Breast-milk fat concentrations of rural African women
1. Short-term variations within individuals

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1. Detailed studies of variations in breast-milk fat concentrations were performed over 12 or 24 h periods on sixty rural Gambian women feeding on demand. The creamatocrit method (Lucas *et al.* 1978) was used.

2. The complex pattern of within-feed and between-feed changes in fat concentration was largely explained by differences in volume per feed and time interval between feeds.

3. No consistent difference in fat production between the breasts of each individual was found despite the local custom of starting all feeds on the right breast.

4. A marked diurnal variation in the mean fat concentration per feed was noted. On average the highest values occurred in the early morning, the lowest values in the late afternoon.

5. There was significantly greater between-individual than within-individual variation in the mean fat concentration per feed over 12 h ($P < 0.001$).

6. A simple, rational and non-intrusive sampling procedure was devised for the estimation of whole-day mean fat levels based on the finding that the mean fat concentration of small samples of milk (0·25 ml) taken from both breasts before and after one specific feed of the day was closely correlated with 12 h and 24 h mean fat concentrations.

During investigations in a rural West African village into the relationship between infant growth and breast-milk intake, considerable variations in breast-milk volume were noted at different times of year, at different stages of lactation and between individual mothers (Whitehead *et al.* 1978; Prentice, 1980). Little was known to what extent variations in breast-milk composition could compensate for these variations in breast-milk volume. Of particular interest in this respect were changes in fat concentration as fat is the major energy component of breast-milk and as fat concentration is independent of milk volume (Hytten, 1954*c*).

To facilitate a long-term investigation of breast-milk fat concentrations in this community a simple, practicable procedure was required which would enable serial milk samples to be obtained from a large number of subjects with the minimum of interference in the normal course of lactation. However, problems in obtaining representative samples of breast-milk caused by sizeable diurnal and within-feed variations in milk fat concentration have been widely documented (e.g. Hytten, 1954*a*, *b*).

The purpose of the study described in this paper was to establish the pattern of breast-milk fat concentration changes that occurs during the day in underprivileged African women feeding on demand and thereby to devise a rational sampling procedure for the estimation of daily mean fat concentrations.

EXPERIMENTAL

Subjects

The mothers involved in this study belonged to the isolated rural farming community of Keneba, The Gambia. The investigation formed part of a long-term study of nutrition and lactation in this community and had the approval of the Ethical Committees of the Dunn Nutrition Laboratory, Cambridge and the Gambian Government.

Sixty individuals took part in this study. The subjects were chosen to represent various stages of lactation (1–18 months post-partum) and parities (1–10). All mothers practised feeding on demand.

Experimental design

Fat concentrations were determined in small samples of milk (approximately 0·25 ml) obtained by maternal expression from both breasts immediately before and after every feed in a 12 h period from 07.00 to 19.00 hours. The duration of the feed was timed, the time of day noted and the order in which the breasts were presented to the child discreetly observed on each occasion. In addition, samples were collected from sixteen of the mothers thoughout the preceding 12 h to enable 12 h results to be related to 24 h values. To avoid any disruption of the normal course of lactation no attempt was made to collect samples during a feed. Although it is known that the variation in fat content during a feed is not linear (Hytten, 1954a; Emery *et al.* 1978), analysis of data presented by Hytten (1954*a*) indicated that a reasonable approximation of the mean feed fat concentration (average error 5%) can be obtained by taking the mean of the fat levels in the first and last milks to be drawn from the breast.

The breast-milk intake of the infants at each feed was determined by test-weighing. In order to achieve an estimate of the relative lactational performance of each mother 12 h breast-milk volumes obtained on the day of the study were corrected for stage of lactation and for season of the year, as both factors are known to influence breast-milk output (Whitehead *et al.* 1978). The mothers were classified into the following groups: very poor (< 70% of mean), poor (70–80% of mean), average (80–120% of mean) and good (> 120% of mean).

The study was carried out during routine breast-milk estimation sessions. These have been described in detail by Prentice (1980).

Fat analysis

Storage. The milk samples were either processed immediately or were stored at $-20°$ and analysed within 1 month of collection.

