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Infant intake of fatty acids from human milk over the first year of lactation

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Despite the importance of human milk fatty acids for infant growth and development, there are few reports describing infant intakes of individual fatty acids. We have measured volume, fat content and fatty acid composition of milk from each breast at each feed over a 24 h period to determine the mean daily amounts of each fatty acid delivered to the infant from breast milk at 1, 2, 4, 6, 9 and 12 months of lactation in five women. Daily (24 h) milk production was 336·60 (SEM 26·21) and 414·49 (SEM 28·39) ml and milk fat content was 36·06 (SEM 1·37) and 34·97 (SEM 1·50) g/l for left and right breasts respectively over the course of the first year of lactation. Fatty acid composition varied over the course of the day (mean CV 14·3 (SD 7·7) %), but did not follow a circadian rhythm. The proportions (g/100 g total fatty acids) of fatty acids differed significantly between mothers ($P < 0·05$) and over the first year of lactation ($P < 0·05$). However, amounts (g) of most fatty acids delivered to the infant over 24 h did not differ during the first year of lactation and only the amounts of 18 : 3*n*-3, 22 : 5n-3 and 22 : 6n-3 delivered differed between mothers ($P < 0·05$). Mean amounts of 18 : 2*n*-6, 18 : 3*n*-3, 20 : 4*n*-6 and 22 : 6*n*-3 delivered to the infant per 24 h over the first year of lactation were 2·380 (SD 0·980), 0·194 (SD 0·074), 0·093 (SD 0·031) and 0·049 (SD 0·021) g respectively. These results suggest that variation in proportions of fatty acids may not translate to variation in the amount delivered and that milk production and fat content need to be considered.

Fatty acids: Infant nutrition: Human milk lipids: Sampling protocols: Breast-feeding

It is now recognised that fat in human milk contributes significantly to the growth and development of the infant. Whilst fatty acids (FA) provide approximately 50 % of the infant's energy requirements (Jensen *et al.* 1995), the long-chain polyunsaturated FA have been the focus of intense investigation for their roles in neonatal neural and visual development (Gibson *et al.* 1996; Innis *et al.* 2001) and immunological development (Isaacs *et al.* 1986). Despite the importance of these FA, Gibson & Makrides (2000) noted that there is currently a lack of knowledge regarding infant FA intake, but concluded that we may need to rely on the composition of breast milk from well-nourished mothers as a guide to dietary recommendations for infants.

However, the determination of infant FA intake is not straightforward. Fat in human milk is variable, changing in content over the course of a feed (Hytten, 1954), during the day (Daly *et al.* 1993; Hartmann *et al.* 2000) and over the course of lactation (Butte *et al.* 1984; Mitoulas *et al.* 2002). Furthermore, the FA composition itself can vary over the course of the day (Hall, 1979; Daly *et al.* 1993) and throughout the first year of lactation (Idota *et al.* 1991; Luukkainen *et al.* 1994; Makrides *et al.* 1995; Huisman *et al.* 1996), as well as from country to country (Read *et al.* 1965; Kneebone *et al.* 1985; Innis & Kuhnlein, 1988). It is, therefore, imperative that all levels of variation are taken into account in order to accurately determine FA composition and ultimately the FA intake of the breast-fed infant.

We have used a sampling protocol similar to that of Hartmann *et al.* (1986): this takes into account changes in fat content of milk during a feed, differences between breasts and changes over the course of the day, and ensures minimum interference with infant feeding behaviour. Using this technique, we have determined the volume of milk removed by the infant, together with the fat content and FA composition of milk from each breast at each feed over a 24 h period at 1, 2, 4, 6, 9 and 12 months of

Abbreviation: FA, fatty acid.

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lactation for five breast-feeding women. These results were used to determine mean 24 h content of fat in milk and amounts of twenty-nine individual FA consumed by the infant from breast milk over 24 h periods from 1 to 12 months after birth.

Methods

Subjects

Healthy mothers and infants were recruited through the Australian Breastfeeding Association, Western Australian Branch, or from private healthcare centres. All mothers supplied written, informed consent to participate in the study, which was approved by the Human Research Ethics Committee, The University of Western Australia. Details of the subjects have been described previously (Cox *et al.* 1999; Kent *et al.* 1999). Briefly, all infants were fully breast-fed on demand for at least 4 months, with complementary solid foods being introduced between 4 and 6 months of age. All mothers maintained their own breast-feeding patterns throughout study periods and were consuming their own *ad libitum* diets. All study periods were within 1 week of the indicated month of lactation.

Milk sampling

Milk samples ($\leq$ 1 ml) were collected before and after each feed from each breast over a 24–28 h period by either manual breast pump (Kaneson Expression and Feeding Bottle, Yanasem Waitch K.K., Osaka, Japan) or hand expression into 5 ml polypropylene vials (Disposable Products Pty Ltd., Adelaide, South Australia, Australia). Samples were initially stored in a household freezer for a maximum of 24 h and then transported on ice to the laboratory, where they were stored at -20°C until analysis.

Determination of 24 h milk production

Milk yield was determined for each breast by test weighing the mother, accounting for insensible water loss as described by Arthur *et al.* (1987). Test weighing was carried out at each mother's home over the same 24–28 h period that milk samples were collected using an electronic Sauter balance (weighing platform, model EC 240; evaluator unit with data output printer, model ED 3300; FSEM Scientific, Perth, Western Australia, Australia). Briefly, mothers weighed themselves before and after each feed from each breast. To account for the insensible water loss incurring during feeding, mothers were instructed to reweigh themselves 20 min after the end of each feeding session. The rate of water loss for this 20 min period was then used to calculate insensible water loss during the feeding period. Final corrected 24 h milk yield was calculated from the data collected over the 24–28 h period using the method described by Arthur *et al.* (1987).

