*J. biosoc. Sci.* **36**, 573–595

*DOI: 10.1017/S0021932003006382*

# LACTATIONAL AMENORRHOEA IN WELL-NOURISHED TOBA WOMEN OF FORMOSA, ARGENTINA

CLAUDIA VALEGGIA AND PETER T. ELLISON

*Reproductive Ecology Labouratory, Department of Anthropology, Harvard University, Cambridge, MA, USA*

**Summary.** The proximate causes of the contraceptive effect of lactation are still a matter of productive debate. This study sought to disentangle the relative impact that intense breast-feeding practices and maternal nutrition have on the regulation of ovarian function in nursing women. A mixed-longitudinal, direct-observational, prospective study was conducted of the return to postpartum fecundity in 113 breast-feeding, well-nourished Toba women. A sub-sample of 70 women provided data on nursing behaviour, daily activities, diet quality and urinary levels of oestrone and progesterone metabolites. Well-nourished, intensively breast-feeding Toba women experienced a relatively short period of lactational amenorrhoea ($10{\cdot}2 \pm 4{\cdot}3$ months) and a high lifetime fertility (TFR = 6·7 live births/woman). Duration of lactational amenorrhoea was not correlated with any of the nursing parameters under study or with static measures of maternal nutritional status. The results indicated that the pattern of resumption of postpartum fertility could be explained, at least partly, by differences in individual metabolic budgets. Toba women resumed postpartum ovulation after a period of sustained positive energy balance. As the relative metabolic load hypothesis suggests, the variable effect of lactation on postpartum fertility may not depend on the intensity of nursing *per se* but rather on the energetic stress that lactation represents for the individual mother.

## Introduction

One of the most striking aspects of the period of postpartum amenorrhoea is the great variability observed in its duration. Women can resume menstruation as early as two months after childbirth or as late as 3 years postpartum, with a broad range of intermediate durations of lactational amenorrhoea between these extremes (Konner & Worthman, 1980; Jones, 1989; Wood, 1994). It has been well established that lactation is a powerful determinant of the period of amenorrhoea that follows childbirth. The relationship between lactation and postpartum fertility has been the focus of research in many areas, such as reproductive physiology (Glasier *et al.*, 1986; McNeilly, 1993; Diaz *et al.*, 1995), demography (Goldman *et al.*, 1987; Retheford *et al.*, 1989), public health (WHO Task Force, 1998a and b) and family planning studies (Labbok *et al.*, 1994; Peterson *et al.*, 2000).

In spite of the attention received, the specific physiological mechanisms underlying lactational amenorrhoea remain elusive. Originally, as seems to be the case in some other species, the pituitary hormone prolactin was thought to be the link between the suckling stimulus and women's ovarian inhibition. However, recent findings suggest that there may not be any direct relationship between changes in prolactin *per se* and the duration of lactational amenorrhoea (Tay *et al.*, 1996; McNeilly, 2001). Other hormonal pathways are being suggested, and most of these are centred on the factors that might affect the pulsatile secretion of GnRH and the sensitivity to the negative feedback effects of oestradiol (Glasier *et al.*, 1986; Wood, 1994; McNeilly, 2001). A tantalizing line of study links metabolic hormones such as insulin to the regulation of reproduction at a central level (Olson *et al.*, 1995; Brüning *et al.*, 2000; Wu *et al.*, 2000). For example, Brüning *et al.* (2000) suggest that insulin receptors expressed on hypothalamic GnRH-producing neurones may modulate the pattern of GnRH secretion. These findings may point to the neural bases of the controversial relationship between changes in energy balance during lactation and the duration of lactational amenorrhea (Ellison & Valeggia, 2003). However, the current view of the neuroendocrine control of lactational infecundity is still highly speculative and more research is needed to settle some contentious relationships.

At a different level of analysis, several studies attempted to identify the major factors explaining the great variability in the duration of lactational amenorrhoea (Diaz, 1989; Lewis *et al.*, 1991; Wood, 1994; Dewey, 1996; Peng *et al.*, 1998). During the last two decades, the list of candidates seems to have been narrowed to two main factors: nursing intensity and maternal nutrition. One major hypothesis proposes nursing intensity (a combined measure of suckling frequency, duration of the suckling episode and total duration of nursing) as a major controller of the duration of lactational amenorrhoea (Howie & McNeilly, 1982). The evidence suggests that either a high frequency of nursing or a few nursing episodes of long duration are effective in preventing ovulation (Howie & McNeilly, 1982; Wood *et al.*, 1985; Vitzthum, 1989). The great variation in the duration of lactational amenorrhoea has often been interpreted to reflect the wide diversity in nursing behaviour across populations: the more intensive the breast-feeding, the longer the impact on fertility (Wood, 1994). However, a great part of the observed variation in the duration of lactational amenorrhoea remains unaccounted for by the variation in nursing intensity.