Creamatocrit determinations. Milk fat concentrations were determined in triplicate by the creamatocrit method of Lucas *et al.* (1978). Milk was taken into 75 mm non-heparinized microhaematocrit tubes. The tubes were closed at one end by sealant material to ensure a flat base for the milk column and were spun for five minutes in a microhaematocrit centrifuge. The heights of the milk and cream columns were read to the nearest 0·025 mm with vernier calipers against background illumination from a photographic safelight.

Calibration of creamatocrit values. The relationship between creamatocrit (the percentage ratio of cream and milk columns) and fat content was determined gravimetrically by a modification of the British Standard Method (1968) on twenty-three samples of pooled breast-milk. The calibration curve was linear and is illustrated in Fig. 1. The following regression equation was obtained:

$$\text{fat concentration} = \text{creamatocrit} \times 6{\cdot}48 - 1{\cdot}46 \text{ g/l}$$

with 95% confidence limits of $\pm 4{\cdot}4$ g/l. The equation was used throughout this study to convert creamatocrit values to fat concentrations. The relationship is similar to that obtained by Lucas *et al.* (1978); the difference in the intercept value is due to the subjectivity involved in reading to the top of the cream layer and stresses the need for each worker to determine his own calibration equation.

Precision. The range of fat concentrations encountered in this study was large: 2·6–179·3 g/l. The precision of the creamatocrit method was tested on two milk samples,

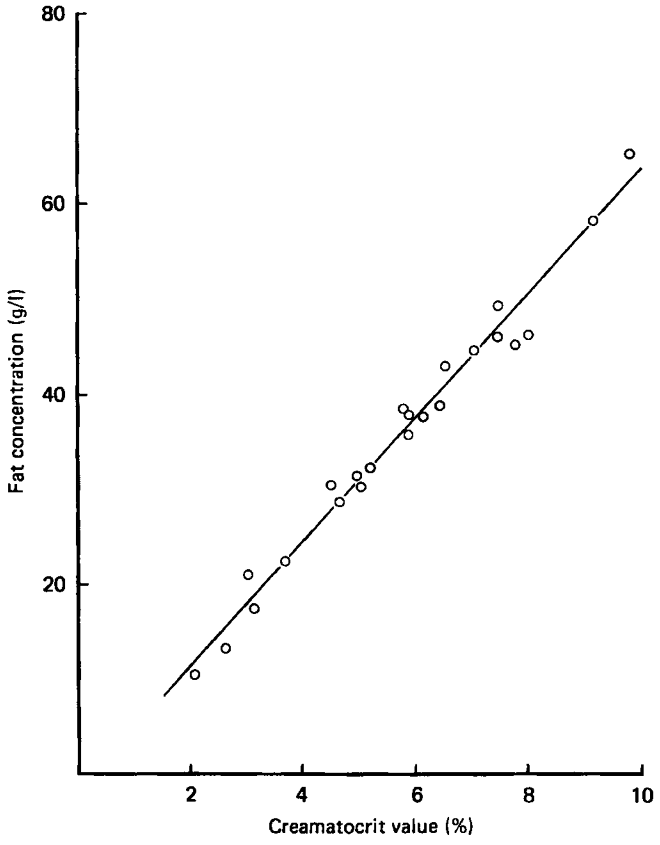

Fig. 1. Relationship between fat concentration (g/l) and creamatocrit value (%) of breast milk of rural African women. Regression equation: $y = 6{\cdot}48x - 1{\cdot}46$ g/l, r 0·99, n 23.

one of average fat content (37·8 g/l) and one of low fat content (7·3 g/l). The coefficient of variation on ten determinations of each sample was 1·3 and 2·8 respectively.

RESULTS

The results from three typical mothers are shown in Fig. 2 and these illustrate the large variations in breast-milk fat concentration that occurred within each feed, between feeds and at different times of the day. This demonstrates the difficulty in obtaining representative breast-milk samples for the analysis of an individual's milk fat levels and shows how true differences in fat intake of breast-fed infants could potentially be obscured.

The observed pattern of breast-milk fat concentration for each woman was found to be influenced by a number of factors. As similar regression equations were obtained when correlations were investigated using either results from a single feed or from every feed per subject, all the values measured in the study were combined to permit various groups of mothers to be analysed separately All regression equations are given in Table 1.

