on FA balance the interrelationships between various breast-milk FA were also investigated. The study subsequently investigated whether the FA composition of a number of term formulae complied with some of these relationships.

Materials and Methods

Milk samples

A total of 455 mature breast-milk samples was collected over 25 y. The countries and places from which these samples derived were: the Netherlands [$n = 222$: 99 collected on postnatal days 14.4 ± 3.5, 98 on day 42.1 ± 2.7 and 25 on day 89.2 ± 5.6 (mean $\pm$ SD)], Antigua ($n = 23$), Belize ($n = 10$), Curaçao ($n = 47$), Dominica ($n = 17$), St. Lucia ($n = 12$), St. Vincent ($n = 30$), Surinam ($n = 20$), Jerusalem ($n = 63$) and Tanzania ($n = 11$). The samples from Antigua, Belize, Curaçao, Dominica, St. Lucia, St. Vincent and Surinam ($n = 159$) will collectively be referred to as deriving from the "Caribbean region". Data on the milk FA compositions in the Netherlands (18), Tanzania and the Caribbean region (19) and Jerusalem (20) have been published before. The Dutch women were of Caucasian origin and those from the Caribbean region were of African origin with eating habits varying between Western and traditional diets. The Jerusalem women were of Middle-Eastern origin (Palestinians) and those from Tanzania of African origin. The latter two groups consumed local traditional diets.

The original data were calculated in mol%, but were recalculated to g% for the present study. Milk from Dutch mothers derived from 24 h samples that were collected by vacuum pump. Ten percent was kept for analyses and the remainder was administered to the baby by bottle or preferably by cup. The milk samples from all other women were midstream samples of about 5 ml that were collected by manual expression. The milk samples were immediately placed in a refrigerator and stored at −20°C. Samples from outside the Netherlands were transported to the Netherlands in dry ice. All FA compositions of the milk samples were analysed by capillary gas chromatography with split injection and flame-ionization detection in the same laboratory (21).

Recommendations

To standardize current recommendations and to enable their comparison with human milk FA data the recommendations were recalculated, if necessary, into g% of total FA. The following recommendations for the formula FA composition were investigated (see Table 1):

- EU Commission directive, 1991 (22): $12{:}0 \leq 15$ g% and $14{:}0 \leq 15$ g%, $18{:}2\omega6 \geq 9$ g% but ≤ 19 g%, as calculated from a minimum fat content of 3.3 and a maximum of 6.5 g 100 kcal^{-1} and a 300–1200 mg 18:2ω6 intake from 100 kcal;
- EU Commission directive (amendment), 1996 (17): LCPω3 ≤ 1 g% and LCPω6 ≤ 2 g%, $20{:}5\omega3 \leq 22{:}6\omega3$;
- ESPGHAN, 1991 (5): $18{:}2\omega6 \geq 11.4$ g% but ≤ 20 g%, as calculated from a minimum fat content of 4.4 and maximum of 6.0 g 100 kcal^{-1} and a 500–1200 mg 18:2ω6 intake from 100 kcal. LCPω3 ≤ 1 g% and LCPω6 ≤ 2 g%, $18{:}2\omega6/18{:}3\omega3 \geq 5$ g g^{-1} but ≤ 15 g g^{-1};
- FAO/WHO, 1994 (13): $18{:}2\omega6 \geq 11.4$ g%, $18{:}3\omega3$

Table 1. Prevalence (%) of human milk samples with fatty acid compositions beyond recommendations.