Biochemical analyses

Milk fat. The fat content of the fore- and hindmilk samples was determined using the modified method of Stern & Shapiro (1953) as described by Cox *et al.* (1996). Briefly, 2·5 μl milk samples (warmed to 37°C) and standards (0–200 mM-triolein) were added to redistilled ethanol (600 μl) and mixed for 10 s. Hydroxylamine hydrochloride (2 M, 100 μl) and NaOH (3·5 M, 100 μl) were then added to each sample and the samples mixed and left to stand at room temperature for 20 min. Each sample was acidified by the addition of HCl (4 M, 100 μl) and colour production achieved by the addition of a $FeCl_3$–TCA solution (7·5 g TCA in 10 ml 0·37 M-$FeCl_3$–0·1 M-HCl, 100 μl). The tubes were mixed and 250 μl was pipetted into duplicate wells on a ninety-six-well microtitre plate. The absorbance of each well was determined at 540 nm using a plate spectrophotometer. The detection limit of this assay was 0·45 (SEM 0·41) g/l (*n* 13) and the interassay CV was 8·1 % (*n* 13).

Fatty acid composition. FA were extracted with chloroform–methanol (2 : 1, v/v) using butylated hydroxyanisole as an antioxidant, according to the modified method of Folch *et al.* (1957; Gibson & Kneebone, 1981). FA methyl esters from milk lipid extracts were prepared by acid transmethylation using H_2SO_4 (10 ml/l in methanol) according to a modified method of Christie (1973). FA methyl esters were separated using a GC (Shimadzu GC-14A, Shimadzu Corporation, Kyoto, Japan) equipped with a 50 m capillary column (0·32 mm internal diameter) coated with BPX-70 (0·25 μm film thickness; SGE Pty Ltd, Ringwood, Victoria, Australia) using a method modified from that of Makrides *et al.* (1995). Each sample (3 μl) was injected onto the column using an automatic injector (Shimadzu AOC-14; Shimadzu Corporation) at a split ratio of 30 : 1. The injector temperature was set at 250°C and the detector (flame ionisation) temperature at 300°C. The initial oven temperature was 140°C and was programmed to rise to 230°C at 5°C/min. The carrier gas was He at a velocity of 4 ml/min. FA were identified based on retention time to authentic lipid standards (Nu-Chek-Prep Inc., Elysian, MN, USA).

The CV of the proportions of 12 : 0, 16 : 0, 18 : 1*n*-9, 18 : 2*n*-6 and 22 : 6*n*-3 over five separate extractions were 6·30, 0·82, 1·08, 0·98 and 3·10 % respectively. The CV of the proportions of 12 : 0, 16 : 0, 18 : 1*n*-9, 18 : 2*n*-6 and 22 : 6*n*-3 over five separate GC injections were 3·49, 0·30, 0·49, 0·60 and 1·90 % respectively. The recovery of 17 : 0 (added as triheptadecanoic acid) to milk prior to extraction with respect to the addition of 15 : 0 (added as a methyl ester after transesterification) was 101·4 (SEM 2·26) % (*n* 6).

Determination of 24 h fatty acid intake

For each feed from each breast, the fat contents of fore- and hindmilk samples were averaged to provide the fat content for each feed. The volume of the feed was then used to determine the amount of fat consumed by the infant at each feed. This amount, together with the FA composition of that feed, as determined by GC, was then used to determine the amount of individual FA consumed by the infant. The sum of the amounts of each FA provided

Table 1. Changes in the volume of milk produced, the fat content and the amount of fat delivered to the infant in 24 h for left and right breasts over the course of the first year of lactation*

(Mean values with their standard errors)

	Breast	1 month			2 months			4 months			6 months			9 months			12 months			1–12 months			Statistical significance of effect: *P*
		Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	
Volume (ml/24 h)	L	337·61	51·73	5	363·69	52·19	5	429·66	50·58	5	373·40	74·75	5	312·02	65·34	5	203·18	69·09	5	336·60	26·21	30	NS
	R	474·52	68·83	5	426·65	42·03	5	481·72	58·14	5	436·80	55·69	5	365·33	94·42	5	301·92	84·87	5	414·49	28·39	30	NS
Fat (g/l)	L	37·71	1·51	5	30·74	2·23	5	32·02	3·33	5	32·90	2·50	5	42·93	2·21	5	40·03	4·82	5	36·06	1·37	30	0·0041
	R	38·32	2·63	5	29·51	2·85	5	29·01	2·57	5	32·77	2·47	5	38·37	3·32	5	41·80	5·00	5	34·97	1·50	30	0·0075
Fat (g/24 h)	L	12·5	1·53	5	10·74	0·55	5	13·34	1·20	5	12·14	2·31	5	13·57	3·18	5	10·19	3·57	5	12·08	0·89	30	NS
	R	17·73	2·43	5	12·16	0·69	5	13·48	0·82	5	14·22	1·75	5	13·70	3·40	5	10·51	2·25	5	13·63	0·88	30	NS

L, left; R, right.
* For details of subjects and procedures, see p. 980.

by all feeds over the study period (24–28 h) and the total volume of milk consumed by the infant over the study period were then used to determine an average concentration (g/l). This concentration and the corrected 24 h volume (Arthur *et al.* 1987) were then used to determine the amount consumed in 24 h by the infant.

Statistical analysis

All longitudinal analyses were performed using the SAS System for Windows, version 6.12 (SAS Institute Inc., Cary, NC, USA) with the general linear means (PROC GLM) procedure. The GLM longitudinal analyses for the composition of FA were weighted for the number of observations for each woman, at each time point. Student's paired *t* tests and other statistics were performed using Statview™ SEM + Graphics (Abacus Concepts Inc., Berkeley, CA, USA). All values are reported as means with their standard errors unless otherwise stated. Differences with *P* values $<0{\cdot}05$ were considered significant.