Change in fat concentration (ΔF) *during a feed*

In general, an increase in fat concentration was observed between milk collected at the beginning and at the end of a feed. A positive relationship was found between ΔF and the

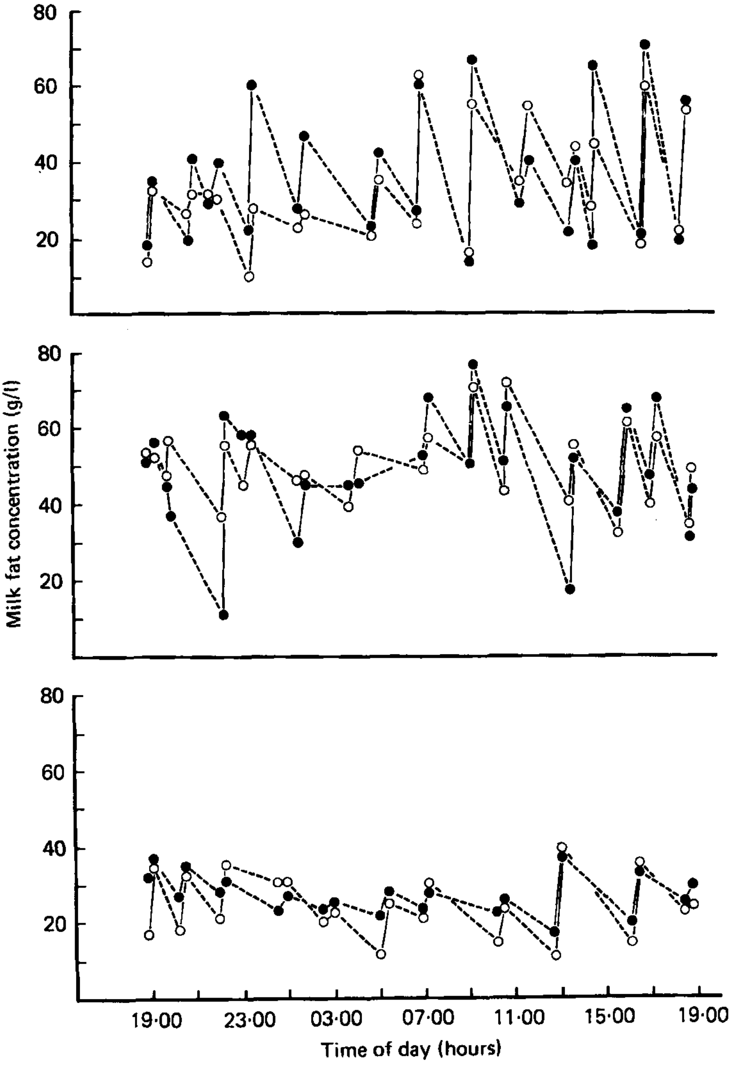

Fig. 2. Variations in breast-milk fat concentration (g/l) of three typical rural Arican women during a 24 h period. ——, Times within feeds. – – –, Times between feeds. ○, Left breast. ●, Right breast.

milk intake of the infant at that feed ($P < 0{\cdot}001$). On average a 100 ml feed was associated with a ΔF of 18 g/l in each breast. The relationship was unchanged when the following were considered separately: day-time and night-time feeds; mothers in different trimesters of lactation; mothers of different parity and mothers with different lactational performance.

Change in fat concentration (ΔF) *between feeds*

A decrease in fat concentration was generally observed between milk collected at the end of one feed and at the beginning of the next. A negative correlation was found between ΔF and the time interval between the feeds ($P < 0{\cdot}001$) during the period 06.00–22.00 hours. On average the fat concentration decreased by 10 g/l per 100 min in each breast. When feeds occurred between 22.00–06.00 hours, approximately the hours of sleep, no relationship was observed.

