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Estimation of 24 h breast-milk fat concentration and fat intake in rural northern Thailand

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1. The present study assesses the accuracy with which mean 24 h breast-milk composition can be estimated if milk samples can only be collected during the daytime.

2. Twenty-five northern Thai mothers, feeding their infants on demand, were studied in their homes for 24 h. All feeds were test-weighed and 0·5 ml pre- and post-feed expressed milk samples taken at each feed.

3. If daytime sampling was restricted to two breast-feeds, it was found that the best estimate of 24 h fat concentration was given by two randomly chosen daytime feeds, predicting 24 h fat concentration with 95% confidence limits of $\pm 7{\cdot}0$ g/l (equivalent to 21% of mean 24 h fat concentration).

4. Alternative sampling methods using the mean fat concentration of the feed after 08.00 hours and after 18.00 hours, or the first two feeds after 12.00 hours, predicted fat concentration with 95% confidence limits of $\pm 9{\cdot}7$ g/l and $\pm 8{\cdot}9$ g/l (28 and 26% of mean 24 h fat concentration) respectively.

5. If well-tolerated by mothers, it would be preferable to sample all daytime feeds, since this reduces the 95% confidence limits to $\pm 3{\cdot}3$ g/l, equivalent to 10% of the mean 24 h fat concentration.

Estimation of the energy intake of the breast-fed infant requires accurate measurement of both the composition and the volume of breast-milk consumed. Since the fat concentration of breast-milk shows circadian variation (Hytten, 1954; Brown *et al.* 1982; Prentice *et al.* 1981; Jackson *et al.* 1988) it is important to obtain milk samples which are representative of the composition of milk consumed over a 24 h period. If repeated measurements are made on the same mother, the samples should be small to avoid interfering with lactation and the infant's feeding pattern (Jackson *et al.* 1988).

Where breast-milk intake is measured by test-weighing the infant before and after each feed, personnel are likely to be on hand for obtaining milk samples. In some societies test-weighing of night-time feeds may be feasible, and nocturnal milk samples can then be collected as part of the protocol. More usually, test-weighing causes unacceptable disruption of normal nocturnal feeding and sleep patterns, particularly in cultures where mother and infant sleep together. Night-time intake can nevertheless be estimated accurately with minimal disturbance by means of a new technique, 'indirect test-weighing', in which night-time milk intake is calculated from the overnight weight changes of the mother and infant, adjusted for insensible water loss (Woolridge *et al.* 1987). As indirect test-weighing does not require any interference with mother and child during the night, taking night-time milk samples would defeat the very purpose for which this technique was devised. We have therefore thought it necessary, in preparation for 24 h studies of dietary intake of breast-fed infants using indirect test-weighing, to establish the accuracy with which mean 24 h breast-milk composition can be predicted from milk samples taken at daytime feeds.

The frequent removal of milk samples may, however, be unacceptable to mothers, and the available laboratory facilities may limit the number of samples that can be analysed. In these cases it would be necessary to restrict sampling of feeds to particular times of the day. The accuracy of estimating fat concentration when only limited daytime samples are available was therefore also assessed.

METHODS

Twenty-five mothers and their babies (aged between 3 weeks and 9 months) were studied in their homes in a rural district of Chiang Mai Province, northern Thailand. All breast-feeds during a 24 h period from 08.00 hours were timed and test-weighed. The majority of the mothers always gave one breast at a feed (80% of all feeds were one-breast-feeds). A breast-feed was therefore defined as milk intake from one breast; the second breast of any two-breast-feeds was treated as a separate feed. Milk samples (0·5 ml) were expressed before and after each feed. Additional 'scheduled samples' (0·5 ml) were expressed from both breasts at 20.00 and 08.00 hours. If the baby happened to be feeding when a scheduled sample was due to be taken, the pre- or post-feed sample from that breast closest to the scheduled time was taken to represent the scheduled sample. The fat concentration of milk consumed during a feed was calculated as the mean fat concentration of the pre- and post-feed samples from that breast. 'Daytime' refers to the period when the mother was awake, 'night-time' to the time when mother and baby were asleep together. Further descriptions of the study group and methods are given in Jackson *et al.* (1988).

Night-time fat concentration

If sampling is not limited, 24 h milk composition and fat intake can be estimated directly from all the daytime feeds. However, a separate estimate of night-time composition used in conjunction with the available daytime data might be more accurate. Night-time fat concentration was estimated using two predictors: (1) the mean fat concentration of the four scheduled samples taken at 20.00 and 08.00 hours, (2) the mean fat concentration of the last feed before the mother went to bed and the first feed after she woke up the following morning.