Results

Breast-feeding characteristics

The mean age and parity of the participating mothers was 33·00 (SEM 1·30) years and 2·60 (SEM 0·24) children respectively. Mean daily milk production for all mothers over the course of the year (five mothers, months 1, 2, 4, 6, 9 and 12, *n* 30) was 336·60 (SEM 26·21) ml for the left breast and 414·49 (SEM 28·39) ml for the right breast and did not differ over the course of the year (Table 1). The mean daily fat content for all mothers over the course of the year was 36·06 (SEM 1·37) g/l for the left breast and 34·97 (SEM 1·50) g/l for the right breast and differed over the course of the year ($P<0{\cdot}05$). This corresponded to the baby receiving 12·08 (SEM 0·89) g fat per 24 h from the left breast and 13·63 (SEM 0·88) g fat per 24 h from the right breast.

Fatty acid composition

There was no significant difference in the proportional composition of FA in fore- and hindmilk. Therefore, subsequent analyses of FA composition for left and right breasts were performed on hindmilk samples only. FA composition did vary over the course of the day: the mean daily CV for all FA identified was 14·3 (SEM 7·7) %. The pattern of daily variation of individual FA was not conserved either between women or over the course of lactation (Fig. 1).

The observed variation in the composition of 10 : 0, 12 : 0, 14 : 0, 16 : 0, 18 : 0, 18 : 1*n*-9, 18 : 2*n*-6, 18 : 3*n*-3, 20 : 4*n*-6 and 22 : 6*n*-3 between women and over the course of lactation was significant ($P<0{\cdot}05$) in all cases (Table 2). Over the course of lactation, the medium-chain FA 10 : 0, 12 : 0 and 14 : 0 all decreased with time to 6 months ($P<0{\cdot}0001$). Following this, 12 : 0 and 14 : 0 increased markedly towards 12 months ($P<0{\cdot}0001$) whilst 10 : 0 remained constant. Palmitic acid (16 : 0)

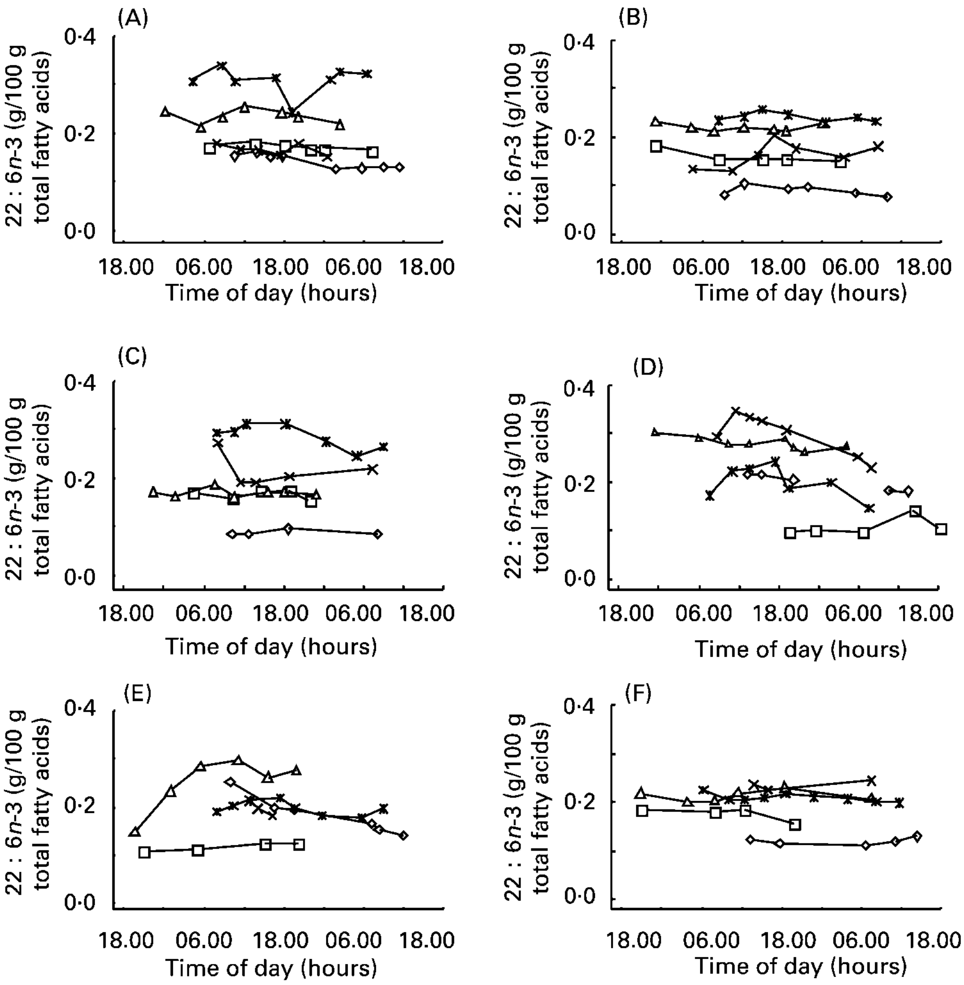

Fig. 1. Variation in the proportion of 22 : 6n-3 in milk from each feed from the left breast over a 24 h period for five mothers at 1 (A), 2 (B), 4 (C), 6 (D), 9 (E) and 12 (F) months of lactation. ◇, Mother 1; □, mother 2; △, mother 3; ×, mother 4; ∗, mother 5. Fatty acid composition varied over the course of the day, but this variation was not consistent between mothers or over the course of lactation. For details of subjects and procedures, see p. 980.

behaved similarly to 10 : 0, showing an initial decrease towards 4 months ($P<0·0001$) and then remaining constant to 12 months of lactation. Although not a medium-chain FA, 18 : 0 followed a similar trend to 12 : 0 and 14 : 0, decreasing towards 9 months ($P<0·0001$) and then increasing from 9 to 12 months of lactation ($P<0·0001$).