Table 1. *Tabulated regression equations*

x	*y*	Subjects‡	Slope	Intercept	*r*	*n*
Volume of feed (ml)	Fat increase during feed (g/l)†	All	0·177	5·9	0·44	410*
Fat decrease between feeds (g/l)†	Time interval (min)	All, 06.00–22.00 hours	0·096	6·4	0·50	318*
		All, 22.00–06.00 hours	—	—	0·07	51
		Good feeders	0·119	7·5	0·57	75*
		Average feeders	0·108	6·7	0·45	127*
		Poor feeders	0·080	2·7	0·59	27*
		Very poor feeders	0·066	6·5	0·49	19
Interval since previous feed (min)	Volume of feed (ml)	All	0·208	29·5	0·41	365*
		All, 22.00–06.00 hours	0·196	29·3	0·42	57*
		Good feeders	0·287	39·3	0·47	74*
		Average feeders	0·315	13·3	0·60	135*
		Poor feeders	0·261	11·4	0·61	30*
		Very poor feeders	—	—	0·19	18
Volume of previous feed (ml)	Corrected mean fat of a feed (%)§	All	0·130	91·0	0·24	263*
		First trimester	0·310	85·0	0·47	81*
Interval since previous feed (min)	Corrected mean fat of a feed (%)§	All, 07.00–19.00 hours	−0·123	113·0	−0·43	271*
		24 h subjects only	—	—	−0·16	173

* $P < 0{\cdot}001$.

† Mean fat change in both breasts.

‡ Good feeders, milk output > 120% of mean; average feeders, milk output 80–120% of mean; poor feeders, milk output 70–80% of mean; very poor feeders, milk output < 70% of mean.

§ The mean fat concentration of each feed of an individual expressed as a percentage of her mean 12 h fat concentration.

The day-time relationship was unaffected by considering mothers of different parity or in different stages of lactation separately. In addition, the correlation existed for mothers of different lactational performance but with a slight increase in the slope of the regression equation for women producing larger quantities of milk (see Table 1), indicating a higher rate of milk fat dilution amongst these women.

Relationship between breast-milk volume and time interval between feeds

For all except the very poor group of lactators, the breast-milk volume of a single feed was found to be positively related to the time interval since the last feed regardless of the time of day ($P < 0{\cdot}001$). The intercept of the regression equation increased with increasing lactational performance (see Table 1). The relationship was unchanged for mothers of different parity and in different trimesters of lactation.

No relationships were detected between the breast-milk volume at one feed and the following variables: ΔF before the next feed, the time interval before the next feed, the size of the next feed and ΔF during the next feed.

Fat levels in the two breasts of an individual

As can be appreciated from Fig. 2, it was rare to observe a similar ΔF during a feed or a similar absolute fat concentration before and after a feed in the two breasts of the same individual. This observation suggested that at any particular feed the two breasts were not suckled to the same extent.

It is a Moslem tradition that all feeds should begin with the right breast. Of the mothers in this study 59% adhered strictly to this custom and 88% of all the feeds observed started on the right. It was thought possible that this practice might place a greater stress on the right breast and might induce physiological differences between the two breasts. However, when the fat levels in the two breasts of individual mothers were considered separately over 12 h, no statistical difference was found using a paired t test between ΔF per feed in each breast. This was the situation both for women who always started with the right breast (t 1·53, df 30) and for all women taken together (t 1·70, df 51). Similarly there was no significant difference between the mean fat concentration per feed in each breast of mothers who always started on the right (t 1·72, df 30) or of all mothers considered together (t 1·70, df 51).

These results indicated that in general there was no consistent bias in fat production between the breasts of an individual as a consequence of this local custom. It was considered, therefore, that a fair approximation of the mean fat concentration of a feed would be given by the mean ($\bar{F}$) of the fat concentrations in milk obtained from both breasts before and after the feed.

Diurnal variation in mean fat concentration of a feed ($\bar{F}$)

The mean milk fat concentration of feeds given by each mother was found to vary considerably with the time of day. Fig. 3 shows the frequency distribution of the times at which the maximum and minimum $\bar{F}$ values were obtained. This demonstrated that in this population there was a marked tendency for feeds of maximum $\bar{F}$ to occur in the early morning and for those of minimum $\bar{F}$ to be found in the late afernoon. Using a paired t test the decrease in $\bar{F}$ between feeds occurring in the periods 07.00–09.00 hours and 17.00–19.00 hours for each individual was shown to be highly significant (t 4·58, df 55, $P < 0{\cdot}001$). On average the $\bar{F}$ value fell 19% between these two periods.

