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NURSING BEHAVIOUR, PROLACTIN AND POSTPARTUM AMENORRHOEA DURING PROLONGED LACTATION IN AMERICAN AND !KUNG MOTHERS

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SUMMARY

In order to determine the effects of protracted nursing in American women, blood was collected hourly for 24 h and nursing periods recorded in 20 mothers, 10 amenorrhoeic, $3\frac{3}{4}$ to $17\frac{1}{4}$ months postpartum (PP), and 10 menstruating, $5\frac{1}{4}$ to 46 months PP. These data were compared to the daytime nursing behaviour and 1000–1100 h PRL of women among !Kung hunter-gatherers of Botswana, a non-contraceptive using population with a birth space interval of >3 years. Intense nursing behaviour maintained amenorrhoea and hyperprolactinaemia for 1 to nearly 2 years PP in both American and !Kung mothers. Among Americans, 80 min of nursing per day, in conjunction with a minimum of six nursing episodes, was highly predictive of remaining amenorrhoeic up to 18 months PP. Amenorrhoea was always accompanied by hyperprolactinaemia, but delay in the onset of menses was related more to nursing behaviour than to a particular 24 h PRL level. The 1000–1100 h sample is equivalent to and about half of the 24 h mean in high and low intensity nursers, respectively. The !Kung women were similar to the high intensity nursing American women in 1000–1100 h PRL, percent amenorrhoeic, and the number of minutes of daytime nursing.

World-wide, breast-feeding may well be the most important determinant of the interval between pregnancies and hence of the birth rate in developing countries (Rosa, 1975). Menses generally return in less than 2 months postpartum (PP) in women who do not nurse their babies (Perez *et al.*, 1971), whereas PP amenorrhoea can last for 11–22 months in populations practising late weaning and frequent nursing (Rosa, 1975). The non-abstinent, non-contraceptive using population group with the longest known intervals

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between births—44·1 months in nomadic, bush-dependent bands—are the !Kung (Bushmen) hunter-gatherers of Botswana and Namibia (Lee, 1979).

Suckling-induced PRL secretion is largely responsible for lactational infertility by inhibition of hypothalamic gonadotrophin regulation (McNeilly, 1980) and of ovarian steroidogenesis (McNeilly *et al.*, 1982a). The amount and frequency of nursing per day are known to contribute to the degree of hyperprolactinaemia and to the duration of PP amenorrhoea, relationships which have been demonstrated with a single daily sample of PRL per subject (Delvoye *et al.*, 1976, 1977; Duchen & McNeilly, 1980; Howie *et al.*, 1981; Andersen & Schioler, 1982). One limitation of these data is that PRL secretion is episodic, circadian (Sassin *et al.*, 1972), responsive to stress (Noel *et al.*, 1972), and occurs with great variability in response to suckling (Bunner *et al.*, 1978; Tyson *et al.*, 1978). Another limitation is that these relationships have not been studied in industrialized populations nursing for 1 to 2 years PP.

!Kung women nurse briefly and very frequently (up to several times per hour), for several years after each birth, and the interval between nursing bouts is inversely related to oestradiol and progesterone levels (Konner & Worthman, 1980). Single daily serum samples from a group of lactating !Kung women whose nursing behaviour had been documented (Konner & Worthman, 1980) were available for PRL assay. To interpret the significance of these, as well as other findings based on single daily samples, and to evaluate conclusions concerning prolonged lactation based on rural populations of developing countries, we studied the 24 h nursing and PRL pattern of lactating (amenorrhoeic or menstruating) American women, including a substantial number with an unusually high frequency of breast-feeding over a much longer time PP than that of most women in industrialized societies.

METHODS

Subjects: American mothers

Twenty paid volunteers were recruited mainly through the Nursing Mother's Council of the Boston Association for Childbrith Education and local chapters of La Leche League International, and in a few instances by other means. All were Caucasian, healthy, well-nourished, non-obese, did not smoke tobacco or take medications (except for one who was taking L-thyroxine for the treatment of hypothyroidism), and had a history of regular menstrual cycles before their recent pregnancy. The mean age of subjects was 28·4 years (range 23–36), all but one had full term pregnancies (three of 20 by caesarean delivery), and the interval after birth ranged from $3\frac{3}{4}$ to 46 months. Five subjects were breast-feeding exclusively (i.e., with no food supplements), one with twins at $6\frac{1}{4}$ months PP and one at $9\frac{1}{2}$ months PP. Half were primiparous. Half were menstruating and were studied during the follicular phase.