For 18 : 1*n*-9, there was no difference in the proportional composition between the values obtained at 1 and 12 months. However, 18 : 1*n*-9 did increase towards 6 months ($P<0·0001$) before decreasing towards 12 months ($P<0·0001$). Linoleic acid (18 : 2*n*-6) also followed this trend by increasing towards 6–9 months ($P=0·0001$) and then decreasing at 12 months of lactation ($P<0·05$). Linolenic acid (18 : 3*n*-3) increased towards 6–9 months ($P<0·0001$) and then remained constant to 12 months. Arachidonic acid (20 : 4n-6) decreased towards 6 months and then remained constant to 12 months ($P=0·0006$). Docosahexaenoic acid (22 : 6*n*-3) decreased from 1–2 months ($P=0·0002$), after which there was an increase towards 6 months ($P<0·0001$) followed by no change through to 12 months.

Amounts of fatty acids consumed by the infant

On average, over the first year of lactation, the greatest amount of any individual FA consumed by the infant over the 24 h period was that of 18 : 1*n*-9 (8·27 (SD 2·84) g/24 h). The infant also consumed both the *n*-6 and *n*-3 essential FA 18 : 2*n*-6 (2·38 (SD 0·98) g/24 h) and 18 : 3*n*-3 (0·19 (SD 0·07) g/24 h) respectively. The infant consumed 92·5 (SD 31·1) mg arachidonic acid (20 : 4*n*-6)/24 h and 49·3 (SD 21·1) mg 22 : 6*n*-3/24 h. Overall, the infant consumed 12·14 (SD 4·29) g saturated FA/24 h and 3·58 (SD 1·07) g polyunsaturated FA/24 h, of which 3·22 (SD 0·95) g was of the *n*-6 family and 0·3 (SD 0·11) g was from the *n*-3 family. This gave a ratio of 8·9 (SD 1·94) for the consumption of the *n*-6 FA : *n*-3 FA. The infant also consumed 0·62 (SD 0·35) g *trans* FA/24 h.

The amounts of major individual FA of interest (10 : 0, 12 : 0, 14 : 0, 16 : 0, 18 : 0, 18 : 1*n*-9, 18 : 2*n*-6, 18 : 3*n*-3, 20 : 4*n*-6 and 22 : 6*n*-3) consumed by the infant during the first year of lactation showed no significant difference with time (Table 3). Only the intakes of 22 : 1*n*-9,

Table 2. Changes in fatty acid composition (g/100 g total fatty acids) of human milk over the course of the first year of lactation*
(Mean values with their standard errors)