In the past few years, several studies have shown no correlation between nursing intensity and the return to postpartum fertility (Fink *et al.*, 1992; Worthman *et al.*, 1993; Tay *et al.*, 1996). At the same time, evidence has accumulated suggesting that maternal nutritional status (Huffman *et al.*, 1987; Peng *et al.*, 1998), energy balance (Lunn *et al.*, 1984) and the introduction of supplementary infant foods (Tay *et al.*, 1996; Rahman *et al.*, 2002) are associated with significant variation in the duration of lactational amenorrhoea, leading some researchers to propose the *relative metabolic*

https://doi.org/10.1017/S0021932003006382 Published online by Cambridge University Press

*load* hypothesis (Lunn *et al.*, 1980, 1984; Ellison *et al.*, 1993; Ellison, 1994). According to this hypothesis, the variable effect of lactation on postpartum fertility depends on the energetic stress that lactation represents for the mother. In some cases the metabolic load of lactation will correlate closely with nursing intensity. As supplementary foods are introduced to the infant, for example, nursing intensity gradually diminishes and so does the relative metabolic load of producing milk. In other cases the metabolic load of lactation may be independent of nursing intensity. Producing a given amount of milk would represent a higher relative metabolic load for poorly nourished mothers or for mothers with high workload demands than for well-nourished mothers or mothers with lesser workloads even when nursing intensity is the same. Thus the relative metabolic load hypothesis subsumes many of the same observations thought to support the nursing intensity hypothesis while generating additional predictions that go beyond the nursing intensity hypothesis.

The metabolic load hypothesis differs from the extreme form of the nursing intensity hypothesis by suggesting that maternal condition, including maternal age, energy status and energy balance, contribute greatly to the contraceptive effect of lactation (Ellison, 1994, 1995). Field studies of very diverse societies support this view. For example, nutritional supplementation of nursing mothers in the Gambia affected prolactin levels and the duration of lactational amenorrhoea (Lunn *et al.*, 1980, 1984). Good maternal nutritional status also mitigated lactational suppression of ovarian function in fully breast-feeding Amele women of Papua New Guinea (Worthman *et al.*, 1993).

This study sought to disentangle the relative impact that intense breast-feeding practices and maternal nutrition have on the regulation of ovarian function in nursing women. According to the nursing intensity hypothesis, given the intensity of their nursing pattern, women in this study should experience a relatively long period of lactational amenorrhoea. In contrast, the relative metabolic load hypothesis would predict that in well-nourished Toba lactation does not represent a high metabolic stress and, therefore, the duration of lactational amenorrhoea would be relatively short. These predictions were evaluated by conducting a mixed-longitudinal, direct-observational, prospective study of the return to postpartum fecundity in breast-feeding, well-nourished Toba women.

## Methods

### *Study population*

The Toba, who belong to the Guaycurúan linguistic family, are one of the three major indigenous groups inhabiting the Gran Chaco region of Argentina. All Gran Chaco indigenous groups share similar subsistence economies despite considerable language variation. These groups were traditionally nomadic or semi-nomadic hunter–gatherers, practising occasional horticulture (Mendoza & Wright, 1989; Braunstein & Miller, 1999). Division of labour was manifest among the Toba (Karsten, 1967). Female gathering played a major role, complementing the almost exclusively male activities of hunting, fishing and honey collecting (Gordillo, 1995; Braunstein & Miller, 1999). The Toba organized themselves in bands composed of groups of extended families. Traditional leadership was limited to extended family heads (Miller, 1980). Monogamy was the main mating pattern.

The Gran Chaco Indians successfully resisted Spanish colonization and Argentine expansion policies, until the late 1800s. Until the 1930s most communities still relied on foraging for their subsistence. During the last century, disruptions to their traditional lifestyle and ecological deterioration of the habitat forced many communities to migrate to urban centres and become settled. At present, indigenous communities in the Gran Chaco fall along an acculturation continuum ranging from the more traditional, living in rural, isolated areas to the more Westernized communities, living in the periphery of most non-indigenous towns in the Gran Chaco and in the cities of Rosario and Buenos Aires (Miller, 1999).

The Toba village of Namqom is located 11 km north-west of the city of Formosa (58 ° 2′ W, 26 ° 2′ S) in the Argentine Chaco. Today, Namqom has a population of approximately 2300 people distributed across 100 hectares. Settled, peri-urban communities, such as Namqom, rely mainly on wage labour of men for their subsistence. Women's activities include household chores, looking after children and basket weaving. Less than 5% of women have paid jobs as cooks or as teaching assistants at the local school. Some women go to the city once a week to sell their weavings or wild herbs door to door (Valeggia & Ellison, 2001).

The provincial government offers pre- and postnatal care programmes, carried out at the village health centre or at the city hospital. At present, 91% of all infants are born at hospitals (Programa NacyDef, 2000). Toba women breast-feed their infants for two to three years, or until the second trimester of their next pregnancy (Valeggia & Ellison, 2001). Co-sleeping and night-time nursing are universal. The current postnatal programme encourages mothers to introduce supplements at 4–6 months of age.

### *Study design*

This study was designed as a two-part research project. The first part consisted of the characterization of reproductive patterns of the Toba population in Namqom. At the beginning of the study, a village-wide demographic survey was conducted, which was followed by the collection of reproductive histories for all women aged 11–65. During the demographic census all households were visited and names, date of birth and place of origin of all household members recorded. From the census data, all women of reproductive age were identified and interviewed at their homes to obtain their reproductive histories. Reproductive history data included current reproductive status (pregnant, non-pregnant/non-lactating, lactating/amenorrhoeic, lactating/menstruating, menopausal) number of pregnancies, number of miscarriages/abortions, number of live births, number and date of birth of live offspring, menstrual history (regular, irregular) and present and past reproductive health status. These data were cross-checked with personal records at the health centre.

The second part of this study evaluated the interaction between nutritional status and breast-feeding intensity on the duration of lactational amenorrhoea. A mixed-longitudinal prospective design was used to conduct this part of the project. Subjects were asked to go once a month to the health centre for anthropometric measurements and were followed until they experienced their third postpartum menses. A short interview was conducted during the monthly measurements. This interview consisted of four structured questions including: (1) breast-feeding behaviour (exclusive, partial, token, no breast-feeding); (2) timing and quality of first introduction of supplements; and (3) timing of first postpartum menstruation.