Procedure

The mother and her child entered the Clinical Study Unit (CSU), Tufts-New England Medical Center, in the morning. After medical examination, an i.v. catheter was placed in the mother's arm (i.v. line with relatively inactive babies; heparin lock otherwise) to permit withdrawal of repeated blood samples with minimal disturbance to the mother. Eight and a half millilitres of blood were drawn at hourly intervals for a 24 h period. In addition, for one or two of the nursing episodes, blood samples were taken before and at three 5-min intervals after the initiation of nursing; 25 episodes from 19 subjects were followed in this way. Five millilitres of the samples were drawn into a heparinized syringe, refrigerated at 4°C, centrifuged after the completion of blood collections for that subject, and the plasma stored at −20°C for subsequent hormone assay. The remainder of the blood sample was treated differently, for subsequent assay of oxytocin and neurophysin (J. M. Stern, A. G. Robinson, T. N. Herman & S. Reichlin, in preparation).

The start and stop times of nursing were recorded by the mothers to the nearest minute while they were in the CSU. Nursing events separated by a non-nursing interval of less than 5 min were considered to be part of the same 'bout'. In addition, nursing events separated by a non-nursing interval of less than 30 min were considered to be part of the same 'breast-feed', a criterion closer to most of the mothers' assessment of their daily breast-feeding frequency. Food supplements were also recorded. The mothers filled out a similar record on a typical day at home close to the time of their CSU visit.

Nursing behaviour and prolactin among !Kung women

As an outcome of a previously reported study serum collected at 1000–1100 h from 10 nursing mothers and from five non-nursing women were available for PRL assay. These samples had been stored in liquid nitrogen for 3 years and then at −70°C for 3 years, except for two prior thaws for assay of ovarian steroids (Konner & Worthman, 1980). In the nursing group, one or two blood samples were assayed from each of seven women who were up to 2 years PP, six of whom were amenorrhoeic. Five to nine blood samples, collected throughout the menstrual cycle, were assayed for each of three nursing women between 2 and 3 years PP and each of five non-nursing women. The daytime nursing behaviour of the women less than 2 years PP had been assessed from 6 h of observations in three 2 h sessions on separate days from 0830–1030 h, 1230–1430 h, and 1630–1830 h. Nursing was recorded to the nearest 30 s, and a nursing session defined by a separation from the adjacent session by at least 30 s (a more stringent definition than that used for the American mothers).

Radioimmunoassay and statistical comparisons

PRL was measured by using antisera and hormone provided by the National Pituitary Hormone Distribution Agency (NPHDA) and Serono PRL standards. The Mann–Whitney U-test (group comparisons with $n=6$), Student's t-test (unpaired and paired), Pearson's correlation coefficient and Fisher's exact probability test (proportion of subjects menstruating) were used for statistical analyses.

RESULTS

Nursing behaviour, prolactin and amenorrhoea in American mothers

Individual patterns during the early months after delivery reveal marked release of PRL beginning within a few min after the start of suckling (Fig. 1, top). In the longest PP intervals studied among mothers still nursing frequently per day, PRL levels follow a