Fatty acid	1 month			2 months			4 months			6 months			9 months			12 months			Statistical significance of effect: *P*
	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	
10 : 0	1·391	0·047	69	1·231	0·046	69	1·105	0·05	65	1·112	0·034	67	1·055	0·026	58	1·14	0·026	61	< 0·0001
12 : 0	5·634	0·214	69	5·242	0·282	69	4·68	0·24	65	4·332	0·121	67	5·56	0·166	59	6·532	0·169	61	< 0·0001
14 : 0	7·221	0·162	69	7·439	0·309	69	6·318	0·251	65	6·092	0·13	67	7·48	0·165	59	9·265	0·22	61	< 0·0001
15 : 0	0·492	0·011	69	0·488	0·01	69	0·425	0·009	64	0·479	0·017	67	0·443	0·013	57	0·453	0·016	57	< 0·0001
17 : 0	0·455	0·007	69	0·431	0·006	69	0·419	0·016	64	0·405	0·018	64	0·362	0·007	57	0·397	0·008	57	< 0·0001
16 : 0	25·375	0·219	69	25·148	0·216	69	24·489	0·119	65	23·807	0·3	67	23·714	0·38	59	24·146	0·222	61	< 0·0001
18 : 0	8·012	0·085	69	9·141	0·127	69	7·496	0·238	65	7·588	0·218	67	7·107	0·174	59	8·432	0·267	61	< 0·0001
20 : 0	0·678	0·017	69	0·716	0·014	69	0·655	0·013	64	0·707	0·019	67	0·617	0·013	57	0·661	0·014	58	< 0·0001
22 : 0	0·079	0·002	66	0·07	0·002	55	0·059	0·003	52	0·057	0·002	43	0·059	0·002	45	0·074	0·003	43	< 0·0001
24 : 0	0·085	0·002	64	0·073	0·003	53	0·065	0·002	49	0·063	0·003	46	0·058	0·002	45	0·053	0·002	37	< 0·0001
14 : 1*n*-9	0·421	0·012	69	0·463	0·01	69	0·367	0·007	64	0·406	0·014	66	0·351	0·014	43	0·397	0·016	31	< 0·0001
16 : *1n*-7	2·457	0·024	69	2·622	0·061	69	2·948	0·105	65	2·722	0·096	67	2·605	0·068	59	2·318	0·073	61	< 0·0001
18 : 1*n*-9	30·85	0·171	69	31·404	0·46	69	34·49	0·422	65	34·264	0·4	67	32·352	0·371	59	30·211	0·267	61	< 0·0001
18 : 1*n*-7	1·502	0·029	68	1·553	0·054	69	1·725	0·071	65	1·739	0·054	67	1·745	0·047	58	1·449	0·043	61	< 0·0001
20 : 1*n*-9	0·291	0·007	69	0·278	0·007	69	0·283	0·006	64	0·261	0·005	67	0·24	0·006	57	0·223	0·005	57	< 0·0001
22 : 1*n*-9	0·04	0·003	60	0·057	0·004	57	0·047	0·004	58	0·041	0·003	50	0·034	0·002	46	0·039	0·002	37	0·0014
trans-18 : 1*n*-9	1·505	0·053	63	1·128	0·076	49	0·784	0·032	55	1·348	0·12	39	0·743	0·05	45	0·746	0·062	30	< 0·0001
trans-18 : 1*n*-7	1·105	0·057	33	1·364	0·044	37	1·249	0·06	36	2·201	0·076	18	1·28	0·109	25	1·351	0·078	34	< 0·0001
trans-18 : 2*n*-6	0·285	0·017	69	0·321	0·018	68	0·257	0·02	64	0·311	0·029	65	0·33	0·019	57	0·327	0·018	57	< 0·0001
18 : 2*n*-6	8·495	0·24	69	8·435	0·276	69	9·285	0·297	65	10·047	0·352	67	10·712	0·304	59	9·281	0·345	61	< 0·0001
18 : 3*n*-6	0·16	0·006	68	0·164	0·005	68	0·165	0·006	62	0·134	0·004	60	0·12	0·005	50	0·125	0·01	53	< 0·0001
20 : 2*n*-6	0·172	0·003	57	0·124	0·002	52	0·109	0·003	50	0·102	0·004	48	0·074	0·004	15	0·056	0·001	16	< 0·0001
20 : 3*n*-6	0·361	0·018	69	0·322	0·01	69	0·296	0·009	64	0·221	0·007	66	0·219	0·009	57	0·191	0·006	56	< 0·0001
20 : 4*n*-6	0·395	0·008	69	0·369	0·006	69	0·388	0·006	64	0·362	0·009	67	0·365	0·01	57	0·338	0·006	57	< 0·0001
22 : 4*n*-6	0·085	0·004	51	0·08	0·005	48	0·077	0·002	43	0·072	0·003	41	0·076	0·003	49	0·068	0·003	43	0·0447
18 : 3*n*-3	0·664	0·013	69	0·698	0·018	69	0·703	0·025	65	0·883	0·022	67	0·871	0·029	58	0·792	0·031	58	< 0·0001
20 : 5*n*-3	0·104	0·007	48	0·086	0·007	46	0·06	0·003	42	0·065	0·004	40	0·071	0·006	44	0·061	0·003	37	< 0·0001
22 : 5*n*-3	0·164	0·003	69	0·164	0·003	69	0·173	0·007	64	0·173	0·003	63	0·187	0·009	55	0·168	0·004	56	NS
22 : 6*n*-3	0·208	0·008	69	0·176	0·007	68	0·185	0·009	62	0·215	0·009	64	0·189	0·007	56	0·183	0·005	55	0·0002
Total	97·661	0·08	69	97·571	0·276	69	97·651	0·207	65	97·795	0·214	67	97·962	0·258	59	98·012	0·161	61	NS
Medium-chain saturated	14·247	0·383	69	13·911	0·62	69	12·038	0·525	65	11·537	0·246	67	14·115	0·335	58	16·968	0·36	61	< 0·0001
Odd-chain saturated	0·947	0·011	69	0·873	0·015	69	0·839	0·022	64	0·844	0·028	67	0·789	0·017	57	0·79	0·018	57	0·0039
Long-chain saturated	34·101	0·264	69	33·739	0·339	56	32·828	0·276	59	31·817	0·616	56	31·458	0·562	50	33·929	0·588	37	0·0019
Monounsaturated	37·458	0·222	64	33·686	0·35	69	38·592	0·525	65	37·545	0·46	57	37·305	0·463	40	33·031	0·545	19	< 0·0001
trans	2·048	0·075	28	2·196	0·121	26	2·205	0·087	29	4·612	0·024	2	1·723	0·159	37	1·769	0·092	37	0·0008
Polyunsaturated	12·707	0·161	27	9·457	0·177	25	11·787	0·446	21	13·373	0·608	26	7·993	0·101	15	6·74	0·027	16	0·0051
Polyunsaturated: saturated	0·269	0·006	27	0·184	0·008	16	0·262	0·015	21	0·326	0·021	26	0·148	0·002	15	0·121	0·001	16	0·0084
n-6	11·199	0·089	40	9·908	0·357	39	11·52	0·222	36	12·568	0·289	38	7·047	0·087	15	5·801	0·023	16	0·0018
n-3	1·178	0·031	56	1·042	0·017	54	1·091	0·027	50	1·322	0·038	54	1·298	0·05	51	1·211	0·037	41	< 0·0001
n-6:*n*-3	10·937	0·283	27	8·093	0·182	25	10·178	0·423	21	9·604	0·578	26	7·469	0·087	15	6·18	0·048	16	0·032

* For details of subjects and procedures, see p. 980.

Table 3. Amounts of fatty acids (g/24 h) delivered to the infant over the course of the first year of lactation*
(Mean values with their standard errors)

