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Variation in fat, lactose and protein in human milk over 24 h and throughout the first year of lactation

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Fat in human milk is extremely variable and can represent up to 50 % of infant energy intake. To accurately determine milk composition and infant intake at 1 (*n* 17), 2 (*n* 17), 4 (*n* 17), 6 (*n* 15), 9 (*n* 6) and 12 (*n* 5) months of lactation, samples of fore- and hind-milk were collected from each breast at each feed over 24 h periods from an initial group of seventeen women. The content of fat in milk varied over 24 h, with a mean CV of 47·6 (SE 2·1) % (*n* 76) and 46·7 (SE 1·7) % (*n* 76) for left and right breasts respectively. The 24 h amounts of fat, lactose and protein in milk differed between women ($P=0{\cdot}0001$), but were consistent between left and right breasts. Daily milk production differed between breasts ($P=0{\cdot}0001$) and women ($P=0{\cdot}0001$). Accordingly, amounts of fat ($P=0{\cdot}0008$), lactose ($P=0{\cdot}0385$) and protein ($P=0{\cdot}0173$) delivered to the infant over 24 h also differed between breasts and women ($P=0{\cdot}0001$). The energy content of milk and the amount of energy delivered to the infant over 24 h were the same between breasts, but differed between women ($P=0{\cdot}0001$). The growth rate of a group of only six infants in the present study was not related to either the concentrations or amounts of fat, lactose, protein and energy in milk over the first 6 months of life. These results show the individuality of milk composition and suggest that only a rigorous sampling routine that takes into account all levels of variation will allow the accurate determination of infant intake of fat, lactose, protein and energy.

Milk fat: Infant intake: Sampling routine: Human lactation

Of the major digestible energy components (fat, lactose and protein) in human milk, fat is the most variable. Woolridge (1995) listed several factors that either individually or in concert could account for the variability in fat content of human milk. Major factors included the amount of milk removed at both the last and current breast-feed, the length of the interval between breast-feeds, and the fat content at the end of the last breast-feed. Daly *et al.* (1993*a*) showed that approximately 70 % of the variation in fat content of breast milk was due to the extent of fullness of the breast (see Cox *et al.* 1996), essentially incorporating all the predictors proposed by Woolridge (1995) and expressing them as one term. Furthermore, the discovery of local (autocrine) control systems for milk synthesis (Henderson & Peaker, 1984) and possibly milk-fat synthesis (Heesom *et al.* 1992), combined with differences in milk production and storage capacity between breasts within mothers (Daly *et al.* 1993*a*), could result in different rates of milk and fat synthesis between breasts. These factors make it difficult to design a sampling protocol, suitable for all women, that will provide a true indication of energy density and intake of breast milk without affecting the natural routine of the demand-fed infant (Prentice & Prentice, 1988; Lucas & Davies, 1990; Prentice *et al.* 1996).

We have used a sampling protocol similar to that of Hartmann *et al.* (1986) that takes into account changes in fat content of milk during a feed, differences between breasts, changes over the course of the day and ensures minimum interference with infant feeding behaviour. We have determined the volume of milk removed together with the fat content, lactose and protein concentrations and the calculated energy content for milk from each breast at each feed over a 24 h period at 1, 2, 4, 6, 9 and 12 months of lactation for women who were breast-feeding. These data were used to determine mean 24 h concentrations

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in milk and amounts of each component delivered to the infant from breast milk from 1 to 12 months after birth.

Methods

Subjects

Healthy mothers and infants were recruited through the Nursing Mothers' Association of Australia, Western Australian Branch, or private health care 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. Subject details have been previously reported (Cox *et al.* 1996, 1999). Briefly, mothers were between 18 and 35 years of age, twelve were multiparous (five mothers having had two children, seven mothers having had three children) and five primiparous, with a mean weight of 68·69 (SE 2·06; range 55·3–84·4) kg (*n* 17). All infants were born at term (except one, born at 31 weeks) and were exclusively 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. 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 by either manual breast pump (Kaneson Expression and Feeding Bottle; Yanase Waitch KK, Osaka, Japan) or hand expression into 5 ml polypropylene vials (Disposable Products Pty Ltd, Adelaide, 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 analysed.

Biochemical analyses

Milk fat. The content of fat in fore- and hind-milk samples was determined using the modified colorimetric spectrophotometric method of Stern & Shapiro (1953), as previously described (Cox *et al.* 1996). Briefly, 2·5 μl portions of the milk samples (warmed to 37°C) and standards (triolein, 0–200 mM) 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 tube contents were mixed and 250 μl from each tube was pipetted into duplicate wells on a ninety-six-well microtitre plate. Absorbance of each well was determined at 540 nm using a plate spectrophotometer (Titertek Multiscan MCC/340; Flow Laboratories, McLean, VA, USA). The detection limit of this assay was 0·45 (SE 0·41) g/l (*n* 13) and the interassay CV was 8·1 % (*n* 13).