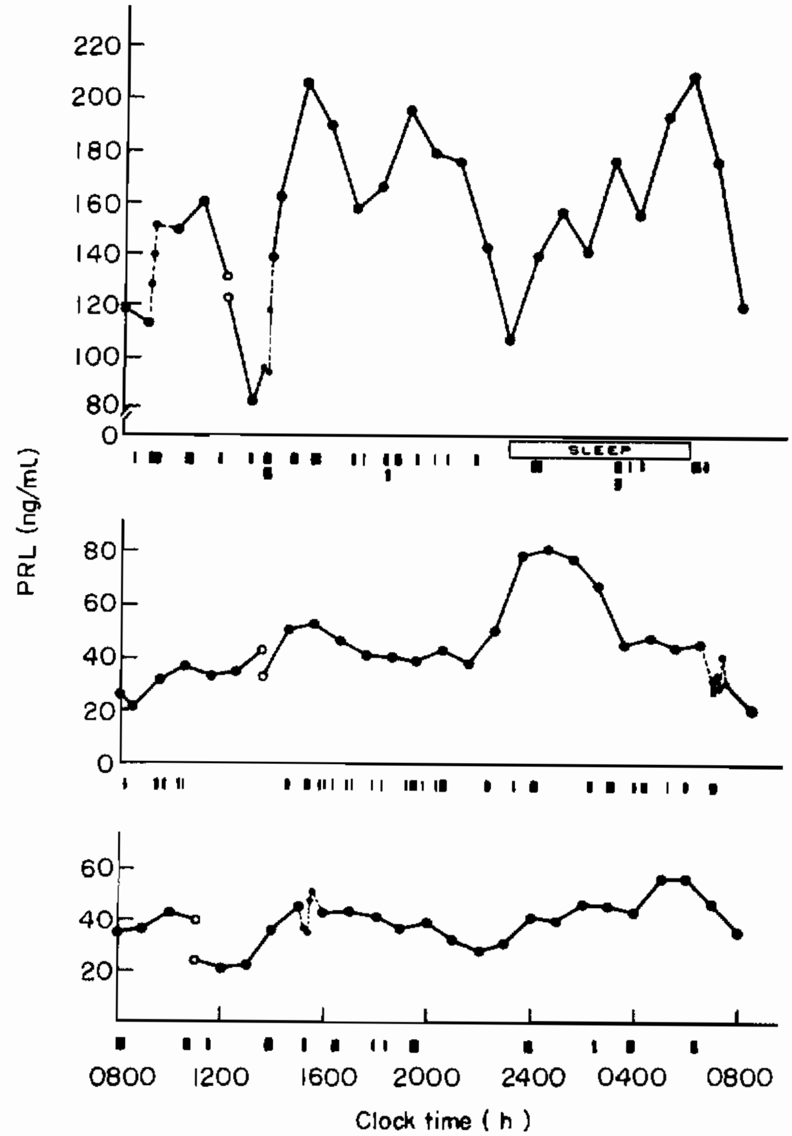

Fig. 1. Plasma PRL (ng/ml) levels at hourly intervals over a continuous 24 h period in three nursing, amenorrhoeic women at varying times postpartum. Nursing bouts are indicated by the black bars beneath each abscissa; two bars at the same time (top) means that both twins were nursed simultaneously. The approximate sleep period of the subjects is indicated beneath the top abscissa. Four nursing bouts followed at 5 min intervals are shown (.....), two with a rise of ~40 ng/ml relatively early postpartum (top) and two with a rise of < 10 ng/ml during late lactation (middle and bottom). Top: subject F, age 27, $6\frac{1}{4}$ months postpartum, $158 \cdot 5 \pm 6 \cdot 5$ ng/ml PRL; amenorrhoeic (twins). Middle: subject O, age 28, $16\frac{1}{2}$ months postpartum, $46 \cdot 8 \pm 3 \cdot 2$ ng/ml PRL; amenorrhoeic. Bottom: subject P, age 31, $17\frac{1}{4}$ months postpartum, $38 \cdot 6 \pm 1 \cdot 8$ ng/ml PRL; amenorrhoeic.

more stable pattern (Fig. 1, middle and bottom). A PRL increment of ≥ 5 ng/ml was observed in 100% of the 25 nursing episodes, 88% within 15 min, and of the remainder, in a sample taken later than 15 min. Thus, even women without hyperprolactinaemia and nursing a child of more than 2 years continue to have modest PRL secretory responses to suckling.

Mean 24 h PRL levels fell over time PP ($r = 0{\cdot}49$; $P < 0{\cdot}05$) (Fig. 2). These levels correlate significantly with both the 24 h peak and nadir levels of PRL ($r = 0{\cdot}75$ and 0·93, $P < 0{\cdot}001$) and with a single sample drawn between 1000 and 1100 h ($r = 0{\cdot}89$, $P < 0{\cdot}001$). Hyperprolactinaemia, defined as a mean 24 h PRL level which is greater than two standard deviations above that of normal non-lactating, non-pregnant women, observed previously to be $13{\cdot}6 \pm 3{\cdot}2$ SD ng/ml (Kapcala *et al.*, 1984), was present in all but three of our subjects and occurred in our sample as late as $22\frac{1}{2}$ months PP.