24 h fat concentration, limited daytime sampling

We have also examined the accuracy with which 24 h milk composition can be estimated when daytime sampling is limited to two feeds.

(1). In this population mean fat concentration of breast-milk is lowest between 04.00 and 08.00 hours while peak values occur between 16.00 and 20.00 hours (Jackson *et al.* 1988). An early and a late feed might therefore be representative of average milk composition over 24 h. Predictor fat concentration was taken as the mean of the first feed of the study day, which began at 08.00 hours ('08.00 hours feed') and the first feed after 18.00 hours ('18.00 hours feed'). All the mothers breast-fed at least once between 18.00 hours and retiring to bed, so this feed could be relied upon to take place, presenting no practical problems.

(2). On average, in this study group, fat concentration is closest to the 24 h mean fat concentration between 08.00 and 12.00 hours and between 12.00 and 16.00 hours (Jackson *et al.* 1988) with less variance in the 12.00–16.00 hours period. The mean fat concentration of the first two feeds after 12.00 hours ('12.00 hours feeds') was therefore used to estimate 24 h concentration.

(3). To see if systematic sampling was in fact any better than random sampling, a third predictor was calculated as the mean fat concentration of two feeds selected randomly from all daytime feeds.

These options were investigated from the regression of observed (reference) fat concentrations (calculated as the mean fat concentration of all feeds given during the night-time or over 24 h) *v.* predictor fat concentrations. Fat intakes were estimated using the regression equation to predict the fat concentration for night-time or 24 h and then multiplying this by the total volume of milk consumed over the corresponding period. Observed fat intakes were then regressed against predicted intakes to obtain a measure of the accuracy of estimation.

RESULTS

Mean milk intakes ranged from 717 g/24 h in the first month of lactation to 498 g/24 h in the third trimester of lactation, with average feed frequency decreasing from twenty-one feeds/24 h to ten feeds/24 h over the same period (Table 1). 24 h fat concentration was highest in the first trimester of lactation (35·7 g/l in the first month of lactation and 37·1 g/l in the second and third months), decreasing to 29·1 g/l by the third trimester. Feed frequency and milk intake were less at night than during the daytime, such that, overall, 28 % of 24 h milk volume intake was consumed at night. The lower night-time milk intakes together with the reduced mean fat concentration of milk taken at night (Table 1) resulted in overall night-time fat intakes which were only 26 % of 24 h fat intakes (Table 1).

The number of daytime feeds was smaller in the older infants (r −0·70, n 25, $P < 0{\cdot}001$) but night-time feed frequency was not affected (r −0·22, n 25, $P > 0{\cdot}2$). Thus, older babies received a greater proportion of feeds during the night (r 0·51, n 25, $P < 0{\cdot}01$), and consumed a greater proportion of 24 h milk intake during the night (r 0·47, n 25, $P < 0{\cdot}02$), than younger babies.

When the proportion of feeds given at night was higher, the fat concentration of the milk was higher at night than during the day. Conversely, when proportionally fewer feeds were given at night, night-time fat concentration was lower than that during the day (r −0·47, n 25, $P < 0{\cdot}02$). We have previously demonstrated that mean fat concentration diminishes with increasing interfeed interval (Jackson *et al.* 1988), which accounts for these observations.

Prediction of night-time fat concentration and intake

Seven of the twenty-five mothers did not breast-feed between waking and the end of the study period at 08.00 hours. To permit a valid comparison of prediction intervals, therefore, equations for predicting night-time fat concentration from scheduled samples and from pre- and post-sleep samples were calculated for the eighteen mothers with complete data. Scheduled samples gave more accurate predictions than pre- and post-sleep feeds, with 95 % confidence intervals of ±12·8 g/l (n 18), corresponding to 41 % of the mean night-time fat concentration. The prediction equation using scheduled samples for the whole study group (n 25) was also calculated (Table 2(*a*)).

Predicted fat concentrations (obtained from the regression equations) were multiplied by night-time volume intake to obtain estimates of actual overnight fat intake. The regression equation for prediction of night-time fat intakes from scheduled samples for all mothers in the study group (n 25) was

observed night-time fat intake =

$$0{\cdot}96 \times (\text{fat intake predicted from samples at 20.00 and 08.00 hours}) + 0{\cdot}2 \quad (r\ 0{\cdot}87),$$

with 95% confidence limits of ±2·4 g, equivalent to 42% of the mean night-time fat intake.