Milk lactose. The concentration of lactose in fore- and hind-milk samples was determined using the modified method of Kuhn & Lowenstein (1967), as described by Arthur *et al.* (1989). Briefly, defatted milk samples and lactose standards (0–300 mM) were diluted 1 in 150 with distilled deionised water. Duplicate portions of diluted standards and samples (5 μl) were pipetted into wells on a flat-bottom ninety-six-well microtitre plate and reagent 1 (8 U β-galactosidase/ml, 0·1 M-$MgCl_2$ in 0·1 M-potassium phosphate buffer, pH 7·2; 50 μl) was added to each well and the plate mixed and incubated at 37°C for 60 min. Following this step, reagent 2 (9·6 U glucose oxidase/ml, 2·5 U peroxidase/ml, 500 μg 2,2-azino-di-(3-ethyl-benzthiazolin-sulfonate)-6-sulfonate/ml in 0·1 M-potassium phosphate buffer, pH 7·2; 200 μl) was added to each well and the absorbance measured at 405 nm on a plate spectrophotometer at 5 min intervals until a peak absorbance was reached at approximately 45 min. The recovery of a known amount of lactose added to milk samples was 102 (SE 1) % (*n* 17). The detection limit of this assay was 16·4 (SE 0·4) g/l (*n* 35) and the interassay CV was 5·6 % (*n* 35).

Milk protein. The concentration of protein in fore- and hind-milk samples was determined using a commercial protein assay kit (Bio-Rad Laboratories, Richmond, CA, USA). The assay procedure was a modification of that of Atwood & Hartmann (1992), in that samples were diluted 1 in 30 with double-deionised water. To overcome the problems inherent in the choice of a milk standard the protein concentration of an aliquot of mature breast milk was determined by the Kjeldahl procedure (Hambræus *et al.* 1978), as described by Atwood & Hartmann (1992). The remaining sample was then diluted with double deionised water to provide a range of standards (0–1 g/l). Briefly, defatted milk samples were diluted 1 in 30 with double-deionised water and pipetted (5 μl) in duplicate, with standards, onto a ninety-six-well microtitre plate. To each well was added 250 μl Bio-Rad protein assay reagent (diluted 1 in 5 with distilled deionised water and filtered through Whatman no. 1 paper) and the plate mixed for 1–2 min and then allowed to stand for 5 min. Absorbance was then measured at 620 nm using a plate spectrophotometer. The recovery of a known amount of protein added to milk samples was 99·96 (SE 1·03) % (*n* 17). The detection limit of this assay was 0·033 (SE 0·002) g/l (*n* 53) and the interassay CV was 4·72 % (*n* 35).

Milk energy. The energy content for each feed was calculated using the conversion factors (Garza *et al.* 1985) of 38·7, 16·5 and 23·7 kJ/g for fat, lactose and protein respectively.

Determination of 24 h milk production

Milk yield was determined for each breast by test weighing the mother, as described by Arthur *et al.* (1987). Test weighing was carried out at each mother's home over a 24–28 h period using an electronic Sauter balance (weighing platform, Model EC 240; evaluator unit with data output printer, Model ED 3300; FSE Scientific, Perth, WA, Australia). Briefly, mothers weighed themselves before and after each feed from each breast. To account for the insensitive water loss, which occurred 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 insensitive water loss during the feeding period.

Determination of 24 h nutrient and energy intake

Fore- and hind-milk concentrations were averaged to provide a concentration of fat, lactose, protein and energy for each feed. The volume of the feed was then used to determine the amount taken by the infant and the total energy provided at each feed. The sum of the amount of each nutrient and the energy provided for all feeds over the study period (24–28 h) and the total volume delivered over the study period were then used to determine an average concentration. This concentration and the corrected 24 h volume (Arthur *et al.* 1987) were then used to determine the amount delivered in 24 h to the infant.

Infant growth rates

Infant growth rates were determined as previously described (Kent *et al.* 1999). Briefly, subjects provided records of birth weights and weight gains up to 6 months of age, as measured by midwives attending the birth and Community Health Nurses respectively.

Statistical analysis

Seventeen mothers initially provided data for milk production and fat content whereas lactose, protein and energy data were initially obtained for only nine of the seventeen mothers (Table 1). For all metabolites the number of mothers decreased as the study progressed, due to the cessation of the collection of samples from eleven mothers at 6 months of lactation. Storage capacity and average feed volumes were determined for only six mothers up to 6 months of lactation, the maximum period of exclusive demand breast-feeding. In all cases left and right breasts were treated separately, therefore *n*, unless otherwise stated, represents the number of individual breasts sampled.

All longitudinal analyses were performed using The SAS System for Windows v6.12 (SAS Institute Inc., Cary, NC, USA) with the general linear means (PROC GLM) procedure. Student's paired *t* tests and other statistics were performed using Statview™ SE+Graphics (Abacus Concepts Inc., Berkeley, CA, USA). All values are reported as means with their standard errors, unless otherwise stated.