In the whole sample, the nocturnal (2300–0700 h) mean PRL percentage of the daytime (0700–2300 h) mean PRL was inversely related to the daytime PRL level ($r = -0{\cdot}512$, $P < 0{\cdot}05$. In 60% of our sample, the nocturnal increment was $\geqslant 20\%$ (mean of $+105{\cdot}2\%$ of daytime PRL) and daytime PRL was $30{\cdot}1 \pm 5{\cdot}2$ ng/ml; in contrast, subjects with little or no increment in nocturnal PRL (mean of $-7{\cdot}7\%$ of daytime PRL) had significantly higher daytime PRL ($71{\cdot}1 \pm 15{\cdot}2$ ng/ml; $t = 2{\cdot}94$, $P < 0{\cdot}01$).

Seventeen of our subjects resumed menses while still nursing; the duration of their PP amenorrhoea ($12{\cdot}7 \pm 1{\cdot}6$ months) was significantly correlated with the number of months of full breast-feeding ($6{\cdot}3 \pm 0{\cdot}7$; $r = 0{\cdot}66$, $P < 0{\cdot}01$), a relationship particularly pronounced for the 10 women who breast-fed with little or no supplementation for 6–12 months ($7{\cdot}9 \pm 0{\cdot}9$; $14{\cdot}9 \pm 1{\cdot}8$ months of amenorrhoea; $r = 0{\cdot}78$, $P < 0{\cdot}01$). In our restricted sample, duration of PP amenorrhoea was not correlated with age or parity. The longest interval without menses observed at the time of study was $17\frac{1}{4}$ months and this subject resumed menses at 21 months; two other subjects experienced longer intervals of 23 and 24 months. At the other extreme, one subject out of 20 (5%) repeatedly resumed menses 6 weeks PP while breast-feeding exclusively.

Amenorrhoea was always accompanied by hyperprolactinaemia, but mean PRL level was not an accurate predictor of the occurrence of amenorrhoea. The lowest 24 h mean PRL level associated with amenorrhoea was $34{\cdot}8 \pm 2{\cdot}4$ ng/ml, but four of the 10 nursing women who had resumed their menses previously had higher values at the time of study (36·4, 41·6, 45·7, 73·1). Mean PRL levels tend to be higher in the amenorrhoeic than in the

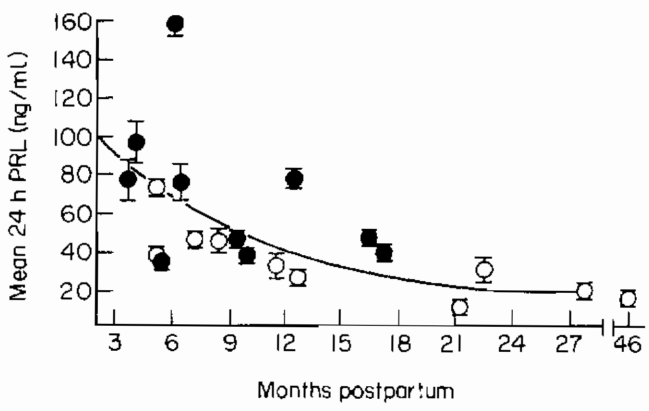

Fig. 2. Mean 24 h PRL levels (±SEM) for 10 amenorrhoeic (●) and 10 menstruating (○) nursing women as a function of months postpartum; line fitted by inspection. Each circle represents the mean of 24 consecutive hourly samples for a single subject. The highest mean was that of a woman nursing twins. The (high-intensity nursing) woman with a hyperprolactinaemic mean at the latest time postpartum ($30{\cdot}3 \pm 6{\cdot}5$ ng/ml at $22\frac{1}{2}$ months) had a daytime mean PRL in the non-lactating range ($14{\cdot}9 \pm 2{\cdot}7$) but very high nocturnal PRL (mean of $57{\cdot}6 \pm 13{\cdot}7$; peak of 125 ng/ml following a nursing bout).

menstruating lactating women we studied (Fig. 2). Because PRL was significantly correlated with time PP statistical comparison was confined only to those amenorrhoeic and menstruating women who fell within the same PP span ($5\frac{1}{4}$–$12\frac{3}{4}$; Table 1). Both the 24 h nursing behaviour and PRL levels were about twice as great in the amenorrhoeic as in the menstruating women, but only the behavioural differences are significant.