Commission	Recommendation	Netherlands ($n = 222$)	Caribbean[a] ($n = 159$)	Jerusalem ($n = 63$)	Tanzania ($n = 11$)	All ($n = 455$)
CD91	12:0 $\leq$ 15 g%	0.0	20.8	0.0	54.5	8.6
CD91	14:0 $\leq$ 15 g%	0.0	17.0	1.6	36.4	7.0
LSRO	18:2ω6 $\geq$ 8 g%	2.3	10.7	0.0	18.2	5.3
CD91	18:2ω6 $\geq$ 9 g%	8.1	16.4	0.0	18.2	10.1
GR, WS	18:2ω6 $\geq$ 10 g%	15.3	24.5	0.0	27.3	16.7
FAO, ESP	18:2ω6 $\geq$ 11 g%	24.3	35.8	0.0	27.3	25.1
CD91	18:2ω6 $\leq$ 19 g%	12.2	8.2	44.4	9.1	15.2
ESP	18:2ω6 $\leq$ 20 g%	8.6	4.4	41.3	9.1	11.6
LSRO	18:2ω6 $\leq$ 35 g%	0.0	0.0	0.0	0.0	0.0
FAO	18:3ω3 $\geq$ 1.0 g%	36.9	80.4	49.2	72.7	51.7
GR	18:3ω3 $\geq$ 1.4 g%	80.2	96.4	76.2	81.8	84.1
SI, WS	18:3ω3 $\geq$ 1.5 g%	85.6	96.4	87.3	90.9	89.0
LSRO	18:3ω3 $\geq$ 1.75 g%	93.2	98.2	95.2	90.9	94.9
LSRO	18:3ω3 $\leq$ 4 g%	0.0	0.0	0.0	0.0	0.0
SI, ESP	18:2ω6/18:3ω3 $\geq$ 5 g g^{-1}	0.5	0.0	0.0	0.0	0.2
LSRO	18:2ω6/18:3ω3 $\geq$ 6 g g^{-1}	0.9	0.0	0.0	0.0	0.5
SI, ESP	18:2ω6/18:3ω3 $\leq$ 15 g g^{-1}	25.2	45.5	73.0	36.4	38.5
LSRO	18:2ω6/18:3ω3 $\leq$ 16 g g^{-1}	19.4	33.9	58.7	27.3	29.9
CHF	20:4ω6 $\geq$ 0.35 g%	14.0	2.5	1.6	0.0	7.9
WS	20:4ω6 $\geq$ 0.5 g%	80.6	21.4	23.8	27.3	50.8
GR	20:4ω6 $\geq$ 0.6 g%	96.8	49.1	63.5	54.5	74.5
FAO	20:4ω6 $\geq$ 0.8 g%	100.0	89.9	95.2	90.9	95.6
SI	20:4ω6 $\leq$ 1 g%	0.0	1.9	0.0	0.0	0.7
CHF	22:6ω3 $\geq$ 0.2 g%	29.7	5.0	44.4	27.3	23.1
WS	22:6ω3 $\geq$ 0.35 g%	86.9	32.1	95.2	72.7	68.6
FAO, GR	22:6ω3 $\geq$ 0.4 g%	92.3	43.4	96.8	81.8	75.6
WS	20:5ω3 $<$ 0.10 g%	8.6	11.3	6.3	18.2	11.4
CD96, SI	20:5ω3 $<$ 22:6ω3	0.0	0.0	0.0	0.0	0.0
SI, ESP, CD96	LCPω6 $\leq$ 2 g%	0.0	4.4	1.6	0.0	1.8
SI, ESP, CD96	LCPω3 $\leq$ 1 g%	1.8	13.8	0.0	0.0	5.7

[a] 18:3ω3 was not investigated in 47 of the 159 Caribbean samples.

CD91: Commission Directive 1991 (22); LSRO: Life Science Research Office (8); GR: Health Council of the Netherlands (Gezondheidsraad) (14); WS: Workshop Statement (12); FAO: Food and Agriculture Organization of the United Nations (13); ESP: ESPGHAN (5); SI: Statutory Instruments (16); CHF: Child Health Foundation (15); CD96: Commission Directive Amendment 1996 (17).

$\geq$ 1.0 g%, 20:4ω6 $\geq$ 0.8 g%, 22:6ω3 $\geq$ 0.4 g%, as calculated from 35 g fat l^{-1}, an intake of 150 ml kg^{-1} body weight and 600 mg 18:2ω6, 50 mg 18:3ω3, 40 mg 20:4ω6 and 20 mg 22:6ω3 kg^{-1} body weight;