Table 1. No of mothers sampled for each part of the study

	Stage of lactation (months)					
Variable measured	1	2	4	6	9	12
Volume	17	17	17	15	6	5
Fat	17	17	16	14	6	5
Lactose	9	9	8	8	6	5
Protein	9	9	9	8	6	5
Energy	9	9	8	8	6	5
Storage capacity	6	6	6	6		

Results

Variation of fat, lactose and protein in human milk over 24 h

The fat content of hind-milk was significantly higher than that of fore-milk ($P<0{\cdot}05$), but there were no significant differences for either lactose or protein. The mean CV (%) in fore- and hind-milk samples collected from left and right breasts for all mothers was 47·6 (SE 2·1) and 46·7 (SE 1·7) for fat (*n* 76), 9·86 (SE 1·66) and 8·37 (SE 1·21) for lactose (*n* 46), and 11·9 (SE 1·2) and 12·3 (SE 1·1) for protein (*n* 46) respectively.

The fat content of fore- and hind-milk samples obtained from an irregular feeding pattern (mean feed volume 60 (SD 35) ml) from breasts with larger storage capacities (an example is shown in Fig. 1(A), storage capacity 271 ml) varied more than the fat content of fore- and hind-milk samples for more regular feeding patterns (mean feed volume 73 (SE 30) ml) from breasts with smaller storage capacities (an example is shown in Fig. 1(B), storage capacity 124 ml). The standard deviation of the mean fat content of each feed over a 24 h period, as a measure of variability, was negatively correlated with the mean feed volume when expressed as a percentage of the storage capacity (Fig. 2; $P<0{\cdot}05$, $r\ -0{\cdot}319$, *n* 44).

Variation between left and right breasts

Milk production (Fig. 3) was found to differ significantly between left and right breasts at 1, 2, 4 and 12 months of lactation ($P<0{\cdot}05$). Overall, the mean 24 h milk production for the left breast was 356 (SD 129) ml and for the right breast was 443 (SD 141) ml.

There were no differences in either the contents of fat and energy, or concentrations of lactose and protein between milk from left and right breasts from 1 to 12 months of lactation (Fig. 4). Although there were some significant differences between the left and right breasts in the amounts of fat, lactose and protein delivered to the infant, there were no significant differences between left and right breasts in the amount of energy delivered to the infant at any stage of lactation (Fig. 4).

Milk production of left and right breasts was consistently different ($P<0{\cdot}05$) for five of the mothers over the first 6 months of lactation. For these mothers the breast that produced more milk was termed the 'preferred' breast (Fig. 5). There was no difference in the fat content of milk between preferred and non-preferred breasts. Nevertheless, the amount of fat delivered to the infant was greater ($P=0{\cdot}005$) from the preferred breast.

Variation over the first year of lactation

The composition and volume of milk removed from each breast over the first 12 months of lactation is shown in Table 2. The mean volume of milk produced per breast from 1 to 12 months of lactation was 399 (SE 11) ml/

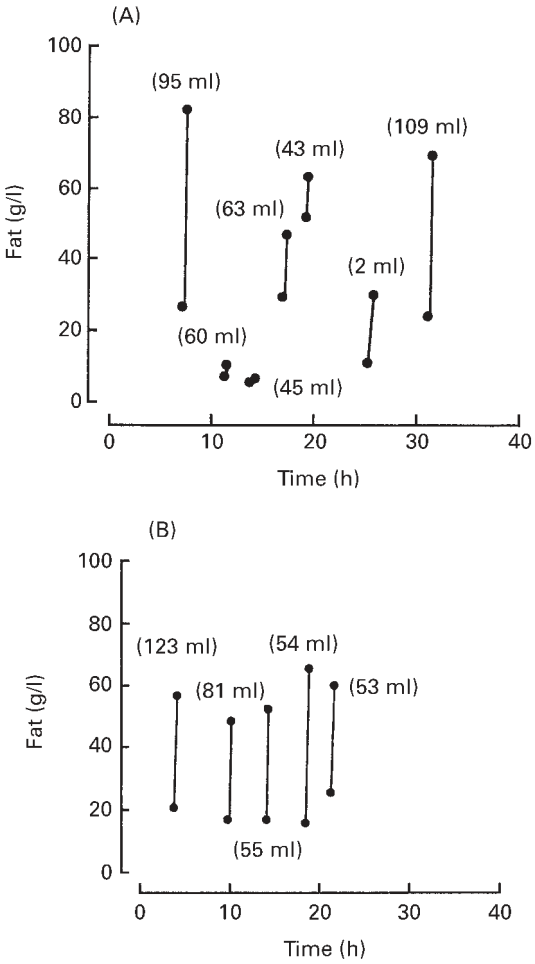

Fig. 1. Differences in the pattern of variation of milk fat content in fore- and hind-milk over 24 h between one breast with a large storage capacity (A: 271 ml; left breast, mother no. 6 at 6 months of lactation) and one breast with a small storage capacity (B: 124 ml; left breast, mother no. 2 at 4 months of lactation). Time 0 at 24.00 hours on the day sampling began. (●), Fore- and hind-milk samples from each feed. The volume of milk taken at each feed is shown in parentheses. For details of subjects and procedures, see p. 30.