Evidence that nursing behaviour is the crucial determinant of both return of menses and PRL levels was gained by comparing the six women with the highest duration (> 180 min) with the six women with the lowest duration (< 90 min) of nursing (up to 2 years PP) in the 24 h period studied (Table 1). In this comparison, the mean nursing behaviour disparity is even more pronounced than that based on menstrual status and all PRL measures, except peak PRL, are significantly different.

The mean 24 h PRL level correlates significantly with the number of minutes of nursing in the 24 h study period for the whole sample as well as for the subsamples of less than and greater than 12 months PP ($n=12$, $r=0{\cdot}62$, $P<0{\cdot}05$; $n=8$, $r=0{\cdot}75$, $P<0{\cdot}05$, respectively; Fig. 3). Because the total time spent nursing was determined by the number of individual nursing episodes times the duration of each episode, an attempt was made to determine the relative importance of these two aspects of nursing (Fig. 4). The number of nursing bouts was highly correlated with time spent nursing in 24 h, but not with the average duration of individual nursing bouts, which remained relatively constant over a wide range of nursing durations per day (13·6 ± 1·1 min/bout).

Table 1. Nursing behaviour and prolactin (mean ± SEM) as a function of menstrual status and nursing duration.

	Menstrual status		Nursing duration	
	Amenorrhoeic	Menstruating	High (> 180 min/24 h)	Low (< 90 min/24 h)
Months after birth	8·4 + 1·1	8·4 ± 1·3	11·9 ± 2·8	11·6 ± 2·2
Nursing behaviour				
Minutes/24 h	183·8 ± 37·0	81·8 ± 15·8*		
Breast-feeds/24 h	9·8 ± 1·3	4·7 ± 0·3*	10·8 ± 1·3	5·8 ± 0·9*
'Bouts'/24 h	13·8 ± 2·8	5·3 ± 0·5*	19·0 ± 2·8	7·2 ± 1·7*
'Bouts'/2300 0700 h	2·8 ± 0·7	1·5 ± 0·4	4·3 ± 0·8	1·7 ± 0·5*
Other meals	1·0 ± 0·4	2·2 ± 0·5*	0·8 ± 0·4	2·5 ± 0·2*
Prolactin (ng/ml)				
24 h mean	71·7 ± 19·0	42·6 ± 6·9	76·0 ± 19·2	30·8 ± 4·8*
1000–1100 h	52·7 ± 20·3	26·7 ± 7·2	72·8 ± 23·4	15·5 ± 4·4*
Nadir	29·5 ± 11·1	15·0 ± 5·3	30·6 ± 10·8	6·3 ± 1·7*
Peak	120·0 ± 23·8	114·6 ± 14·1	137·1 ± 23·2	95·4 ± 20·2
2300–0700 h mean	79·0 ± 19·8	54·0 ± 7·9	80·6 ± 17·2	43·7 ± 7·1*

($n=6$ per comparison group).

*$P<0{\cdot}05$– $<0{\cdot}001$, Mann–Whitney U-test

Comparison groups do not differ in age, height, weight or parity. There is some overlap in the constitution of groups between the two comparisons.

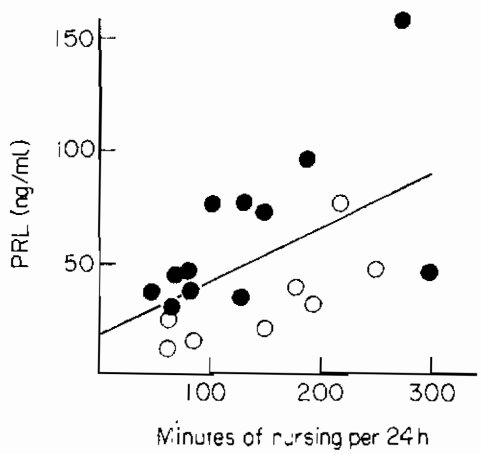

Fig. 3. Mean 24 h PRL levels as a function of number of minutes of nursing in the same 24 h period. Each circle represents a single woman. Although the same amount of nursing results in higher levels of PRL in the 1st year than in the 2nd year postpartum, more of the variance in PRL is due to nursing duration in the 2nd year than in the 1st (56% vs 38%). The woman exclusively breast-feeding her 9½-month-old baby (far right) had lower PRL than expected, probably related to her premature delivery. The woman fully breast-feeding her 6½-month-old twins (top right) had higher PRL than expected, indicating a role for nursing intensity in addition to duration. (●) < 12 months; (○) > postpartum. $r = 0{\cdot}54$; $P < 0{\cdot}02$.