Table 1. *Milk intakes, number of breast-feeds, fat concentrations of breast-milk and fat intakes during the daytime, night-time and over 24 h of breast-fed infants of mothers from a rural northern Thai population*

(Mean values and standard deviations for twenty-five mothers at different stages of lactation: six mothers in first month of lactation, eight mothers in 2nd to 3rd month, six mothers in 4th to 6th month and five mothers in 7th to 9th month)

Stage of lactation (months)...	≤ 1		2–3		4–6		7–9		Overall	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Milk intake (g)										
Daytime	513·3	144·5	452·3	69·8	514·3	117·4	337·2	144·8	458·8	129·4
Night-time	203·5	60·9	146·1	57·1	227·0	75·6	160·8	64·7	182·2	69·0
24 h	716·8	192·5	598·4	90·1	741·3	150·7	498·0	182·0	641·0	170·8
Number of breast-feeds										
Daytime	16·3	5·7	12·3	4·1	10·0	3·2	7·0	3·6	11·6	5·2
Night-time	4·8	1·3	2·6	1·1	3·7	1·0	3·0	1·2	3·5	1·4
24 h	21·1	6·6	14·9	4·6	13·7	3·7	10·0	4·5	15·1	6·1
Fat concentration (g/l)										
Daytime	36·2	7·8	37·8	7·6	32·9	4·2	28·4	7·6	34·4	7·5
Night-time	33·8	8·1	32·4	6·6	30·3	7·5	28·0	1·6	31·4	6·6
24 h	35·7	7·9	37·1	7·3	32·3	4·7	29·1	6·4	34·0	7·0
Fat intakes (g)										
Daytime	19·0	7·4	17·0	4·6	16·9	4·4	9·5	2·8	15·9	5·9
Night-time	7·0	2·5	4·8	2·5	6·4	1·9	4·5	1·5	5·7	2·3
24 h	26·0	9·1	21·8	4·6	23·3	4·5	14·0	3·1	21·6	6·9

Table 2. *Estimation of breast-milk fat concentration of twenty-five mothers from a rural northern Thai population*

Fat concentration and predictor	Equation	r	95% confidence limits	% of mean
(*a*) Mean fat concentration during the night-time				
Scheduled samples*	$y = 0{\cdot}36x + 18{\cdot}3$	0·56	12·8	41
Pre- and post-sleep*	$y = 0{\cdot}39x + 18{\cdot}3$	0·48	13·8	44
Scheduled samples	$y = 0{\cdot}37x + 18{\cdot}9$	0·59	11·3	36
(*b*) Mean 24 h fat concentration				
All daytime feeds	$y = 0{\cdot}92x + 2{\cdot}4$	0·98	3·3	10
(*c*) Mean 24 h fat concentration (limited daytime samples)				
Two random feeds	$y = 0{\cdot}53x + 14{\cdot}5$	0·88	7·0	21
08.00 and 18.00 hours feeds	$y = 0{\cdot}57x + 13{\cdot}8$	0·76	9·7	28
12.00 hours feeds	$y = 0{\cdot}49x + 17{\cdot}0$	0·80	8·9	26

y, observed fat concentration; x, predictor fat concentration. *n 18.

24 h fat concentration and intakes, sampling not limited

The mean of all daytime feeds predicted 24 h fat concentration with 95% confidence limits of $\pm 3{\cdot}3$ g/l, equivalent to 10% of the mean (Table 2(*b*)). The corresponding 24 h fat intakes were predicted by the equation

$$\text{observed 24 h fat intake} = 0{\cdot}96 \times (\text{fat intake predicted from daytime feeds}) + 0{\cdot}6 \; (r \; 0{\cdot}98),$$

with 95% confidence limits of $\pm 2{\cdot}8$ g (13% of mean 24 h fat intake). A slight improvement in accuracy was obtained by adding the fat intake estimated from the best night-time method (scheduled samples) to the observed daytime fat intake:

$$\text{observed 24 h fat intake} = 0{\cdot}99 \times (\text{predicted fat intake}) + 0{\cdot}5 \; (r \; 0{\cdot}99, \; n \; 25),$$

estimating 24 h fat intake to within $\pm 2{\cdot}4$ g (11% of the mean) in 95% of cases.

24 h fat concentrations and intakes, sampling limited to two daytime feeds

The best estimate of 24 h fat concentration was obtained using two randomly selected feeds with 95% confidence limits of ± 7 g/l (21% of mean 24 h fat concentration). Feeds at 08.00 and 16.00 hours, and the first two feeds after 12.00 hours, estimated 24 h fat concentration to within $\pm 9{\cdot}7$ g/l (28% of the mean) and $\pm 8{\cdot}9$ g/l (26% of the mean) respectively (Table 2(*c*)).