24 h. Although there was no significant difference in milk production from 1 to 6 months of lactation, milk production at 12 months was significantly less than that at 6 months of lactation ($P<0{\cdot}05$; Table 2).

The fat content of milk and the amount of fat delivered in 24 h per breast over the first 12 months of lactation were 37·4 (SE 0·6) g/l and 14·8 (SE 0·5) g respectively. The fat content of milk differed greatly between women ($P=0{\cdot}0001$), with some mothers producing milk with a fat content >50 g/l, while in others it was <35 g/l at all stages of lactation (Fig. 6). Mean fat content showed marked differences over the 12 months of lactation ($P=0{\cdot}0001$), decreasing from 39·9 (SE 1·4) g/l at 1 month to 35·2 (SE 1·4) g/l at 2 months and then increasing again to 40·7 (SE 1·7) g/l at 9 months. In contrast, the mean amount of fat delivered to the infant did not change with stage of lactation.

The mean concentration of lactose and protein in milk from 1 to 12 months of lactation was 61·4 (SE 0·6) g/l and 9·2 (SE 0·2) g/l respectively (Table 2), but there were

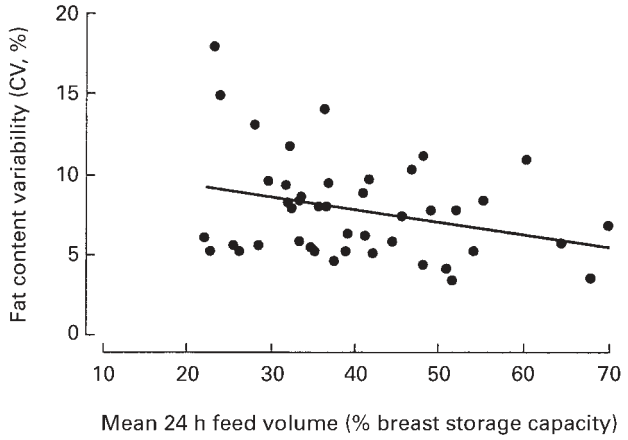

Fig. 2. Relationship between the mean 24 h feed volume (expressed as a percentage of breast storage capacity) and the standard deviation of daily milk fat content as an indicator of the variability of milk fat content over 24 h for left and right breasts of six mothers from 1 to 6 months of lactation. $P<0{\cdot}05$, r −0·319, n 44. For details of subjects and procedures, see p. 30.

significant differences ($P=0{\cdot}0001$) between women. The concentration of lactose in milk did not change with stage of lactation, whereas the concentration of protein decreased from 10·5 (SE 0·4) g/l at 1 month to 8·0 (SE 0·4) g/l at 6 months, and then remained steady. The amount of lactose and protein delivered to the infant (Table 2) differed between women ($P=0{\cdot}0001$) and declined with stage of lactation ($P<0{\cdot}0253$).

The energy content and amount delivered to the infant was 2·65 (SE 0·04) kJ/ml and 1007 (SE 39) kJ per breast respectively. Both the energy content and amount delivered to the infant differed between women ($P=0{\cdot}0001$). However, only the energy content differed with stage of lactation ($P=0{\cdot}0001$), decreasing from 2·7 (SE 0·1) kJ/ml at 1 month to 2·5 (SE 0·1) kJ/ml at 2 months and then increasing to 2·8 (SE 0·1) kJ/ml at 9 months.

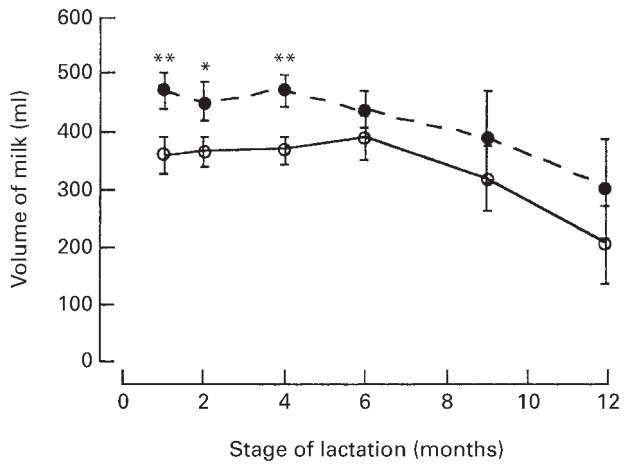

Fig. 3. Volume of milk produced over a 24 h period for left (○—○) and right (●– –●) breasts from 1 to 12 months of lactation. Differences at individual time points between left and right breasts are indicated, *$P<0{\cdot}05$, **$P<0{\cdot}01$, ***$P<0{\cdot}001$. Values are means with their standard errors represented by vertical bars. For details of subjects and procedures, see p. 30.