- UK Statutory Instruments (amendment), 1997 (16): 18:3ω3 $\geq$ 1.5 g%, as calculated from a minimum fat content of 3.3 g 100 kcal^{-1} (taken from ref. 22) and the recommendation of 50 mg 100 kcal^{-1}; 20:4ω6 $\leq$ 1 g%, LCPω3 $\leq$ 1 g% and LCPω6 $\leq$ 2 g%, 20:5ω3 $\leq$ 22:6ω3, 18:2ω6/18:3ω3 $\geq$ 5 g g^{-1} but $\leq$15 g g^{-1};
- LSRO, 1998 (8): 18:2ω6 $\geq$ 8 g% but $\leq$ 35 g%, 18:3ω3 $\geq$ 1.75 g% but $\leq$ 4 g%. With a fat content between 4.4–6.4 g 100 kcal^{-1} this corresponds to 350–2240 mg 100 kcal^{-1}18:2ω6, 77–256 mg 100 kcal^{-1} 18:3ω3; 18:2ω6/18:3ω3 $\geq$ 6 g g^{-1} but $\leq$16 g g^{-1};
- Workshop Statement, 2000 (12): 18:2ω6 $\geq$ 10.0 g%, 18:3ω3 $\geq$ 1.50 g%, 20:4ω6 $\geq$ 0.50 g%, 22:6ω3 $\geq$ 0.35, 20:5ω3 $<$ 0.10 g%;
- Health Council of the Netherlands (Gezondheidsraad), 2001 (14): 18:2ω6 $\geq$ 10 g%, 18:3ω3 $\geq$ 1.4 g%, 20:4ω6 $\geq$ 0.6 g%, 22:6ω3 $\geq$ 0.4 g%, as calculated from an estimated FA intake of 600 mg 18:2ω6, 80 mg 18:3ω3, 40 mg 20:4ω6 and 20 mg 22:6ω3 kg^{-1} body weight;
- Child Health Foundation, 2001 (15): 20:4ω6 $\geq$ 0.35 g%, 22:6ω3 $\geq$ 0.2 g%.

Fatty acid composition of commercially available formulae

The FA compositions of 30 formulae for term infants were used for comparison with current recommendations and the FA compositions of human milk. Ten of these formulae were previously investigated (18). To allow comparison, their data were converted from mol% to g%.

Data evaluation

Recommendations regarding infant formula contents of 12:0, 14:0, 18:2ω6, 18:3ω3, 20:4ω6, 20:5ω3, 22:6ω3, LCPω6 and LCPω3, and the 18:2ω6/18:3ω3 and 20:5ω3/22:6ω3 ratios were investigated. The numbers of human milk samples with contents beyond recommended amounts were calculated. The outcome was