Fatty acid	1 month			2 months			4 months			6 months			9 months			12 months			Statistical significance of effect: *P*
	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	
10:0	0·432	0·098	5	0·279	0·040	5	0·303	0·067	5	0·293	0·041	5	0·293	0·053	5	0·233	0·062	5	NS
12:0	1·701	0·347	5	1·181	0·235	5	1·297	0·312	5	1·132	0·155	5	1·521	0·322	5	1·269	0·333	5	NS
14:0	2·123	0·249	5	1·666	0·238	5	1·729	0·343	5	1·562	0·139	5	2·076	0·465	5	1·842	0·588	5	NS
15:0	0·147	0·019	5	0·111	0·010	5	0·112	0·011	5	0·122	0·019	5	0·128	0·030	5	0·099	0·036	5	NS
17:0	0·136	0·016	5	0·098	0·004	5	0·109	0·014	5	0·102	0·011	5	0·102	0·024	5	0·083	0·028	5	NS
16:0	7·623	0·893	5	5·742	0·168	5	6·544	0·492	5	6·160	0·645	5	6·523	1·474	5	4·908	1·464	5	NS
18:0	2·376	0·241	5	1·827	0·098	5	2·026	0·265	5	1·946	0·264	5	2·060	0·536	5	1·625	0·477	5	NS
20:0	0·199	0·017	5	0·163	0·010	5	0·188	0·014	5	0·181	0·016	5	0·175	0·044	5	0·140	0·048	5	NS
22:0	0·024	0·003	5	0·015	0·002	5	0·017	0·003	5	0·017	0·003	5	0·015	0·004	5	0·013	0·004	5	NS
24:0	0·026	0·004	5	0·017	0·001	5	0·017	0·002	5	0·016	0·003	5	0·014	0·003	5	0·014	0·004	5	NS
14:1*n*-9	0·140	0·023	5	0·104	0·007	5	0·097	0·009	5	0·103	0·013	5	0·107	0·030	4	0·463	0·232	5	NS
16:1*n*-7	0·738	0·094	5	0·649	0·053	5	0·759	0·048	5	0·704	0·130	5	0·673	0·089	5	0·481	0·122	5	NS
18:1*n*-9	9·339	1·151	5	7·212	0·617	5	9·000	0·645	5	9·224	1·587	5	8·828	1·555	5	6·038	1·547	5	NS
18:1*n*-7	0·468	0·073	5	0·357	0·054	5	0·441	0·046	5	0·461	0·103	5	0·439	0·049	5	0·294	0·077	5	NS
20:1*n*-9	0·087	0·008	5	0·064	0·004	5	0·076	0·003	5	0·070	0·012	5	0·065	0·011	5	0·046	0·012	5	NS
22:1*n*-9	0·018	0·004	5	0·012	0·002	5	0·013	0·001	5	0·012	0·001	5	0·008	0·002	5	0·009	0·001	4	0·0419
18:1*n*-9	0·353	0·086	5	0·206	0·068	5	0·202	0·009	5	0·348	0·095	4	0·221	0·056	5	0·102	0·051	4	NS
18:1*n*-7	0·320	0·042	3	0·369	0·033	3	0·348	0·048	3	0·506	0·150	2	0·423	0·203	3	0·280	0·142	4	NS
18:2*n*-6	0·080	0·010	5	0·070	0·009	5	0·064	0·008	5	0·067	0·011	5	0·124	0·045	5	0·104	0·050	5	NS
trans-18:2*n*-6	2·706	0·568	5	1·945	0·275	5	2·447	0·327	5	2·758	0·631	5	2·684	0·302	5	1·753	0·417	5	NS
18:3*n*-6	0·051	0·006	5	0·037	0·004	5	0·047	0·006	5	0·035	0·006	5	0·031	0·007	5	0·026	0·007	5	0·0415
20:2*n*-6	0·056	0·010	4	0·029	0·003	4	0·031	0·003	4	0·028	0·007	4	0·033		1	0·020		1	0·0331
20:3*n*-6	0·110	0·029	5	0·072	0·006	5	0·079	0·011	5	0·057	0·009	5	0·054	0·004	5	0·037	0·009	5	0·0167
20:4*n*-6	0·119	0·016	5	0·083	0·005	5	0·100	0·007	5	0·098	0·019	5	0·092	0·011	5	0·065	0·015	5	NS
22:4*n*-6	0·027	0·003	4	0·018	0·001	4	0·021	0·002	4	0·020	0·002	4	0·018	0·002	5	0·016	0·005	5	NS
18:3*n*-3	0·205	0·034	5	0·162	0·018	5	0·187	0·026	5	0·242	0·049	5	0·221	0·026	5	0·148	0·034	5	NS
20:5*n*-3	0·026	0·002	5	0·021	0·005	4	0·015	0·003	5	0·021	0·005	5	0·018	0·006	5	0·021	0·008	5	NS
22:5*n*-3	0·048	0·004	5	0·037	0·002	5	0·044	0·002	5	0·045	0·007	5	0·049	0·009	5	0·034	0·009	5	NS
22:6*n*-3	0·060	0·007	5	0·040	0·006	5	0·048	0·007	5	0·057	0·013	5	0·051	0·010	5	0·040	0·012	5	NS
Total	29·593	3·630	5	22·425	0·803	5	26·214	1·950	5	26·003	3·417	5	26·830	4·898	5	20·108	5·230	5	NS
Medium-chain saturated	4·256	0·678	5	3·126	0·499	5	3·329	0·708	5	2·987	0·313	5	3·891	0·832	5	3·344	0·970	5	NS
Odd-chain saturated	0·283	0·031	5	0·210	0·013	5	0·221	0·023	5	0·224	0·028	5	0·230	0·054	5	0·182	0·064	5	NS
Long-chain saturated	10·247	1·147	5	7·764	0·224	5	8·793	0·684	5	8·320	0·867	5	8·786	2·053	5	6·701	1·992	5	NS
Monounsaturated	10·790	1·318	5	8·399	0·700	5	10·387	0·670	5	10·574	1·789	5	10·099	1·713	5	8·949	1·046	4	NS
trans	0·695	0·179	3	0·586	0·101	3	0·622	0·040	3	1·323		1	0·728	0·327	3	0·390	0·216	3	NS
Polyunsaturated	2·776	1·037	5	2·242	0·308	2	3·477	0·304	3	4·155	0·886	3	3·225	0·357	5	2·142	0·497	5	NS
Polyunsaturated:saturated	0·164	0·058	5	0·182	0·026	2	0·260	0·043	3	0·322	0·069	3	0·282	0·041	5	0·220	0·038	5	NS
n-6	4·061	0·111	3	2·374	0·412	3	3·151	0·296	3	3·719	0·808	3	2·886	0·318	5	1·900	0·446	5	NS
n-3	0·339	0·044	5	0·241	0·016	4	0·294	0·024	5	0·364	0·071	5	0·340	0·047	5	0·242	0·054	5	NS
n-6:*n*-3	10·195	0·979	3	8·143	0·628	2	9·699	0·999	3	8·741	1·596	3	8·732	0·632	5	7·563	0·653	5	NS

*For details of subjects and procedures, see p. 980.

18 : 3*n*-6, 20 : 2*n*-6 and 20 : 3*n*-6 differed with time over the first year of lactation ($P<0{\cdot}05$). Furthermore, there were no differences between infants in amounts of the FA consumed, except for the *n*-3 FA : 18 : 3*n*-3, 22 : 5*n*-3 and 22 : 6*n*-3.