A sub-sample of women was selected from the larger pool for a more detailed study of nursing behaviour and reproductive changes. Home behavioural observations for each of these women were scheduled twice a month until the woman had experienced her third menses postpartum. During the home visits data were collected on type and duration of daily activities of the mother, temporal pattern of nursing, food consumption, morbidity of mother and infant and physical activity. In addition, urine samples were collected weekly for measurements of oestrogen and progesterone conjugates.

### *Subjects*

Demographic information was collected from a total of 1942 villagers. A total of 395 women (ages 11–65) provided reproductive history data.

Subjects in the lactational amenorrhoea study were 113 breast-feeding mothers and their infants. Most subjects were recruited during the demographic census. The research team approached the remaining subjects during their first postnatal visit to the local health centre. Subjects were recruited within six months of their last delivery. With one exception, they were all still breast-feeding at the end of the study (March 2000). All participating women had a full-term birth (birth weight>2500 g, gestational age>37 weeks), were amenorrhoeic at the beginning of the study, and were not using contraceptives of any kind. None of the subjects smoked or was voluntarily dieting to lose weight.

Subjects in the sub-sample group were 70 women selected from the larger pool. These women had infants who were 4 months old or younger at the beginning of the study and were breast-feeding them exclusively. Participating breast-feeding mothers represented 90% of all the breast-feeding, amenorrhoeic women in the village at the beginning of the study. The remaining 10% included women who did not meet the full-term birth criterion (4), declined to participate in the study (3), had had a twin birth (1), were planning to foster away their babies (3), or had symptoms of mastitis (2). Informed consent was obtained from all the subjects after the objectives and the methods of the study were explained to them. The protocol for this study was approved by the Harvard University Standing Committee on the Use of Human Subjects in Research (1998).

### *Anthropometry*

Maternal pre-pregnant weights and infant birth weight data were obtained from the village health centre records and from a preliminary study conducted in 1997 (Valeggia & Ellison, 1998). Weight and height were measured using a beam balance and a portable aluminium standiometer, respectively. Triceps and subscapular skinfold thickness was measured in triplicate on the left arm using a calibrated Lange skinfold calliper accurate to 0·5 mm. Percentage body fat was estimated using a BEI meter (BIA RJL Model 101S, RLJ-Systems Inc., Detroit, MI, USA). Percentage of body fat was estimated from bioimpedance values using previously derived equations (Lukaski & Bolonchuk, 1987). Anthropometric measures are explained in detail elsewhere (Valeggia & Ellison, 2003).

### *Behavioural observations*

Each mother–infant pair in the sub-sample ($n=70$) was observed an average of 1·7 times per postpartum month. Behavioural observations were conducted at the subject's home and were separated by at least 2 weeks. Observation periods lasted 2 hours and were scheduled in the morning or in the afternoon. Morning and afternoon periods were balanced within subjects and across the study. Early morning periods started between 7 and 8 a.m. and late morning periods started between 10 and 11 a.m. Early afternoon periods started between 12 and 1 p.m. and late afternoon periods started between 3 and 5 p.m. In all, 1852 hours of observation were recorded. Prior to data collection, each mother–infant pair was visited twice to habituate the participants to the recording activities. One of the researchers and three trained research assistants performed all behavioural observations. The criterion of inter-observer reliability was 85% concordance and it was obtained within four observation sessions.

During each visit, the observer recorded the temporal pattern of nursing. Periods 'on' and 'off' the nipple were recorded to the nearest second with a stopwatch. A nursing episode began when the infant's mouth covered the nipple and finished when the nipple was visible again. All occurrences of nursing during the 2-hour observation visit were recorded. Intervals between nursing episodes that lasted 5 seconds or less were discarded and the duration of episodes was added.

The activities that the mother and her infant engaged in were recorded using focal sampling. The type of activity performed by the mother was recorded to the nearest minute using continuous recording (Martin & Bateson, 1993) during the entire observation period. As an approximation to the mother's level of physical activity, postures and activities such as standing, sitting, croaching, walking, walking with load and chopping firewood were recorded using instantaneous sampling every five minutes, for a total of 24 observation points per session (Martin & Bateson, 1993). All instances of food consumption by the mother as well as any supplementary food consumed by the infant were also recorded on the check-sheet. Food type, quantity, and time of consumption of food items were included in the recording. Self-report of the health status of the mother, and of her report on the infant's health, were recorded at the beginning of each observation.

### *Urine sample collection and hormone analysis*

First-void morning urine samples were collected once a week from subjects in the sub-sample ($n=70$) until they experienced three menstrual periods. The mean interval between successive samples was 7·6 ($\pm$1·2) days. Samples were aliquoted in triplicates and frozen at $-20$ °C within 2 hours of collection. Urine samples corresponding to the third week of each postpartum month were analysed for oestrone-1 conjugates ($E_1C$), pregnanediol-3a-glucuronide (PdG) and C-peptide. Steroid hormone levels were estimated using enzyme-immunoassays (EIAs) previously characterized (Lasley & Shideler, 1994). Antibodies and reagents were kindly provided by Dr B. Lasley (ITEH, University of California, Davis). Urinary creatinine concentrations were estimated using Taussky's method (Taussky, 1954). Hormone concentrations are expressed as nanograms or picograms of hormone per milligram of creatinine. Intra- and interassay coefficients of variation for $E_1C$ averaged 6·4% and 8·5%, respectively. The average intra- and interassay coefficients of variation for PdG were 8·1% and 13·5%, respectively. Three women became pregnant during the urine-sampling period. Post-conception samples from these women were not included in the analyses of ovarian function.