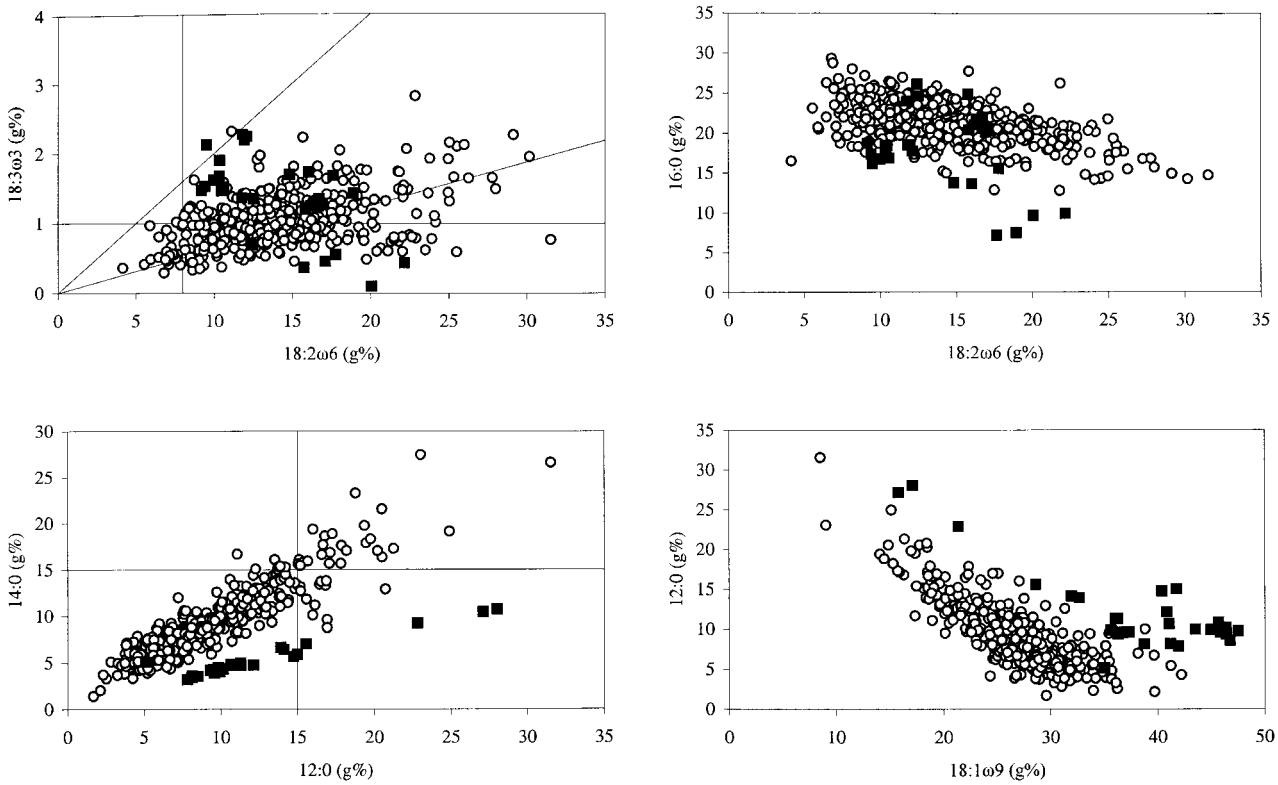

Fig. 1. Relations between selected fatty acids in human milk and formula. ○: human milk ($n = 455$); ■: formula ($n = 30$). Recommendations for the 18:2ω6 lower limit vary between 8 and 11 g%, and for the upper limit between 19 and 35 g%. The 18:3ω3 lower limit recommendation varies between 1.0 and 1.75 g% and the upper limit is 4 g%. The lowest and the highest limits for the 182ω6/18:3ω3 ratio are 5 g g^{-1} and 16 g g^{-1}, respectively. Recommendations for 12:0 and 14:0 are $\leq$15 g%. For abbreviations and detailed recommendations see Table 1.

expressed as a percentage of all samples within a region, and as percentages of all samples. FA interrelationships were calculated with Spearman rank tests, using SPSS 8.0 for Windows (SPSS, Chicago, IL, USA).

Results

Prevalence of human milk samples with fatty acid contents beyond formula recommendations

Table 1 shows the percentages of human milk samples with FA contents beyond those recommended for formula. The magnitudes of the deviations for 12:0, 14:0, 18:2ω6, 18:3ω3 and 18:2ω6/18:3ω3 may be derived from Fig. 1. It was found that 12:0 and 14:0 exceeded the $\leq$15 g% recommendation in about 21 and 17% of the milk samples from the Caribbean and in about 55 and 36% of those from Tanzania, respectively. The lowest prevalence of 12:0 $>$ 15 g% in the Caribbean amounted to 9% in Curaçao and the highest to 41% for Dominica (data not shown). The milk 18:2ω6 content was below 8 g% in only 2% of the Dutch human milk samples, but in about 11 and 18% of the samples from the Caribbean and Tanzania, respectively. The prevalence of 18:2ω6 $<$ 8 g% in the Caribbean region ranged from 0% in Curaçao to 50% in St. Lucia (not shown). Virtually none of the human milk samples met the 18:3ω3 $\geq$ 1.75 g% criterion of the LSRO and 30% of all investigated human milk samples had 18:2ω6/18:3ω3 $>$ 16 g g^{-1}. Only three samples (0.7%) contained more than 1 g% 20:4ω6, while only 4% met the 20:4ω6 $\geq$ 0.8 g% criterion of the FAO. Around 24% reached the highest recommendation for 22:6ω3 (i.e. $\geq$ 0.4 g%), while 77% met the 22:6ω3 $\geq$ 0.2 g% criterion. All human milk samples had 20:5ω3/22:6ω3 ratios below 1, but 11% had 20:5ω3 $\geq$ 0.10 g%. Around 2 and 6% of the milk samples exceeded the criteria of LCPω6 $\leq$ 2 g% and LCPω3 $\leq$ 1 g%, respectively.

Milk fatty acid interrelationships

Correlation coefficients for the association between selected milk FA of the whole dataset are shown in Table 2. These FA were chosen because of either their quantitative or presumed qualitative importance in breast milk. Correlations between all 28 analysed FA are available on request. The strongest positive correlation was observed between 12:0 and 14:0 ($r = 0.896$, $p = 0.0001$), whereas the strongest negative correlation was observed between 12:0 and 18:1ω9 ($r = -0.779$,

Table 2. Correlations between selected fatty acids in breast milk.