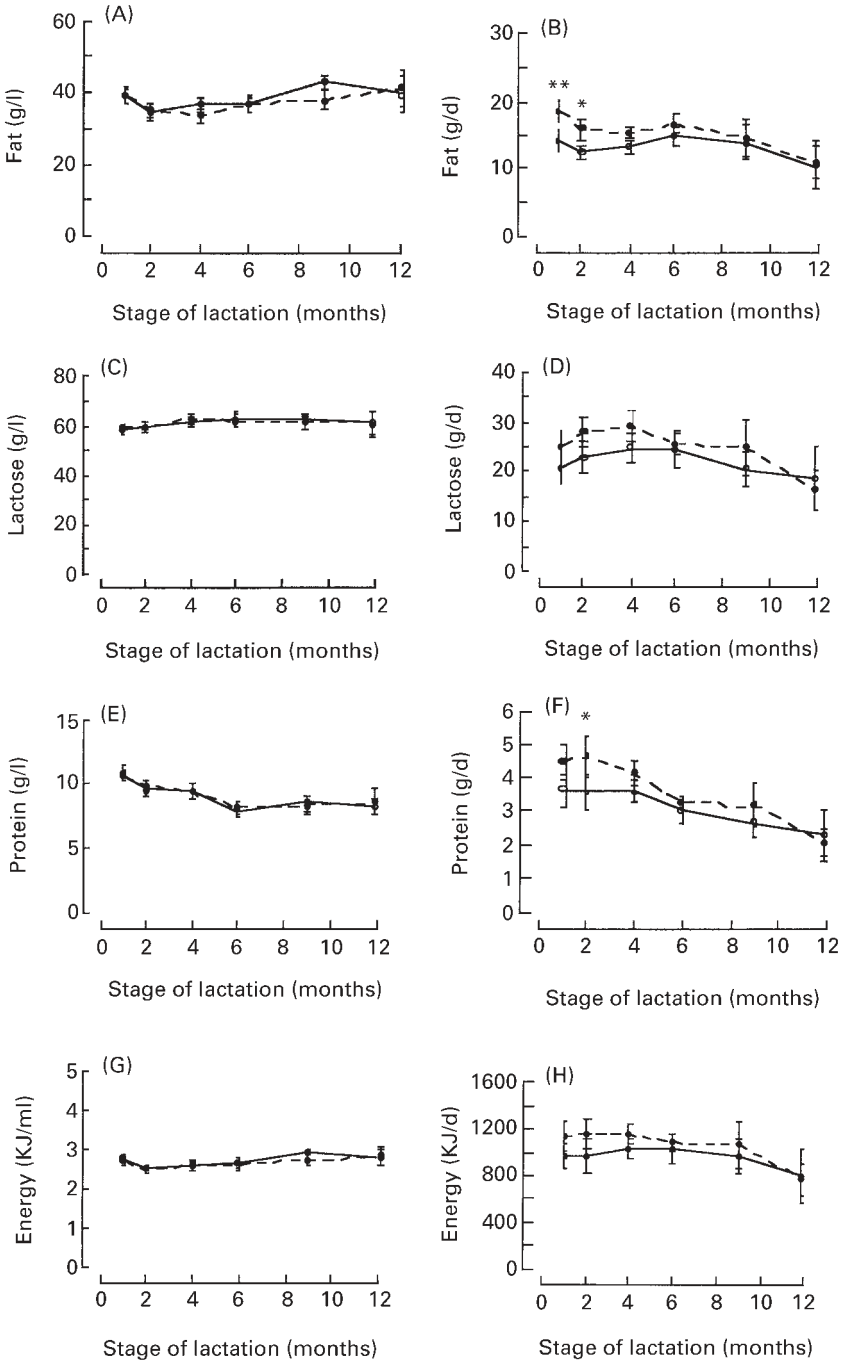

Fig. 4. Milk fat content (A), amount of fat delivered over 24 h (B), milk lactose concentration (C), amount of lactose delivered over 24 h (D), milk protein concentration (E), amount of protein delivered over 24 h (F), milk energy content (G) and amount of energy delivered over 24 h (H) for left (○—○) and right (●---●) breasts from 1 month to 12 months of lactation. Differences at individual time points between left and right breasts are indicated, $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$. Values are means with their standard errors represented by vertical bars; *n* at each time point is shown in Table 1. For details of subjects and procedures, see p. 30.

Infant growth

Growth rates for six infants were 20·4 (SE 2·0) g/d from birth for the first 6 months of life. No significant relationships were found between growth rate of the infants and either the amount taken in by the infant or the mean milk concentrations of fat, lactose, protein and energy for the first 6 months of lactation. Energy intake (kJ/kg body weight) from breast milk for four of the infants decreased significantly ($P=0{\cdot}0006$) from 1 month (456 (SE 64)) to 6 months of lactation (268 (SE 33)).