Figs 4 and 5 show the mean $\bar{F}$ in milk produced by all the mothers, together with the mean volume of milk consumed and the calculated mean milk fat intake of their infants at different times of the day. It can be seen that on average the time of highest $\bar{F}$ coincided

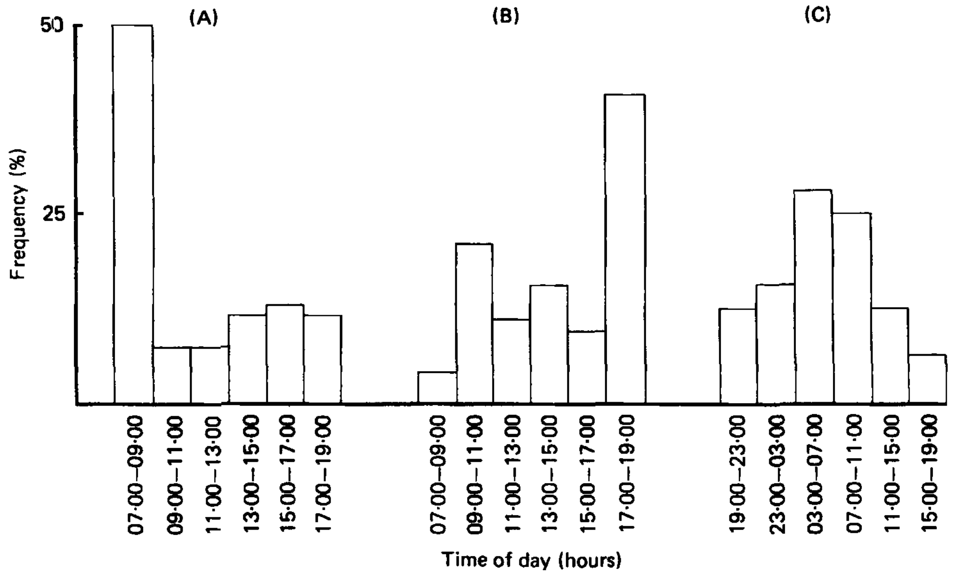

Fig. 3. Frequency distributions of occurrence of maximum (A, C) and minimum (B) mean fat concentrations in breast milk of individual rural African women. (A, B) are the maximum and minimum values occurring during a 12 h period respectively (*n* 54). (C) is the maximum values occurring during a 24 h period (*n* 16).

with the period of lowest milk output. However, the increase in $\bar{F}$ only marginally compensated for the decrease in breast-milk consumption leading to an over-all decrease in milk fat intake at this time.

The factors influencing the diurnal variation in $\bar{F}$ could not be fully elucidated. No relationship was found between $\bar{F}$ of one feed and the volume of that feed, even when night-time values were excluded or when $\bar{F}$ values for each woman were expressed as a fraction of her average $\bar{F}$ value over 12 h to take individual differences in milk fat levels into account. When $\bar{F}$ values were corrected in this way, a correlation was found between $\bar{F}$ and the volume of the preceding feed for mothers in the first trimester of lactation ($P < 0{\cdot}001$); the correlation coefficient decreased when all subjects were considered together although the relationship was still highly significant (see Table 1). A negative correlation was found between corrected $\bar{F}$ values and the time interval since the last feed provided only day-time values were considered ($P < 0{\cdot}001$) but was not significant for values in the 24 h study. The effect of the maternal feeding pattern was not examined. However, in this community it is rare for women to eat early in the day and it is unlikely, therefore, that the marked increases in $\bar{F}$ noted at this time were caused by a recent meal.

Characteristic mean fat concentration of an individual and development of the sampling procedure

Despite the marked diurnal variation in an individual's $\bar{F}$ values, it was apparent, as can be seen from Fig. 2, that certain mothers consistently produced feeds of higher $\bar{F}$ during the day than others. One-way analysis of variance revealed a significantly greater between-mother than within-mother variation in $\bar{F}$ values obtained during the 12 h period (F 8·73 on 59 and 296 df, $P < 0{\cdot}001$).