Fatty acid		12:0	14:0	16:0	18:0	18:3ω3	22:6ω3	18:2ω6	20:4ω6
14:0	*r*	0.896							
	p	0.0001							
16:0	*r*	−0.410	−0.159						
	p	0.0001	0.001						
18:0	*r*	−0.544	−0.364	0.558					
	p	0.0001	0.0001	0.0001					
18:3ω3	*r*	−0.403	−0.394	−0.097	0.244				
	p	0.0001	0.0001	0.050	0.0001				
22:6ω3	*r*	0.364	0.356	0.018	−0.190	−0.205			
	p	0.0001	0.0001	0.698	0.0001	0.0001			
18:2ω6	*r*	−0.329	−0.507	−0.494	−0.149	0.433	−0.288		
	p	0.0001	0.0001	0.0001	0.001	0.0001	0.0001		
20:4ω6	*r*	0.227	0.107	−0.242	−0.412	−0.229	0.486	0.155	
	p	0.0001	0.023	0.0001	0.0001	0.0001	0.0001	0.001	
18:1ω9	*r*	−0.779	−0.756	0.219	0.348	0.212	−0.407	0.143	−0.216
	p	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.002	0.0001

Spearman's correlation coefficient for 455 mature human milk samples from the Netherlands ($n = 222$), the Caribbean region ($n = 159$), Jerusalem ($n = 63$) and Tanzania ($n = 11$).

$p = 0.0001$). The two FA that did not exhibit interrelationships were 16:0 and 22:6ω3 ($r = 0.018$, $p = 0.698$).

Figure 1 shows the relationship between 18:2ω6 and 18:3ω3, 12:0 and 14:0, 18:2ω6 and 16:0, and 18:1ω9 and 12:0. The data derive from all investigated human milk samples. The FA contents of 30 infant formulae are also shown. Although only 4 of the 30 investigated infant formulae had 12:0 beyond the recommendations, only one proved in agreement with the genuine physiological relationship between human milk 12:0 and 14:0. For the relationships between 18:2ω6/18:3ω3, 18:2ω6/16:0 and 18:1ω9/12:0, it was observed that many formulae had compositions that were not in line with human milk when considered from a two-dimensional point of view.

Discussion

This study investigated whether current recommendations for formula FA composition comply with the FA composition of a large dataset of human milk samples derived from geographically distinct populations. The aim of the study was not to disqualify human milk on the basis of its FA composition, nor to disqualify current recommendations, but rather to point out discrepancies and their possible causes. By emphasizing the interrelationships between human milk FA, a more integrated approach is proposed that may be of use for future recommendations for formula FA composition.

The data showed that, in particular, breast milk from women living in non-Western communities did not meet the recommendations for 12:0, 14:0 and 18:2ω6 (Table 1). This is not surprising in view of the fact that current recommendations for formula FA composition are mainly based on milk from Caucasian mothers who predominantly consumed typically Western diets. The discrepancies are probably caused by the higher intakes of carbohydrates of women eating non-Western diets, which are known to increase mammary gland *de novo* synthesis of 12:0 and 14:0 (23). These dietary habits are apparently associated with lower milk 18:2ω6 contents, as illustrated by the inverse relations between 12:0 and 14:0 on the one hand and 18:2ω6 on the other (Table 2).

Another discrepancy between recommendations and actual milk FA composition regards the 18:3ω3 content. A high percentage of human milk samples did not reach the $18{:}3\omega3 \geq 1.75$ g% and $18{:}2\omega6/18{:}3\omega3 \leq 15$ g g^{-1} criteria, and even the less stringent recommendations of $18{:}3\omega3 \geq 1.0$ g% and $18{:}2\omega6/18{:}3\omega3 \leq 16$ g g^{-1} were met by no more than 48 and 70% of all investigated samples, respectively. A high 18:3ω3 content and a low 18:2ω6/18:3ω3 ratio stimulates, to a certain extent, the synthesis of LCPω3 in newborns (24). In contrast to many formulae, human milk contains LCPω3 and breastfed babies have consequently better LCPω3 status than babies receiving formula without LCPω3 (25, 26). This implies that, from the point of view of balance, there may be a need in the future to define some relationships between 18:2ω6, 18:3ω3 and their long-chain metabolites, notably for those formulae containing each of these FA.