Table 2. Production, composition and infant intake of fat, lactose, protein and energy from human milk during the first 12 months of lactation*
(Mean values with their standard errors for no. of samples per breast shown)

Stage of lactation (months)…	1			2			4			6			9			12			1–12		
	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*
Volume (ml/24 h)	416^{a}	24	34	408^{ab}	23	34	421^{a}	20	34	413^{ac}	25	30	354^{bcd}	47	12	252^{d}	51	10	399	11	154
Fat: g/l	39·9^{a}	1·4	34	35·2^{b}	1·4	34	35·4^{b}	1·4	32	37·3^{ab}	1·4	28	40·7^{c}	1·7	12	40·9^{c}	3·3	10	37·4	0·6	150
g/24 h	16·4^{a}	1·2	34	14·2^{ab}	0·95	34	14·3^{ab}	0·6	32	15·7^{ab}	0·9	28	14·3^{ab}	1·9	12	10·4^{b}	2·0	10	14·8	0·5	150
Lactose: g/l	59·7^{a}	0·8	18	60·4^{ab}	1·1	18	62·6^{b}	1·3	16	62·5^{ab}	1·7	16	62·8^{b}	1·5	12	61·4^{ab}	2·9	10	61·4	0·6	90
g/24 h	22·9^{ab}	2·0	18	25·4^{ac}	2·2	18	27·0^{a}	2·2	16	25·1^{ad}	2·1	16	22·4^{bcde}	3·2	12	17·4^{e}	3·5	10	23·86	1·0	90
Protein: g/l	10·5^{a}	0·4	18	9·6^{a}	0·37	18	9·33^{a}	0·42	18	8·03^{b}	0·38	16	8·34^{bc}	0·45	12	8·34^{ac}	0·57	10	9·16	0·19	92
g/24 h	4·0^{a}	0·4	18	4·05^{a}	0·41	18	3·83^{a}	0·23	18	3·13^{b}	0·2	16	2·94^{b}	0·39	12	2·14^{b}	0·41	10	3·5	0·15	92
Energy: kJ/ml	2·72^{a}	0·06	18	2·5^{b}	0·06	18	2·58^{ab}	0·09	16	2·62^{ab}	0·09	16	2·81^{c}	0·09	12	2·79^{c}	0·14	10	2·65	0·04	90
kJ/24 h	1030^{a}	89	18	1047^{ab}	88	18	1081^{a}	62	16	1040^{ac}	75	16	995^{ac}	132	12	738^{bc}	141	10	1007	39	90

^{a,b,c,d,e}Mean values with unlike superscript letters were significantly different ($P<0·05$).
* For details of subjects and procedures, see p. 30.

Discussion

A number of protocols have been employed to measure the variation in the fat content of milk both during a breast-feed and between breasts. These procedures range from the removal of milk from the non-feeding breast at