### *Data analysis*

To allow for comparisons with other studies on breast-feeding and lactational amenorrhoea, the following definitions were used in the analysis of data. Following Labbok and Krasovec's definitions (Labbok & Krasovec, 1990), breast-feeding was considered exclusive when breast milk was the infant's only source of nourishment (no water, teas or juices were given). Partial breast-feeding was taken as any breast-feeding supplemented by other liquid, semi-solid or solid food items such as formula, soups, juices, purées and fruits.

Menstruation was defined as any vaginal bleeding or spotting that lasted three days or more and that occurred after day 45 postpartum.

Intensity of nursing was analysed using four parameters: frequency (nursing episodes per hour), duration of individual nursing episodes, duration of inter-episode intervals, and total duration of nursing per hour. For each 2-hour observation session, the mean frequency of nursing episodes per hour was calculated as the number of instances the infant was on the nipple divided by two. The mean duration of individual nursing episodes was calculated as the average duration of all nursing episodes observed during the session. The mean duration of inter-episode intervals was calculated as the average duration of all closed intervals between nursing episodes observed during the session. Finally, total duration of nursing per hour was computed as the sum of minutes the infant was on the nipple during the entire observation divided by two. When subjects had more than one observation for a given postpartum month, nursing parameters were averaged. Thus, each subject had four nursing parameters for each postpartum month in which she was observed. These parameters were used to evaluate changes in nursing behaviour.

Each subject's nursing style was characterized by the grand mean of each of the four nursing parameters mentioned above. The association between nursing behaviour and lactational amenorrhoea was evaluated using nursing behaviour both as a time-dependent variable and as a single, average index of individual nursing style.

The association between energetics and duration of lactational amenorrhoea was also analysed using 'static' measures of nutritional status and 'dynamic' measures

**Table 1**. Age-specific fertility rates of Namqom Toba women (TFR = 6·71)

| Age (years) | Women-years at risk | No. offspring born | 5-year mean $m(x)$ |
|---|---|---|---|
| 10–14 | 1983 | 27 | 0·014 |
| 15–19 | 1643 | 317 | 0·193 |
| 20–24 | 1221 | 359 | 0·294 |
| 25–29 | 862 | 241 | 0·280 |
| 30–34 | 586 | 140 | 0·239 |
| 35–39 | 387 | 71 | 0·183 |
| 40–44 | 205 | 23 | 0·112 |
| 45–49 | 111 | 3 | 0·027 |

that reflect maternal energy balance. The following static measures were analysed for each individual woman: pre-pregnancy BMI, average postpartum BMI, average postpartum percentage body fat, average postpartum arm circumference, BMI at 1 month before resumption, and average BMI of the 3 months before resumption. Anthropometric and body composition measures were also taken as time-dependent variables and analysed against time to resumption of menses. In addition, ΔBMI was calculated as an index of energy balance that reflected the more dynamic nature of maternal postpartum energetics. ΔBMI was calculated as $\text{BMI}(t_x) - \text{BMI}(t_{x-1})$, where $x$ = postpartum month.

The effect of time postpartum (in months) on hormonal levels, anthropometric measures and nursing variables was tested using one-way ANOVAs. Duration of the period of lactational amenorrhoea was analysed using survival analysis. To assess the impact of independent variables on the duration of lactational amenorrhoea the Cox proportional hazards regression model was used. Differences were considered statistically significant at $p<0{\cdot}05$ in all the tests performed. SPSS Base 10·0 (Windows) was used for all data analysis.

## Results

### *Fertility patterns*

Fertility among Toba women of Namqom was relatively high. The mean number of live births for women over 45 years of age was 6·8 ± 3·4. This value agrees well with the total fertility rate, which equals 6·7 live births (Table 1). Only eight (0·7%) of the 1139 births recorded were reported as twin births. Three women over 45 years of age did not have any pregnancies in their lifetime (10% infertility).

Age at first birth has been declining steadily during the last few decades (Cox regression analysis, $\chi^2 = 5{\cdot}9$, $p = 0{\cdot}013$). A survival analysis indicated that the mean age at first birth declined from 21·5 ± 4·5 years for women born in the 1930s and 40s to 15·5 ± 1·0 years for women born in the 1980s (Fig. 1).