Thus, the milk produced by a woman at each feed during the day had a mean fat concentration representative of the individual on that day. Correlations between the average

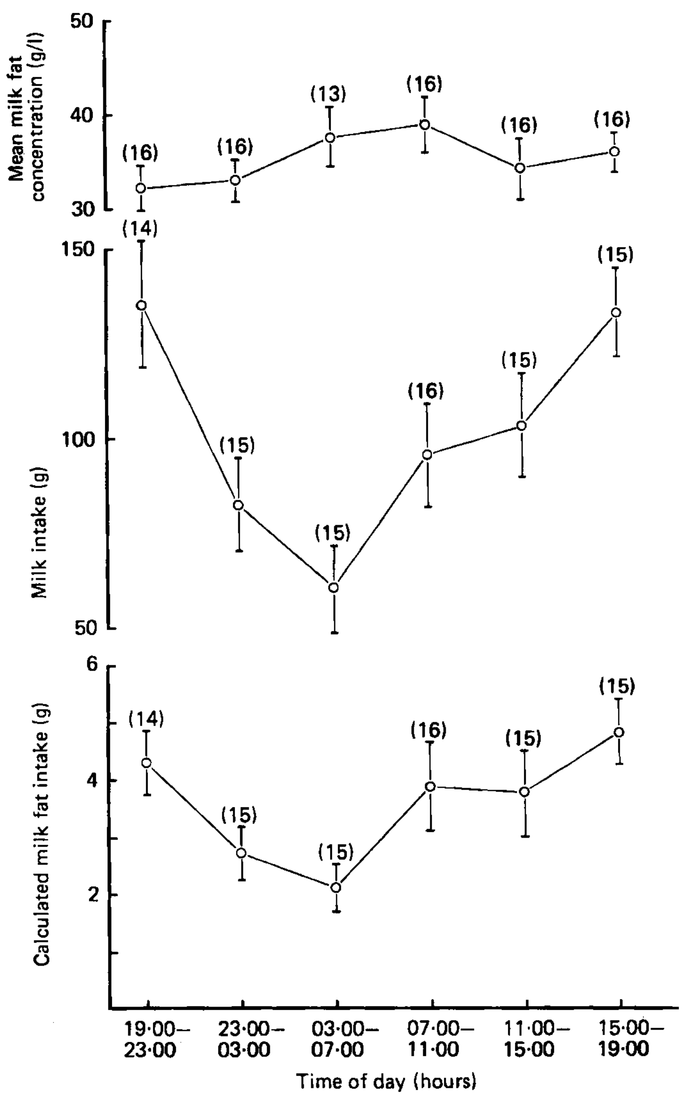

Fig. 4. Variations in breast-milk fat concentration (g/l), breast-milk intake (g) and calculated milk fat intake (g) during a 24 h period for rural African women. The points represent mean values, with their standard errors represented by vertical bars, for number of subjects indicated in parentheses.

F value over 12 h and $\bar{F}$ of single feeds occurring at specified times of day were investigated to determine whether 12 h mean fat concentrations could be predicted from $\bar{F}$ of one particular feed. The closest correlation with 12 h values was obtained at the first feed occurring after 13.00 hours (r 0·89, n 56, $P < 0·001$, residual standard deviation 5·45 g/l), possibly as the diurnal variation in $\bar{F}$ is only slight in the early afternoon (Fig. 5). The relationship is illustrated in Fig. 6 and provided the following regression equation with 95% confidence limits of ±11·0 g/l:

$$12 \text{ h mean } \bar{F} = \bar{F}\,(13.00 \text{ hours}) \times 0·66 + 14·4 \text{ g/l}.$$