Although the nursing behaviour assessed in the CSU is highly correlated with the home record (bouts: $r = 0{\cdot}76$; min: $r = 0{\cdot}84$, $P < 0{\cdot}001$), for 15 of 19 women whose home record was available (78·9%), nursing frequencies and total durations were somewhat higher in the CSU than at home (bouts: $t = 2{\cdot}76$, $P < 0{\cdot}02$; min: $t = 2{\cdot}78$, $P < 0{\cdot}01$). Therefore the nursing behaviour at home was used to show that a nursing duration of ⩾80 min or a nursing frequency of ⩾6 bouts in a 24 h period was highly associated with being amenorrhoeic, at least up to 18 months PP. Specifically, of six women nursing <80 min, five were menstruating (the exception resumed menses 17 days after the study), while of 10 women nursing ⩾80 min, 9 were amenorrhoeic (the exception having resumed menses at 6 weeks PP after each of her three births while breast-feeding exclusively) ($P < 0{\cdot}01$). All six women nursing <6 bouts were menstruating and all 10 nursing ⩾6 bouts were amenorrhoeic ($P < 0{\cdot}001$). Using cut-offs of 120 min and 9 bouts, this categorical breakdown is essentially the same with the CSU record.

Significance of the 1000–1100 h sample in relation to !Kung women

The 1000–1100 h sample accurately reflects 24 h secretion in the high duration nursers (Table 2; 72·8 vs 76·0 ng/ml), but in low duration nursers the 1000–1100 h measure is approximately half the 24 h mean (15·5 vs 30·8 ng/ml), i.e., a single sample underestimates the daily PRL secretion. Further, the nadir-peak excursion is four times greater among the low than the high nursing duration mothers.

The 1000–1100 h sample of PRL was compared in the low and high nursing duration American and the !Kung mothers, all nursing a child less than 2 years of age (Fig. 5). In two of the amenorrhoeic !Kung women, PRL values were undetectable, presumably due to sample damage. In the four remaining !Kung amenorrhoeic women, the PRL levels were comparable to those of our Boston-area women engaging in high duration-high

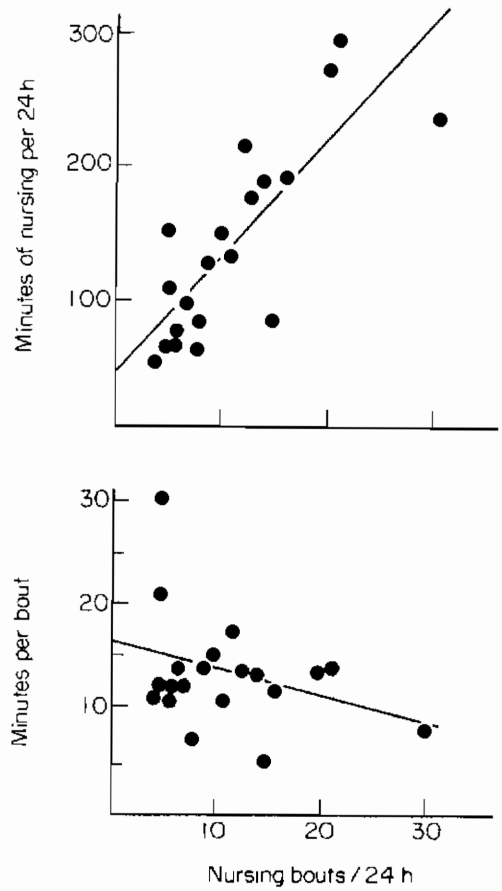

Fig. 4. Relationship between number of nursing bouts per 24 h to number of minutes spent nursing per 24 h (top: $r = 0{\cdot}81$; $P < 0{\cdot}001$) and to number of minutes per bout (bottom: $r = -0{\cdot}33$; $P > 0{\cdot}1$). Each circle represents a single woman.

frequency nursing. The PRL level of the single cycling !Kung mother was comparable to those of the low duration-low frequency Boston women, although her nursing behaviour was similar to that of the amenorrhoeic !Kung women. The 1000–1100 h PRL levels of two American and three !Kung menstruating women nursing a child over 2 years of age were low (10·4 and 9·1 ng/ml, respectively) and similar to those of non-lactating, non-pregnant healthy American women (Kapcala *et al.*, 1984) and to those of five non-nursing !Kung women (6·1 ng/ml, grand mean).