**Fig. 1.** Mean age (in years) at first birth for women born in different decades since 1930.

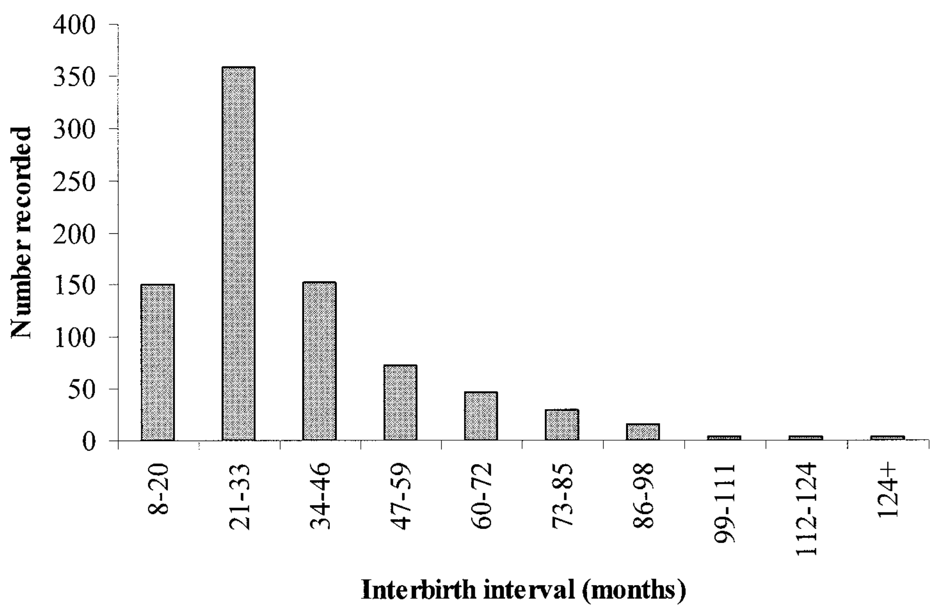



**Fig. 2.** Frequency distribution of 835 inter-birth intervals (IBI) from 236 Toba women.

A total of 236 women provided data on 835 closed inter-birth intervals (IBI). The median IBI was 29·2 months (mean = 35·6 ± 21·2, Fig. 2). There was no association between age of the mother and the duration of the IBIs ($R^2_{(829)} = 0{\cdot}00$, $p = 0{\cdot}92$). However, women born in the 1930–40s tended to have longer IBIs than women born in the 1950s and later ($F_{(4)} = 10{\cdot}5$, $p < 0{\cdot}005$).

https://doi.org/10.1017/S0021932003006382 Published online by Cambridge University Press

**Table 2**. Selected characteristics of participating breast-feeding women

| | *n* | Mean ± SD (range) |
|---|---|---|
| Age (years) | 113 | 24·5 ± 6·2 (14·1–40·6) |
| Parity | 113 | 3·7 ± 2·4 (1–10) |
| Pregnancy weight gain (kg) | 75 | 9·9 ± 4·0 (5·0–27·2) |
| Pre-pregnancy BMI (kg/m$^2$) | 113 | 25·3 ± 3·0 (19·0–32·5) |
| Height* (cm) | 113 | 155·9 ± 4·7 (142·0–166·5) |
| Birth weight of index infant (g) | 111** | 3415 ± 459 (2500–4400) |

*Height was recorded at the beginning of the study.
**The sample included two home deliveries for which the birth weight was not recorded, but which was certainly>2500 g.

### *Anthropometry*

Subjects were well-nourished before initiating the pregnancy with the index infant and presented a normal weight gain during pregnancy (Table 2). Breast-feeding women remained in good nutritional status during the entire postpartum period (Table 3).

Maternal age had a significant effect on changes in BMI ($F_{(2)}=19·2$, $p<0·001$), triceps skinfold ($F_{(2)}=5·4$, $p=0·004$), subscapular skinfold ($F_{(2)}=4·3$, p = 0·01), and percentage body fat ($F_{(2)}=6·9$, $p=0·001$). For each postpartum month observed, on average, 36% of the participating women were adolescents, i.e. women 19 years old and younger. These young women tended to lose weight and fat mass during the first 6 months postpartum. They regained the early postpartum values by the end of the study (Table 3). In adolescent women, changes in all the measures with time postpartum fitted a quadratic regression (BMI: $F_{(309)}=9·65$, $p<0·001$; percentage body fat: $F_{(216)}=4·17$, $p=0·016$; triceps thickness: $F_{(256)}=3·86$, $p=0·022$; subscapular thickness: $F_{(256)}=9·86$, $p<0·001$). Adult women showed no changes with time postpartum for any of the anthropometric measures. A more detailed analysis of postpartum anthropometric changes is presented elsewhere (Valeggia & Ellison, 2003).