Discussion

The FA composition of milk was found to vary over the course of the day (Fig. 1) and this finding is in agreement with previous results from this laboratory (Daly *et al.* 1993), but in contrast to reports in the literature (Hall, 1979; Jensen, 1989). Hall (1979) found no evidence of a circadian rhythm in FA composition in human milk samples taken at regular time points over 3 d. However, upon closer inspection of Hall's results, FA composition did vary, with some FA showing daily CV of up to 40 %. The current results agree with those of Hall (1979); however, the interpretation differs, as Hall was specifically looking for a circadian rhythm. The lack of a circadian rhythm, however, does not preclude the FA composition from changing in a regulated manner over the course of the day, as was the case with the results of Daly *et al.* (1993).

The FA composition varied between the five mothers in the present study and with time over the first year of lactation (Table 2). Differences between mothers were expected and are well documented, and are most likely to be due to differences in maternal diet (Jensen, 1989). Reports on longitudinal changes in FA composition over the course of lactation are not as common; furthermore, there is a lack of consistency in the results in the literature. For example, the two essential FA (18 : 2*n*-6 and 18 : 3*n*-3) increased at 6–9 months of lactation. Similar trends were observed up to 3 (Huisman *et al.* 1996), 6 (Luukkainen *et al.* 1994) and 8 (Idota *et al.* 1991) months of lactation. However, Makrides *et al.* (1995) showed no change in either FA over 7·5 months (30 weeks) of lactation, whereas Marangoni *et al.* (2000) showed differences over 12 months but no specific trend. Similar differences in the literature can be found for other FA and these results highlight the complex nature of the relationships between individual FA in human milk and clearly suggest a need for future research investigating the regulation of FA incorporation into breast milk.

Whilst much recent interest has focused on infant long-chain polyunsaturated FA status, few studies have actually investigated infant FA intake (Marangoni *et al.* 2000). An important feature of the current study was the ability of the sampling method of Hartmann *et al.* (1986) to allow the conversion of concentrations of individual FA into amounts consumed by the infant. Changes in the amounts of specific FA (10 : 0, 12 : 0, 14 : 0, 16 : 0, 18 : 0, 18 : 1*n*-9, 18 : 2*n*-6, 18 : 3*n*-3, 20 : 4*n*-6 and 22 : 6*n*-3) delivered to the infant in a 24 h period over the first year of life (Table 3) showed no significant differences with stage of lactation. Furthermore, of these FA, only the *n*-3 FA differed between women. This is in contrast to changes in the proportional composition of FA: all of these FA were different both between women and over the course of lactation, as was also shown by Marangoni *et al.* (2000). These results suggest that variation in the proportional composition of individual FA cannot be translated to variation in infant intake. Intake needs to be determined directly using FA concentration and milk production. Therefore, changes in the composition of FA (either between women or with time) can be compensated for by changes in milk intake by the infant. It is interesting to note that only the *n*-3 long-chain polyunsaturated FA were different between women, perhaps suggesting the dependence of these FA on immediate diet as opposed to body depots (Del Prado *et al.* 2000).

Estimated whole-body 22 : 6*n*-3 requirement rates of 20 (Cunnane *et al.* 2000) and 30 (Farquharson *et al.* 1993) mg/24 h are below the mean infant intakes recorded in the current study (Table 3). Furthermore, the mean 24 h infant intake of 22 : 6*n*-3 of 49 (SEM 21) mg/24 h determined in the current study agrees with the reported estimated intakes of 50 (Hachey, 1994) and 60 (Cunnane *et al.* 2000) mg/24 h by the breast-fed infant. These results suggest that the five breast-fed infants in the present study had access to a surplus of preformed 22 : 6*n*-3 and in this regard they are probably representative of the average, term-delivered, exclusively breast-fed infant from 1 to 6 months of age. However, the present study cannot establish whether or not these intake levels are sufficient or indeed 'optimal', because of the limited number of subjects and the lack of any outcome measures. Further studies are required to associate actual infant intakes of specific FA, as opposed to breast-milk concentrations, with specific functional outcomes before formal dietary recommendations can be made.

In conclusion, the FA composition of milk determined in the current study was similar to that previously reported (Jensen *et al.* 1995). In contrast to the study of Jensen *et al.* (1995), we observed that the FA composition did vary over the short-term; however, there was no consistent pattern (circadian or otherwise) in FA variation. It is, therefore, recommended that this variation together with the variation in total fat content (Mitoulas *et al.* 2002) be accounted for by the use of a suitable sampling protocol, i.e. similar to that of Hartmann *et al.* (1986), when investigating infant FA intakes. FA composition also varied over the long-term; however, the variation in FA composition both between women and with stage of lactation was not conserved when amounts of FA consumed by the infant per 24 h were investigated. Therefore, it is important to recognise that changes in the proportions of individual FA may not necessarily result in commensurate changes in 24 h infant intake of those FA, and that intake must be measured directly if the effects of altering milk FA composition are to be correlated with infant functional outcomes.

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References

Arthur PG, Hartmann PE & Smith M (1987) Measurement of the milk intake of breast-fed infants. *J Pediatr Gastroenterol Nutr* **6**, 758–763.

Butte NF, Garza C, Smith EO & Nichols BL (1984) Human milk intake and growth in exclusively breast-fed infants. *J Pediatr* **104**, 187–195.

Christie WW (1973) *Lipid analysis. Isolation, Separation, Identification and Structural Analysis of Lipids*. Oxford: Pergamon Press.

Cox DB, Kent JC, Casey TM, Owens RA & Hartmann PE (1999) Breast growth and the urinary excretion of lactose during human pregnancy and early lactation: endocrine relationships. *Exp Physiol* **84**, 421–434.

Cox DB, Owens RA & Hartmann PE (1996) Blood and milk prolactin and the rate of milk synthesis in women. *Exp Physiol* **81**, 1007–1020.