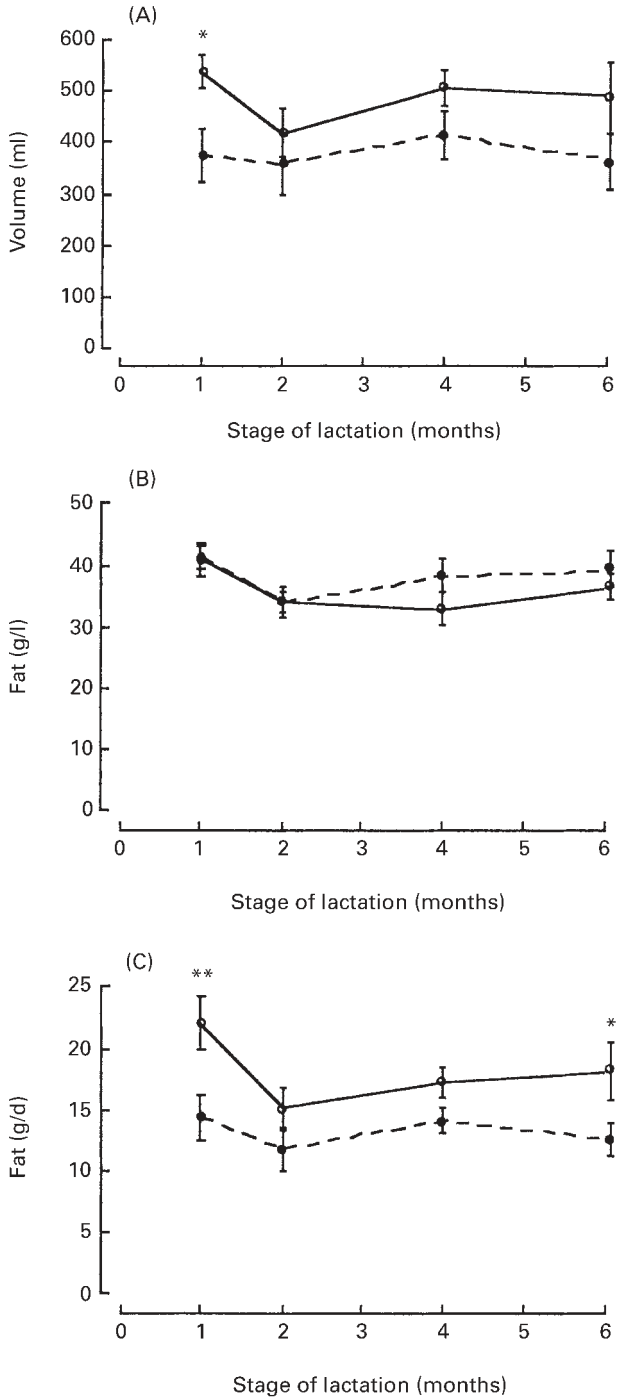

Fig. 5. Volume of milk produced (A), milk fat content (B) and amount of fat delivered (C) over 24 h for preferred (○—○) and non-preferred (●---●) breasts for five mothers at 1 – 6 months of lactation. Differences at individual time points between left and right breasts are indicated, *$P<0·05$, **$P<0·01$, ***$P<0·001$. Values are means with their standard errors represented by vertical bars. For details of subjects and procedures, see p. 30.

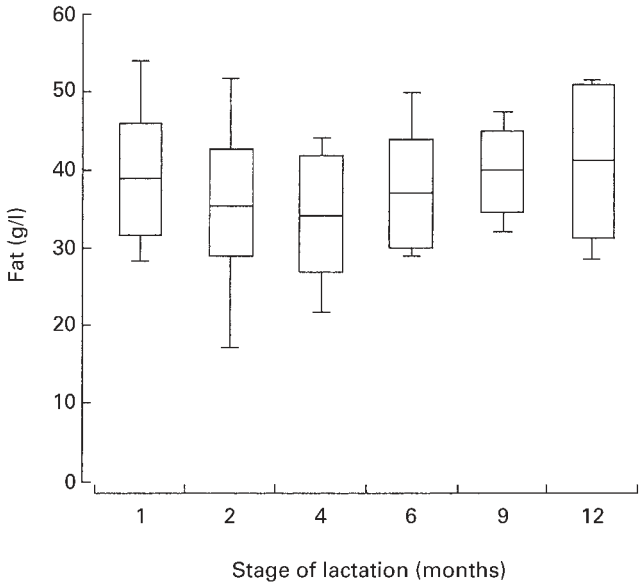

Fig. 6. Daily fat content of milk from all mothers at 1, 2, 4, 6, 9 and 12 months of lactation. Values are mean (—) and standard deviations (□) and ranges, represented by vertical bars. For details of subjects and procedures, see p. 30.

alternate breast-feeds over the course of the day (Butte *et al.* 1984; Garza & Butte, 1986; Nommsen *et al.* 1991) to the collection of either random or timed milk samples (Hall, 1979; Lauber & Reinhardt, 1979; Bitman *et al.* 1983; Allen *et al.* 1991). We used a sampling protocol similar to that of Hartmann *et al.* (1986) that involved the collection of fore- and hind-milk samples and the measurement of the production of milk from each breast at each feed over a period of 24 h at 1, 2, 4, 6, 9 and 12 months of lactation to account for the variation in fat and to accurately determine the concentrations of fat, lactose, protein and energy in human milk from an initial group of seventeen mothers. Whilst the total number of mothers recruited may seem low it must be stated that this demanding sampling protocol involved the collection and multiple analysis of over 2000 milk samples, as well as requiring mother participation for a minimum of 6 months and a maximum of 12 months.

The maximum amount of milk that can be stored in the breast and is available to the infant, storage capacity (Daly *et al.* 1993*b*; Kent *et al.* 1999), and the infant's appetite indirectly influenced the content of fat in milk. For the breast with a large storage capacity ($\geq$200 ml) the volume of each feed over the course of the day can vary greatly, with the infant rarely draining the breast at any one feed (Fig. 1(A)). This factor allows for less regularity in timing and volume of milk removed for each feed, and results in increased variation in the content of fat in fore- and hind-milk (Fig. 1(A)). Thus, any one feed is unlikely to approximate the daily average. On the other hand, for a breast with a small storage capacity ($\leq$150 ml) the feed volume more closely approximates the storage capacity, and the variation between the fat content of fore- and hind-milk will be low (Fig. 1(B)) and is more likely to be representative of the daily average. Consequently, the collection of milk samples either randomly or at particular times of the day will be unrepresentative for women with larger storage capacities because of the variability exhibited both in the fat content of fore- and hind-milk and in the intake of milk at each breast-feed.

The significant difference in milk production between breasts (P=0·0001; Fig. 3) is in agreement with previous findings (Morrison, 1952; Hytten, 1954; Cox *et al.* 1996). The daily mean content of fat and energy together with the concentrations of lactose and protein in milk were found to be the same between breasts within women over the course of the study (Fig. 4(A, G, C and E respectively)). Furthermore, when left and right breasts were reclassified as preferred and non-preferred, based on the consistent differences (P<0·05) in milk production (Fig. 5(A)), no differences between breasts were found in the fat content of milk (Fig. 5(B)). This similarity in the daily mean composition of milk between breasts (either left or right, or preferred or non-preferred) within women validates those methods that remove milk from the non-feeding breast at alternate breast-feeds over the course of the day for the determination of milk composition. In addition, the finding that there was no significant difference between the mean 24 h content of fat in milk in each breast may reflect an additional level of control on milk synthesis in women. Apart from the endocrine and autocrine control mechanisms of milk synthesis and production (Hartmann *et al.* 1998) there may also exist a 'metabolic' level of control based on the homeorhetic model proposed by Bauman & Currie (1980).