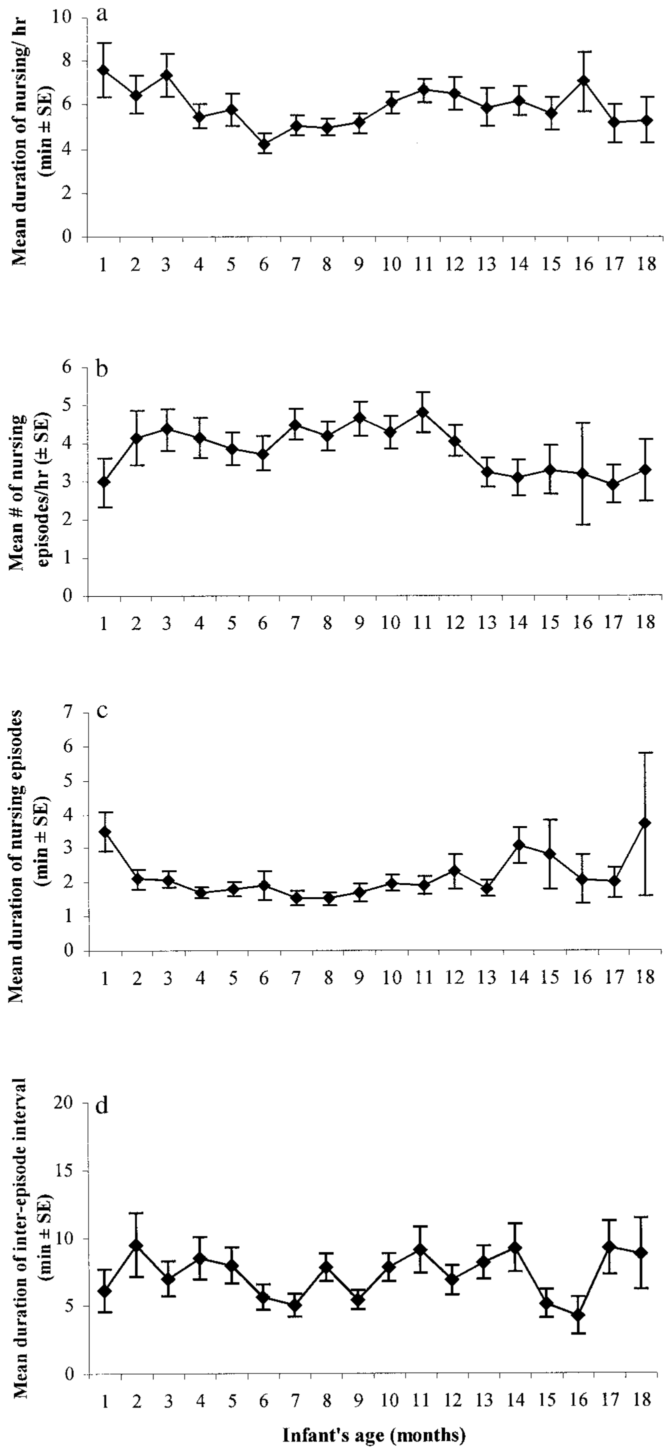
### *Behavioural observations*

*Nursing behaviour.* The mean duration of exclusive breast-feeding was 5·7 ± 1·8 months. Seven women (10%) introduced cow's milk (prepared from powdered milk) when the infant was 3 months old or younger. Between 4 and 6 months of age subjects introduced light broths, white breads and mashed starchy vegetables. By 9 months of age infants also received rice, noodles and cornmeal. Usually by 12 months of age infants relied on breast-feeding supplemented by the usual Toba diet. *Torta frita* (fried dough), sweet biscuits and white bread were always the snack of choice for children 6 months old and older. Subjects reported that weaning was started when the mother became pregnant again or when the children 'themselves' decide to stop nursing (usually around the third year of age).

**Table 3**. Summary of maternal anthropometric measures (means ± SE) for adolescent (<20 years) and adult (≥20 years) breast-feeding mothers at selected months postpartum

| | Adolescents | | | | Adults | | | |
|---|---|---|---|---|---|---|---|---|
| Postpartum month | 1 | 6 | 12 | 18 | 1 | 6 | 12 | 18 |
| Mean BMI (kg/$m^2$) | 27·9 ± 1·2 | 23·9 ± 0·7 | 25·1 ± 0·9 | 26·0 ± 1·7 | 24·5 ± 1·1 | 27·2 ± 1·0 | 26·6 ± 1·1 | 25·1 ± 1·6 |
| Mean % fat | 38·5 ± 3·6 | 33·8 ± 1·8 | 35·0 ± 2·8 | 37·6 ± 5·2 | 36·4 ± 1·7 | 38·5 ± 2·1 | 36·8 ± 3·1 | 36·2 ± 3·0 |
| Mean triceps skinfold (cm) | 20·3 ± 2·8 | 18·5 ± 1·3 | 19·7 ± 1·6 | 21·6 ± 1·0 | 18·1 ± 0·8 | 21·8 ± 1·6 | 24·1 ± 2·6 | 21·9 ± 2·2 |
| Mean subscapular skinfold (cm) | 29·0 ± 3·6 | 20·6 ± 1·8 | 24·3 ± 2·2 | 25·8 ± 4·2 | 27·8 ± 1·5 | 26·31 ± 2·1 | 28·0 ± 3·1 | 26·9 ± 3·0 |
| Sample size (*n*) | 20 | 26 | 20 | 13 | 37 | 42 | 34 | 23 |

https://doi.org/10.1017/S0021932003006382 Published online by Cambridge University Press




https://doi.org/10.1017/S0021932003006382 Published online by Cambridge University Press

Overall, Toba mothers nursed their infants intensively. A total of 6200 nursing episodes were recorded (mean number of episodes per subject ($\pm$SD) = 8·1 ± 6·7, range 0–37). The number of mother–infant pairs observed in each postpartum month averaged 42 ± 20 and ranged between seventeen pairs observed during the 18th month and 70 pairs observed during the 6th, 7th, 8th and 9th months. During the first 18 months postpartum infants were nursed, on average, between 5 and 8 minutes per hour (Fig. 3a). The frequency of nursing episodes averaged 2·9–4·8 times per hour, for approximately 2 minutes each time (Fig. 3b and c). Mean inter-episode interval oscillated between 5·0 ± 6·9 and 9·2 ± 7·1 minutes (Fig. 3d). There was no change with time postpartum for any of the nursing variables analysed (all $p$ values > 0·05).

Nursing frequency in the early postpartum period may have been affected by the age of the mother. During the first 6 months postpartum, the frequency of nursing episodes was negatively correlated with maternal age ($r^2 = -0{\cdot}173$, $p = 0{\cdot}004$). There was no correlation with maternal age beyond 6 months postpartum.

Pre-pregnancy BMI was not associated with nursing variables when taken as single, average measures per individual (all $p$ values > 0·05). Although differences were not statistically significant, nursing women with higher pre-pregnancy BMI tended to breast-feed more minutes per hour than those with lower pre-pregnancy BMI.

Time of the day had an effect on nursing frequency. Toba women breast-fed their babies more frequently (3·8 ± 3·2 vs 4·3 ± 3·5 episodes/hour, $F_{(763)} = 3{\cdot}9$, $p = 0{\cdot}049$) and for longer (5·4 ± 3·9 vs 6·2 ± 4·8 min/hour, $F_{(763)} = 9{\cdot}4$, $p = 0{\cdot}002$) in the afternoon than in the morning. Duration of episodes did not differ between morning and afternoon observation sessions. None of the nursing parameters analysed was significantly affected by sex of the infant, day of the week, calendar month or ambient temperature (all $p$ values > 0·05).