Cunnane SC, Francescutti V, Brenna JT & Crawford MA (2000) Breastfed infants achieve a higher rate of brain and whole body docosahexaenoate accumulation than formula-fed infants not consuming dietary docosahexaenoate. *Lipids* **35**, 105–111.

Daly SEJ, Di Rosso A, Owens RA & Hartmann PE (1993) Degree of breast emptying explains changes in the fat content, but not fatty acid composition, of human milk. *Exp Physiol* **78**, 741–755.

Del Prado M, Villalpando S, Lance A, Alfonso E, Demmelmair H & Koletzko B (2000) Contribution of dietary and newly formed arachidonic acid to milk (secretion in women on low fat diets). In *Short and Long Term Effects of Breast Feeding on Child Health*, pp. 407–408 [B Koletzko, KF Michaelson and O Hernell, editors]. New York: Kluwer Academic/Plenum Publishers.

Farquharson J, Cockburn F, Patrick WA, Jamieson EC & Logan RW (1993) Effect of diet on infant subcutaneous tissue triglyceride fatty acids. *Arch Dis Child* **69**, 589–593.

Folch J, Lees M & Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**, 497–509.

Gibson RA & Kneebone GM (1981) Fatty acid composition of human colostrum and mature breast milk. *Am J Clin Nutr* **34**, 252–257.

Gibson RA & Makrides M (2000) *n*-3 Polyunsaturated fatty acid requirements of term infants. *Am J Clin Nutr* **71**, Suppl., 251S–255S.

Gibson RA, Neumann MA & Makrides M (1996) Effect of dietary docosahexaenoic acid on brain composition and neural function in term infants. *Lipids* **31**, Suppl., S177–S181.

Hachey DL (1994) Benefits and risks of modifying maternal fat intake in pregnancy and lactation. *Am J Clin Nutr* **59**, Suppl., 454S–463S.

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

Hartmann PE, Mitoulas LR & Sherriff JL (2000) Synthesis and secretion of fat in human milk and its role in infant development. *J Integr Study Diet Habits* **10**, 65–73.

Hartmann PE, Morgan SEG & Arthur PG (1986) Milk let-down and the concentration of fat in breast milk. In *Human Lactation 2: Maternal and Environmental Factors*, pp. 275–281 [M Hamosh and AS Goldman, editors]. New York: Plenum Press.

Huisman M, van Beusekom M, Lanting CI, Nijeboer HJ, Muskiet FA & Boersma ER (1996) Triglycerides, fatty acids, sterols, mono- and disaccharides and sugar alcohols in human milk and current types of infant formula milk. *Eur J Clin Nutr* **50**, 255–260.

Hytten FE (1954) Clinical and chemical studies in human lactation: I. Collection of milk samples. II. Variation in major constituents during feeding. III. Diurnal variation in major constituents in milk. *Brit Med J* **23**, 175–182.

Idota T, Sakurai M, Sugawara Y, *et al.* (1991) The latest survey for the composition of milk obtained from Japanese mothers. Part II. Changes of fatty acid composition, phospholipid and cholesterol contents during lactation. *Jpn J Pediatr Gastroenterol Nutr* **5**, 159–173.

Innis SM, Gilley J & Werker J (2001) Are human long-chain polyunsaturated fatty acids related to visual and neural development in breast-fed term infants? *J Pediatr* **139**, 532–538.

Innis SM & Kuhnlein HV (1988) Long chain *n*-3 fatty acids in breast milk of Inuit women consuming traditional foods. *Early Hum Dev* **18**, 185–189.

Isaacs CE, Thormar H & Pessolano T (1986) Membrane-disruptive effect of human milk: inactivation of enveloped viruses. *J Infect Dis* **154**, 966–971.

Jensen RG (1989) *The Lipids of Human Milk*. Boca Raton, FL: CRC Press.

Jensen RG, Bitman J, Carlson SE, Couch SC, Hamosh M & Newberg DS (1995) Milk Lipids. In *Handbook of Milk Composition*, pp. 495–576 [RG Jensen, editor]. San Diego, CA: Academic Press.

Kent JC, Mitoulas LR, Cox DB, Owens RA & Hartmann PE (1999) Breast volume and milk production during extended lactation in women. *Exp Physiol* **84**, 435–447.

Kneebone GM, Kneebone R & Gibson RA (1985) Fatty acid composition of breast milk from three racial groups from Penang, Malaysia. *Am J Clin Nutr* **41**, 765–769.

Luukkainen P, Salo MK & Nikkari T (1994) Changes in the fatty acid composition of preterm and term human milk from 1 week to 6 months of lactation. *J Pediatr Gastroenterol Nutr* **18**, 355–360.

Makrides M, Simmer K, Neumann MA & Gibson RA (1995) Changes in the polyunsaturated fatty acids of breast milk from mothers of full term infants over 30 wk of lactation. *Am J Clin Nutr* **61**, 1231–1233.

Marangoni F, Agostoni C, Lammardo AM, Giovannini M, Galli C & Riva E (2000) Polyunsaturated fatty acid concentrations in human hindmilk are stable throughout 12-months of lactation and provide a sustained intake to the infant during exclusive breastfeeding: an Italian study. *Brit J Nutr* **84**, 103–109.

Mitoulas LR, Kent JC, Cox DB, Owens RA, Sherriff JL & Hartmann PE (2002) Variation in fat, lactose and protein in human milk over 24 h and throughout the first year of lactation. *Brit J Nutr* **88**, 29–37.

Read WWC, Lutz PG & Tashjian A (1965) Human Milk Lipids II. The influence of dietary carbohydrates and fat on the fatty acids of mature milk. A study in four ethnic groups. *Am J Clin Nutr* **17**, 180–183.

Stern I & Shapiro B (1953) A rapid and simple method for the determination of esterified fatty acids and for total fatty acids in blood. *J Clin Pathol* **6**, 158.