The changes observed for all measured components over the first 12 months of lactation (Table 2) were similar to those reported previously. Milk production was constant for the first 6 months (Dewey & Lonnerdal, 1983; Hartmann *et al.* 1995; Cox *et al.* 1996), after which there was a steady decline (Neville *et al.* 1991). The fat content of milk decreased between 1 and 4 months (Butte *et al.* 1984), before increasing by 12 months of lactation (Ferris & Jensen, 1984; Allen *et al.* 1991). The concentration of protein in milk decreased by 6 months of lactation (Hytten, 1954; Prentice *et al.* 1981; Butte *et al.* 1984; Allen *et al.* 1991) and then remained constant (Neville *et al.* 1991), whereas the concentration of lactose remained constant throughout the first year of lactation (Hartmann & Kulski, 1978). The decrease in the energy content at 2 months and the subsequent increase by 9 months can be attributed to the changes in fat content of the milk (Fig. 5).

The minimum requirements for the estimation of the energy intake of the breast-fed infant are the measurement of milk production over a 24 h period together with the accurate determination of the average composition of the breast milk consumed by the infant over the same period (Hartmann *et al.* 1998). The mean total 24 h milk production from both breasts was 798 (SD 232) ml (Table 2), which is consistent with previous reports for milk production in women (Butte *et al.* 1984; Dewey *et al.* 1986; Hartmann *et al.* 1995; Cox *et al.* 1996). The mean fat, lactose and protein contents (g/l) of breast milk (37·4 (SE 0·6), 61·4 (SE 0·6), 9·16 (SE 0·19) respectively; Table 2) was determined by averaging the fore- and hind-milk

concentrations of fat, lactose and protein for each breast-feed from each breast over the 24 h period and weighting the values for the amount of milk consumed from each feed from each breast at 1, 2, 4, 6, 9 and 12 months of lactation (Picciano, 1984). Although these values are similar to those reported previously (Jensen *et al.* 1995), there was considerable variation about the means (CV 21·2, 9·3 and 19·9 % respectively). Thus, the concentrations of fat, lactose, protein, and energy, as well as milk production, differed significantly between women ($P=0{\cdot}0001$), resulting in infants of the same age receiving different daily intakes. These results highlight the differences in milk composition between women and emphasise the inadequacy of using population averages of milk composition to determine either the intakes of individual breast-fed infants or the dietary requirements for lactation of individual mothers (Hartmann *et al.* 1995).

Growth rates over the first 6 months of life for the six infants monitored were not related to either the concentrations or amounts of fat, lactose, protein and energy in milk. Indeed, the only factor to have an effect on growth rate was milk intake (Kent *et al.* 1999). These results, from a small group of infants, are supported by the findings of a larger study (Butte *et al.* 1984), and together they highlight the importance of initially addressing milk intake by the infant and the possible mismanagement of breast-feeding, rather than questioning milk quality or composition, during the clinical treatment of slow-weight-gain infants (Lawrence & Lawrence, 1999).

Previous reports have shown energy intakes of formula-fed infants to be greater than those of exclusively breast-fed infants (Garza & Butte, 1990; Heinig *et al.* 1993). In the current study the increase in body weight between 1 and 6 months of age was obtained for four of the infants. For these breast-fed infants energy intake (kJ/kg body weight) at 1 month of age was not different from what they would have received if they had been fed formula, as directed by the manufacturer. However, at 6 months of age the energy intake from breast milk had decreased significantly ($P<0{\cdot}05$), whereas that for formula did not. These results, obtained with a different sampling protocol, corroborate those of Heinig *et al.* (1993) and show, as expected, that each infant received less energy (kJ/kg body weight) as lactation progressed (Dewey & Lonnerdal, 1983). When combined with the findings of Kramer (1981) and von Kries *et al.* (1999) that breast-feeding has a protective effect against childhood obesity, these results, albeit from a small group of only four subjects, add to the dispute over the current recommendations for the energy intake for formula-fed infants, and further support the establishment of new dietary guidelines based on the energy intakes of breast-fed infants.

In conclusion, from our studies using an initial group of seventeen women we have found that milk composition does not differ between either left and right breasts or between preferred and non-preferred breasts. It is due to this factor that the results on milk composition obtained by the current study (using a rigorous sampling routine) are similar to those of previous studies, indicating that it is possible to determine population averages by a variety of sampling schedules. However, the finding that the daily variation in milk fat content together with average milk composition and production differ significantly between women shows that all other sampling schedules cannot provide an accurate indication of the intake of fat, lactose, protein and energy of the individual infant and must, therefore, be interpreted cautiously.

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