The daytime nursing behaviour (Table 2) of the low and high nursing duration American women differed significantly in frequency, duration and average number of minutes between nursing bouts, but not in the number of minutes per bout or in the maximal interval between bouts. The very high frequency, very short duration per bout nursing pattern of the !Kung women is clearly different from even that of the high nursing duration American mothers. The latter fall between the low duration and the !Kung mothers with respect to number of bouts per hour, minutes between bouts, and mean maximal interval between bouts, but they do not differ from the !Kung mothers in number of minutes per hour, an indicator of total nursing duration per 24 h.

The high and low nursing duration mothers differed significantly in % who were amenorrhoeic at the time of study (Table 2; Fig. 5) and subsequent follow-up revealed a mean menstrual onset of $15{\cdot}2 \pm 2{\cdot}2$ and $8{\cdot}6 \pm 0{\cdot}9$ months, respectively.

Table 2. Daytime nursing behaviour (mean ± SEM) and percent amenorrhoeic of American (low vs high nursing duration) and !Kung mothers < 2 years postpartum

	Low ($n = 6$)	High ($n = 6$)	!Kung ($n = 7$)
Nursing behaviour			
Minutes per hour	4·0 ± 0·5	11·1 ± 1·8*	10·2 ± 2·5
'Bouts' per hour	0·4 ± 0·1	0·9 ± 0·1*	4·8 ± 0·6
Minutes per 'bout'	9·9 ± 1·2	11·4 ± 1·8	2·7 ± 1·0
Minutes between 'bouts'	157·5 ± 0·7	55·4 ± 13·1*	10·2 ± 1·1
Maximum interval (min)	184·0 ± 23·5	123·5 ± 30·6	47·8 ± 5·2
Percent amenorrhoeic	16·7	83·3†	85·7

0830 1830 h in the Clinical Study Unit vs three 2 h sessions on three separate days for the !Kung: 'bouts' were defined as nursing events separated by non-nursing intervals of 5 min and 30 s for American and !Kung women, respectively. Using a 1 min criterion for the American women, the data for only 35% is affected, and for these there are only 1·1 more bouts and 2·7 fewer min of nursing.

*$P < 0·02$–$P < 0·01$, low vs high; Mann Whitney U-test.

†$P < 0·05$; Fisher exact probability test. The one amenorrhoeic woman in the low group resumed menses 17 d after the study.

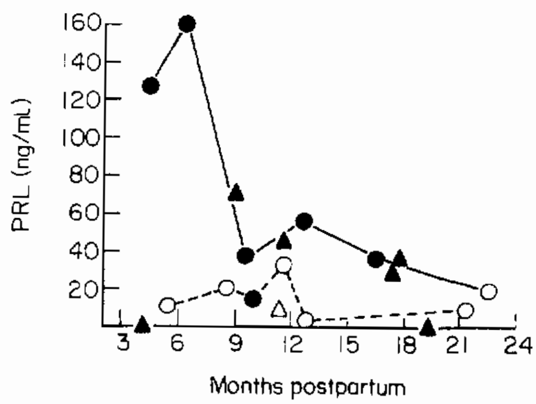

Fig. 5. The PRL level between 1000 1100 h among 'high' (—) and 'low' (- - -) nursing duration Boston-area mothers (circles) and among !Kung nursing mothers (triangles) up to 2 years postpartum. Each symbol represents a single PRL sample from one woman; filled and open symbols indicate whether the woman was amenorrhoeic or menstruating, respectively, at the time the blood sample was taken.

DISCUSSION

Intense nursing behaviour maintains amenorrhoea and hyperprolactinaemia for 1–2 years PP among both American and !Kung women. Our finding of a mean lactational amenorrhoeic interval of 15·2 months among high intensity nursers and of 21–24 months as the longest amenorrhoeic intervals experienced by our American subjects are comparable to those based on a sample of 29 American women with similar nursing behaviour (22·8 and 14·6 months of breast-feeding and amenorrhoea, respectively, and only 7% with amenorrhoea >24 months; (Kippley & Kippley, 1977)). In several rural Asian and African populations, with an average breast-feeding duration of 21–28 months (24·8 months, grand mean), mean PP amenorrhoea lasted 11–22 months (15·8 months, grand mean) (Rosa, 1975). Our limited data and these comparisons suggest that the duration of PP amenorrhoea in traditional !Kung populations is similar to that of the longest mean intervals reported previously.