Fat intakes were given by:

$$\text{observed 24 h fat intake} = \times 0{\cdot}89 \times (\text{fat intake predicted from feeds at 08.00 and 16.00 hours}) + 2{\cdot}2 \; (r \; 0{\cdot}88),$$

with 95% confidence limits of ± 7 g (32% of mean 24 h fat intake), and

$$\text{observed 24 h fat intake} = 0{\cdot}89 \times (\text{fat intake predicted from feeds at 12.00 hours}) + 2{\cdot}2 \; (r \; 0{\cdot}92),$$

with 95% confidence limits of $\pm 5{\cdot}8$ g (27% of mean intake).

DISCUSSION

Analysis of variation in fat concentration between feeds has shown that, in this northern Thai population, the fat concentration of a feed is correlated with the time elapsed since the previous feed, the fat concentration at the end of the previous feed, the volume of the previous feed and the volume of the current feed (Jackson *et al.* 1988). Each mother has an individual pattern of timing of feeds and feed volume which contributes to the variance in the circadian pattern of changes in milk composition observed in this population. Even though statistically significant circadian variation was found at the population level, there is evidently considerable variation between mothers, shown by the fact that two feeds chosen randomly from all daytime feeds predicted mean 24 h fat concentration at least as accurately as sampling feeds at specific times. Random sampling may, however, be impractical, requiring advance knowledge of the total number of feeds to be given to calculate the sampling probability or, if feeds are sampled at randomly chosen times, the different sampling timetable for each subject may present a logistical problem in deployment of field personnel. In this population, using the alternative methods of first feed after 08.00 hours and the first feed after 18.00 hours, or the first two feeds after 12.00 hours, estimated 24 h fat concentrations with 95% confidence limits of 9·7 and 8·9 g/l, were equivalent to 28 and 26% of mean 24 h fat concentration respectively. The corresponding fat intakes were estimated to within 32 and 27% of mean 24 h fat intakes in 95% of cases.

For comparison, in a study (using similar methods) of sixteen mothers from The Gambia, samples taken from both breasts before and after the first feed after 13.00 hours showed the best correlation with observed 24 h fat concentration, with 95% confidence limits of $\pm 10{\cdot}8$ g/l at the mean (Prentice *et al.* 1981). The population mean of the sixteen Gambian women appears to be about 35 g/l, so that 24 h fat concentration was estimated to within approximately 30% of the mean 24 h fat concentration.

In the northern Thai population, the older the baby the greater the proportion of feeds and milk intake taken at night, and therefore the greater the volume of milk of unknown composition (if night-time milk samples cannot be taken). This may be a common phenomenon in cultures where mothers leave their babies to go out to work during the day, and the infant sleeps next to his or her mother during the night. In a separate longitudinal study of lactation and infant growth, in the same area of northern Thailand, we have observed that as the baby gets older, the number of daytime feeds may decrease to two or one, or even zero during the day, yet substantial amounts of milk may still be taken at night, and the bulk of the infant's fat intake therefore has to be estimated from milk samples taken outside the night-time period. This process is likely to compromise the accuracy of estimating 24 h fat intakes.

In this region of Thailand, collecting milk samples at every daytime feed is culturally acceptable, since the amount expressed is not so large as to make mothers feel their babies are being deprived, yet is adequate for measurement of fat and protein contents. The mothers prefer to express the samples themselves and taking samples as part of the test-weighing procedure appears to cause little or no disruption of the mother's normal daily routine. Under these circumstances it is possible to obtain a complete record of the daytime milk intake of the breast-fed baby, enabling fat concentration to be estimated with 95% confidence limits of ± 10% of mean 24 h fat concentration and fat intakes to within ± 13% of mean 24 h fat intake. Calculating night-time fat intakes separately from the best available method (samples taken from both breasts at 20.00 and 08.00 hours) and adding these to the observed daytime intakes gave a slight improvement in estimation (11% of mean).

If it is not possible to sample breast-milk at every daytime feed, preliminary studies must be carried out on the particular study population in question to determine the best sampling strategy (Prentice *et al.* 1981; Jackson *et al.* 1988). If the number of feeds that can be sampled is limited, the accuracy with which fat concentrations are predicted is likely to be of the order of $\pm$20–30% of mean 24 h fat concentrations in 95% of cases.

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