*Maternal activities.* Toba mothers spent, on average, 49·1 ± 23·2% of their daytime involved in infant and childcare activities. The second most frequent category was social activity such as chatting with relatives and friends (18·5 ± 19·5% of the observation time). The rest of the day they devoted to miscellaneous household activities, predominantly washing (8·8 ± 16·3%) and cooking (4·4 ± 11·1%).

Breast-feeding women had a low level of physical activity. On average, they were observed in low-energy activities (i.e. sitting, standing) for more than 85% of observation points (Fig. 4). The level of physical activity was influenced by time postpartum. As the infant grew older, Toba mothers tended to sit more ($F_{(18)} = 1{\cdot}67$, $p = 0{\cdot}04$) and walk less ($F_{(18)} = 1{\cdot}76$, $p = 0{\cdot}02$). There was no correlation with calendar month, day of the week or ambient temperature (all $p$ values > 0·05).

*Maternal diet.* Maternal food intake observations allowed a qualitative analysis of the breast-feeding mothers' diet in this peri-urban setting. Table 4 shows the

**Fig. 3.** Changes in nursing behaviour with time postpartum: (a) mean total duration of nursing per hour of observation (minutes at the breast per hour); (b) mean frequency of nursing episodes per hour of observation; (c) mean duration of individual nursing episodes; and (d) mean duration of intervals between nursing episodes.

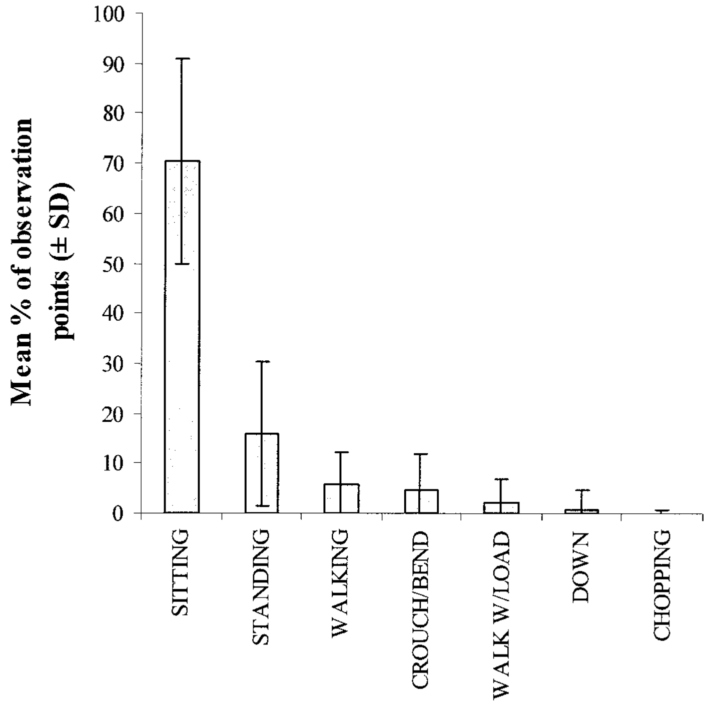



**Fig. 4.** Main categories of maternal activities during the postpartum period and percentage of records in each category.

categories of food items recorded and the percentage of the total food intake entries that each item represents. In general, their diet was relatively monotonous and consisted of calorie-dense food items. The typical day included a breakfast with *mate* (a kind of herbal tea extracted from *Ilex paraguayensis*) and *torta frita* (fried dough). The main meal of the day consisted of a stew prepared with noodles, rice or cornmeal with potatoes, onions, carrots and squash. Some pieces of beef or chicken were added to the stew in approximately half of their main meals. During the day, women snacked on white bread, *torta frita*, crackers, sweet biscuits and sugared milk.

*Ovarian function*

Toba women experienced a relatively short period of lactational amenorrhoea. A survival analysis of the reported intervals to first menses postpartum (Fig. 5) yielded a median survival time of 9·75 months (mean (corrected for censored data) ± SD = 10·2 ± 4·3, range = 2·5–29·5). The analysis of monthly menstrual status reports included a total of 103 closed lactational amenorrhoea intervals and ten right-censored intervals (10%). The censored intervals corresponded to six women who were still amenorrhoeic at the end of the study and four women who left the study area before their first postpartum menses.

**Table 4.** Categories of main food items consumed in a typical diet of breast-feeding Toba women and percentage of diet record entries each category represented

| Food | % of entries |
|---|---|
| White breads | 33·0 |
| *Torta frita* | 22·7 |
| Stew with beef or chicken | 13·6 |
| Stew with noodles, rice or cornmeal | 6·9 |
| Sugared milk | 4·7 |
| Candy | 3·6 |
| Fruits | 3·6 |
| *Mate* with sugared milk | 3·0 |
| Soda | 2·8 |
| Biscuits | 2·2 |
| Miscellaneous | 2·1 |
| Eggs | 1·6 |