Although PRL declines with time PP, suckling continues to elicit a detectable PRL increment for over 2 years after delivery and contributes to 24 h hyperprolactinaemia of ⩾30 ng/ml for approximately 1 and 2 years among low and high nursing duration American mothers, respectively, extending reports, based on single daily samples, on several African populations (Delvoye *et al.*, 1976, 1977; Duchen & McNeilly, 1980). Eventually, PRL does decline to low, non-nursing, cycling levels in spite of continued lactation (Tyson *et al.*, 1978; Hennart *et al.*, 1981), levels apparently necessary to maintain lactation (Brun del Re *et al.*, 1973).

Our analysis of the 1000–1100 h PRL levels indicates that a single daily sample, taken without regard to the prior suckling interval, is highly correlated with the 24 h PRL mean, being similar to it among high intensity nursers and half of it among low intensity nursers. The latter finding suggests that the degree of hyperprolactinaemia may have been underestimated for low intensity (Caucasian) nursing mothers in previous studies based on single samples (Gross & Eastman, 1979; Howie *et al.*, 1981; Andersen & Schioler, 1982; Hennart *et al.*, 1985), and may have contributed to erroneous conclusions about the relationship between PRL levels and other variables, such as return of menses. Also, because of differences in suckling frequency, the variability in a single PRL sample is much greater for low than for high frequency nursers. Nevertheless, the use of a single sample does provide a useful estimate of 24 h PRL, especially if nursing behaviour is taken into account.

Eighty minutes of nursing per day, in conjunction with a minimum of six nursing episodes, was highly predictive of persisting amenorrhoea among Americans, at least up to 18 months PP, similar to conclusions based on studies of Danish (Andersen & Schioler, 1982) and Scottish (McNeilly *et al.*, 1983) women nursing for less than 1 year. Our results confirm that the maintenance of high nursing levels is associated with a limitation on the frequency of supplementary feeding (Howie *et al.*, 1981; Andersen & Schioler, 1982). To the extent that hyperprolactinaemia *per se* contributes to lactational infertility, nocturnal breast-feeding probably does not play a large role since PRL rises proportionately more at night in women whose daytime (suckling-induced) PRL levels are relatively low.

Delay in the onset of menses was associated more with high suckling duration and frequency than with a particular level of PRL. Amenorrhoea was always accompanied by hyperprolactinaemia, but the latter occurred in some menstruating women as well. In large groups of African women, PRL (based on one sample per woman) was found to be significantly higher amongst amenorrhoeic than menstruating nursing women, but there was considerable overlap between groups (Delvoye *et al.*, 1976, 1978). In a small percentage of full breast-feeding women, menses return in the early months PP (Perez *et al.*, 1971); one such woman, studied herein at $5\frac{1}{4}$ months after birth, was found to have high 24 h PRL (73·1 ng/ml). Therefore, even the very high PRL levels found early PP provide only a relative, not an absolute, block to the onset of menses. Elevated PRL levels are a necessary, but not a sufficient condition for the maintenance of lactational amenorrhoea.

The !Kung women did not differ from the high intensity nursing American women (both up to 2 years PP) in 1000–1100 h PRL, percent amenorrhoeic, or number of min of daytime nursing. Thus, the very frequent, short nursing bout aspect of the !Kung nursing pattern (a pattern inconsistent with the lifestyle of even the most devoted breast-feeding women in industrialized societies) does not appear to be critical for maintaining hyperprolactinaemia and amenorrhoea. However, this pattern may contribute to reduced fertility once menses resumes, such as anovulation and luteal insufficiency (Howie *et al.*, 1982; McNeilly *et al.*, 1982b), perhaps by a direct effect of the suckling stimulus on gonadotrophin regulation (Smith, 1978; Plant *et al.*, 1980; Schallenberger *et al.*, 1981). Further, these similarities between American and !Kung nursing women suggest that deficient maternal diet *per se* (Howell, 1979; Lunn *et al.*, 1984; Hennart *et al.*, 1985) is not a critical determinant of lactational amenorrhoea; rather, maternal dietary inadequacy associated with reduced rate of milk secretion may ultimately affect PRL levels and fertility indirectly through increased suckling stimulation.

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