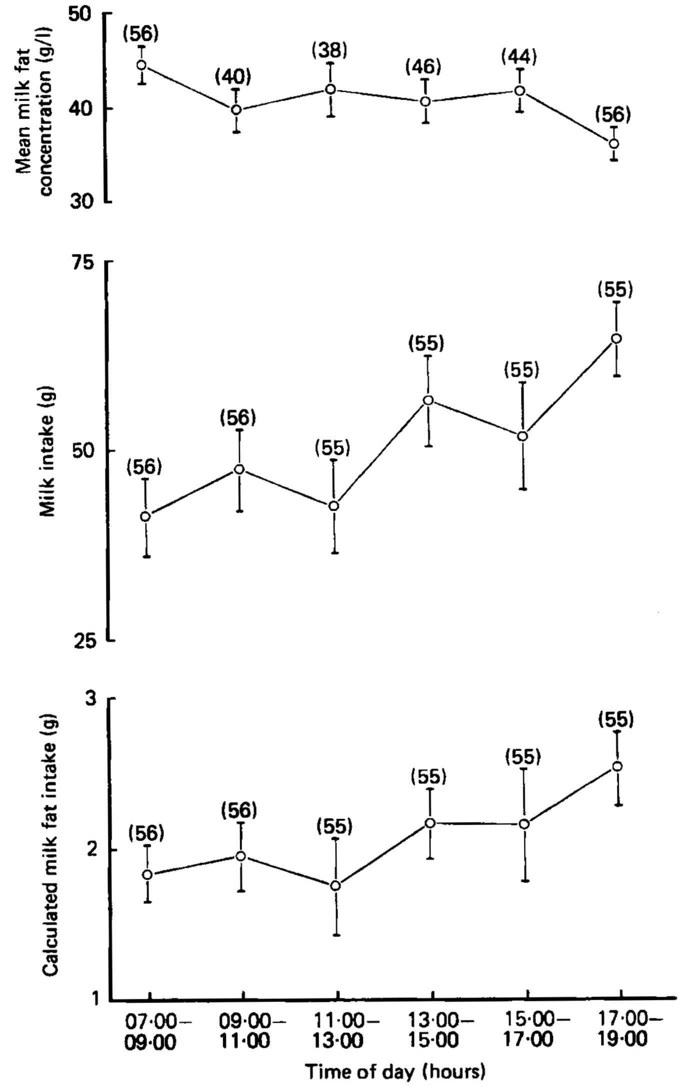

Fig. 5. Variations in breast-milk fat concentration (g/l), breast-milk intake (g) and calculated milk fat intake (g) during a 12 h period for rural African women. The points represent mean values, with their standard errors represented by vertical bars, for no. of subjects indicated in parentheses.

A similar correlation was found between 24 h mean values and the first feed after 13.00 hours (r 0·75, n 16, $P < 0{\cdot}001$, residual standard deviation 4·89 g/l), although as there was a sizeable contribution to the error of prediction caused by the small number of points involved the 95% confidence limits ranged from $\pm 10{\cdot}8$ g/l at the mean to $\pm 12{\cdot}7$ g/l at the mean ± 30 g/l. The regression equation was:

$$24\text{ h mean } \bar{F} = \bar{F}\text{ (13.00 hours)} \times 0{\cdot}43 + 21{\cdot}0\text{ g/l.}$$

On the basis of these results it was evident that the mean fat concentration of small samples of milk collected from both breasts immediately before and after the first feed occurring after 13.00 hours could be used for the estimation of 12 h and 24 h mean fat

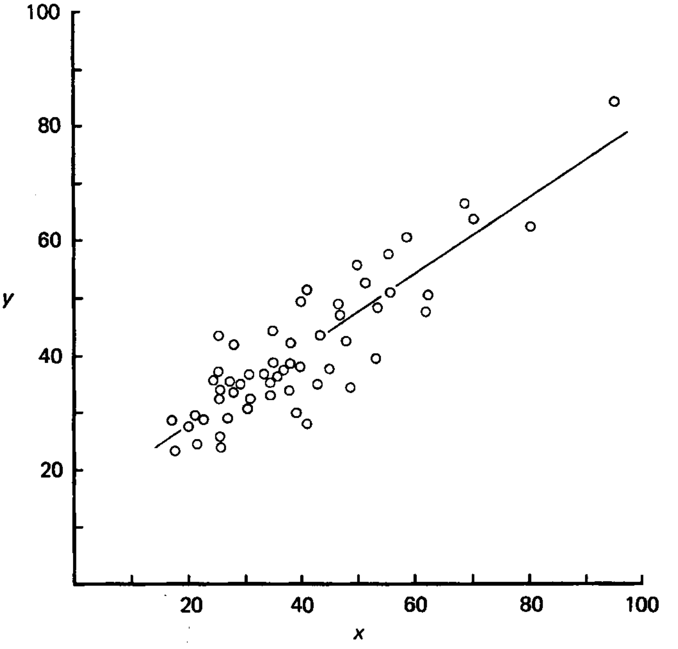

Fig. 6. Relationship between 12 h (07.00–19.00 hours) mean breast-milk fat concentration (g/l; y) and mean breast-milk fat concentration of the first feed occurring after 13.00 hours (x) in rural African women. Regression equation: $y = 0{\cdot}66x + 14{\cdot}4$ g/l, r 0·89, n 56.

concentrations and that this measurement would constitute a simple and practicable procedure for long-term studies of breast-milk fat concentration.