The most striking discrepancies between recommendations and actual breast-milk FA composition were observed for 20:4ω6 and 22:6ω3. These are caused by the wide differences in present recommendations. The LSRO does not advise the addition of 20:4ω6 and 22:6ω3 (8), whereas the FAO, the International Workshop on the essentiality of and recommended dietary intakes for ω6 and ω3 FA, and the Health Council of the Netherlands recommend rather high amounts (12–14). For 20:4ω6, the upper limit of 1 g% as set by the UK Statutory Instrument (16) is close to the lower limit of

0.8 g% issued by the FAO (13). The FAO criteria for 20:4ω6 (i.e. $\geq$ 0.8 g%) and 22:6ω3 ($\geq$ 0.4 g%) were met by only 4 and 24% of all milk samples, respectively. The observation that fish intake increases milk 22:6ω3 (27, 28) is, for instance, reflected in the samples of Caribbean women. In particular, women from the island of Dominica had high milk 22:6ω3 (up to 2.1 g%), which by far exceeded the UK Statutory Instrument recommendation of LCPω3 $\leq$ 1 g%. By contrast, about 10% of the Dutch and Palestinian women had milk 22:6ω3 as low as 0.1 g%, probably reflecting low fish intake by these women. In contrast to 22:6ω3, milk 20:4ω6 seems almost unaffected by either short- or long-term dietary changes (20, 28, 29). Both the lowest and the highest milk 20:4ω6 (0.3 and 1.1 g%, respectively) were found in the Caribbean women (data not shown). Recently the Child Health Foundation (15) advised amounts of $\geq$ 0.35 g% for 20:4ω6 and $\geq$ 0.2 g% for 22:6ω3, which are around half the amounts recommended by the FAO and the International Workshop (12, 13). It seems that there is little agreement at present on the recommended 20:4ω6 and 22:6ω3 contents. As suggested previously, future recommendations could be based on the human milk balance of parent essential FA and their metabolites, or on randomized controlled trials that show benefits of the investigated compositions, e.g. on the growth and neurodevelopment of formula-fed infants (4, 11).

The breast-milk FA composition differs between various populations and these differences are probably mainly due to different diets. To the authors' knowledge there are no reports that ascribe interindividual variation in the human milk FA composition to ethnic background. Therefore, interrelationships between human milk FA could be used as the basis for future recommendations, since they reflect the naturally occurring physiological balance. Table 2 shows that almost all of the qualitatively and quantitatively most important FA were either positively or negatively related. This is partially caused by a closure effect (i.e. all FA add up to 100%). It may, however, be proposed that a humanized formula FA composition would be any composition that can not be distinguished from that of human milk on the basis of this FA balance. Examples of non-balanced FA compositions in this sense are shown in Fig. 1, which shows that many formulae complied with recommendations in a univariate sense, but not so when studied in a two-dimensional model. Proof of balance would become more complicated if all current 28 milk FA were to be taken into account simultaneously. The solution might be the construction of a multivariate model in which the relations between all FA are studied simultaneously by means of principal component analysis, for example. However, even if the FA composition of formula resembled that of human milk, many other factors would remain unaccounted for. Examples are the FA (stereo) chemical sn-locations and combinations on the glycerol moieties of either phospholipids or triglycerides, the specific packaging of the fat within the milk fat globules, the complex interaction with other components in human milk, and the immunological and psychological aspects of breastfeeding (1, 4, 30, 31).

In conclusion, this study has shown that the human milk FA composition of different populations is often beyond FA recommendations for infant formula. These discrepancies are mainly caused by FA recommendations being based on breast milk of mothers living in Western countries (i.e. 12:0, 14:0 and 18:2ω6). Furthermore, current recommendations compensate for the lack of 22:6ω3 by increasing the recommended amounts of 18:3ω3. Finally, recommendations for LCP are poorly developed because of a lack of solid long-term evidence. In view of the encountered strong human milk FA interrelationships, it is proposed that future recommendations should also derive from this physiological balance, which reflects the outcome of mammary gland *de novo* synthesis, transport, metabolism, competition and many other complex, mostly genetically determined, biochemical processes. A humanized formula FA composition would, in that sense, be any composition that cannot be distinguished from that of human milk by techniques such as principal component analysis.

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