DISCUSSION

The women who participated in the present study were representative of mothers in many parts of the developing world in so far as they maintained long periods of lactation in spite of the multiple handicaps associated with poor nutritional status and underprivileged living conditions. Among such women the practice of demand-feeding results in a high feed frequency and in low mean feed volumes. In Keneba the mean feed frequency decreases from fifteen to ten feeds per 24 h as lactation progresses while the mean feed volume remains close to 55 ml per feed throughout lactation (Whitehead *et al.* 1978).

In spite of the fundamental differences in feeding habits, many similarities were found between the diurnal pattern of milk fat changes demonstrated in this study and in studies of well-nourished women feeding on fixed schedules. For example, with scheduled feeders the milk fat concentration is known to rise during a feed (Macy *et al.* 1931; Morrison, 1952; Hytten, 1954*a*; Hall, 1979), to decrease between feeds by an amount that is possibly related to the time interval (Hytten, 1954*a*) and to differ between the breasts of an individual at any one feed (Hytten, 1954*a*; Hall, 1979). Similarly, the time interval between scheduled feeds is thought to affect the mean fat concentration (Kon & Mawson, 1950) and the breast-milk yield (Deem, 1931; Hytten, 1954*b*) of the next feed. In addition, although there have been no detailed reports in the literature of a relationship between the fat change during a feed and the volume of that feed for women feeding on schedule, such a relationship was implied by Newton (1952) who described a test for lactational success which involved measuring the milk fat change occurring during a single feed.

One striking difference between the pattern of milk fat changes found for mothers feeding on demand in this village and for those feeding at fixed intervals in more prosperous communities was the diurnal variation in mean fat concentration per feed. In Keneba the highest mean fat concentration generally occurred in the early morning. For women on fixed schedules feeds at this time are associated with the lowest mean fat concentration of the day (Deem, 1931; Hytten, 1954*b*) even when an extra feed is included at night (Nims *et al.* 1932; Gunther & Stanier, 1949).

In spite of the marked diurnal variation in fat concentrations, the individuals in this study exhibited a characteristic level of mean milk fat concentration per feed thoughout the day. This may have been a consequence of the high feed frequency associated with demand-feeding as this has not been observed with women feeding on fixed schedules (Hytten, 1954*b*; Picciano & Guthrie, 1976). This finding led to the conclusion that in this community the determination of the mean fat concentration of a single feed could provide an estimate of the 12 h and 24 h milk fat levels of a mother and would allow differences in milk fat levels of individuals to be assessed.

The large variations in milk fat concentration which occur during the day have posed severe sampling problems in previous studies of breast-milk quality. In an attempt to overcome these problems several authors advocate the analysis of milk collected by breast-pump or by manual expression from both breasts over a full 24 h period (Hytten, 1954*b*; Janz *et al.* 1957; Picciano & Guthrie, 1976; Jensen *et al.* 1978). However, information obtained in this way can bear little relationship to the milk fat ingested by a breast-fed infant during normal lactation especially in communities where demand-feeding is practised. Also, such procedures are neither practicable nor ethically justifiable when repeated sampling from an individual is required. In particular when repeated measurements are to be made on the same subjects the sampling procedure must avoid any disruption of the normal course of lactation. The volume of the sample must be kept to a minimum as the volume of milk produced at each feed may be small. The sampling procedure must not interrupt the flow of milk and, as the procedure must be well accepted by the mother, it must be quick and easy to implement, requiring only a minimum of subject involvement.

The sampling procedure developed during this study, in which small samples of milk (approximately 0·25 ml) are taken from both breasts of a mother before and after the first feed occurring after 13.00 hours, meets all the above requirements. This, coupled with the use of the creamatocrit method of fat analysis which is ideally suited to work in the field, makes it possible to determine the milk fat levels of a large number of individuals on specified days and to repeat the determinations as necessary. It must be stressed, however, that the relationship between whole-day values and the mean fat concentration at the first feed after 13.00 hours may only hold for mothers in this community and may not be applicable to demand-feeding communities elsewhere.

The sampling procedure described in this paper has been used on a routine basis in the Keneba study for many months. The long-term variations in milk fat levels of mothers in this community are described in the accompanying paper.

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