The resumption of postpartum menses was not associated with nursing behaviour either taken as a summary index per woman or taken as a time-dependent variable. None of the four nursing parameters selected to define individual nursing styles had a significant association with the duration of lactational amenorrhoea (regression statistics: nursing frequency, $r^2=0{\cdot}001$, $p=0{\cdot}77$; duration of nursing bouts, $r^2=0{\cdot}035$, $p=0{\cdot}113$; total duration of nursing/hour, $r^2=0{\cdot}032$, $p=0{\cdot}128$; and interval duration, $r^2=0{\cdot}08$, $p=0{\cdot}23$). When nursing parameters were analysed as time-dependent variables, i.e. when monthly values were analysed against time to resumption, none of the parameters was found to be significantly associated with duration of lactational amenorrhoea. Pearson correlation values between months from first postpartum menses and nursing parameters were the following: for duration of nursing bouts, $r^2=-0{\cdot}062$, $p=0{\cdot}14$; for total duration of nursing/hour, $r^2=-0{\cdot}07$, $p=0{\cdot}11$; for frequency of bouts/hour, $r^2=-0{\cdot}08$, $p=0{\cdot}08$; and for interval duration, $r^2=-0{\cdot}028$, $p=0{\cdot}51$.

Timing of introduction of supplements was a mild predictor of duration of lactational amenorrhoea ($r^2=0{\cdot}08$, $p=0{\cdot}015$). Maternal age was negatively, but not significantly, correlated with duration of lactational amenorrhoea (Pearson correlation $=-0{\cdot}15$, $p=0{\cdot}21$). Maternal morbidity and parity did not affect the timing of postpartum resumption of menses (all $p$ values$>0{\cdot}05$).

None of the static measures of nutritional status (see Data Analysis) was a good predictor of duration of lactational amenorrhoea (all $p$ values$>0{\cdot}05$). However, when anthropometrics and body composition measures were analysed as time-dependent variables, there was an association between those measures and time to resumption of menses. BMI, percentage body fat and arm circumference increase as women approach resumption of menses (regression parameters: for BMI, $r^2=0{\cdot}05$, $p=0{\cdot}059$; for percentage body fat, $r^2=0{\cdot}08$, $p=0{\cdot}006$; for arm circumference; $r^2=0{\cdot}09$, $p=0{\cdot}003$).

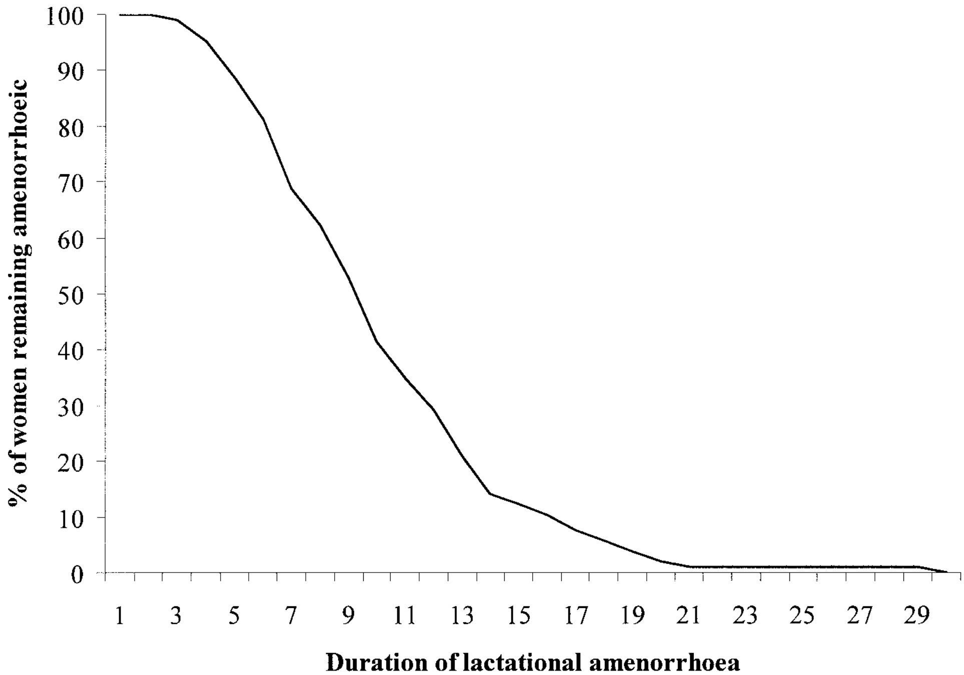



**Fig. 5.** Survival curve of breast-feeding women who had not experienced their first menses with time postpartum.

Lactating women tend to be in a sustained period of positive energy balance by the time they resume postpartum menses. There was a significant correlation between ΔBMI and time to resumption of menses ($r^2=0{\cdot}11$, $p=0{\cdot}002$). Figure 6 shows changes in maternal energy balance as women approach time of first postpartum menses. Due to the nature of the mixed-longitudinal design, sample size for each month prior to resumption of menses varied between 12 (15 months to resumption) and 70 (1 month to resumption), with an average of 45 women sampled.

As expected, urinary metabolites of oestrogen and progesterone increased with time postpartum ($E_1C$: $F=22{\cdot}9$, $p<0{\cdot}001$; PdG: $F=9{\cdot}69$, $p=0{\cdot}002$). Mean $E_1C$ levels showed a steady increase as breast-feeding women approached resumption of menses, suggesting an increasing number of women resuming follicular development (Fig. 7). PdG levels increased in a step-wise fashion, probably reflecting luteal activity in some women during the first cycles postpartum (months to resumption −1 to 1, Fig. 7).

## Discussion

The proximate causes of the contraceptive effect of lactation are still a matter of productive debate. Most evidence has pointed to nursing intensity and maternal nutrition as the major variables affecting the duration of lactational amenorrhoea (Ellison, 1995). However, most discussions seem to be focused on which of these variables is more important in determining fertility postpartum. This study attempted

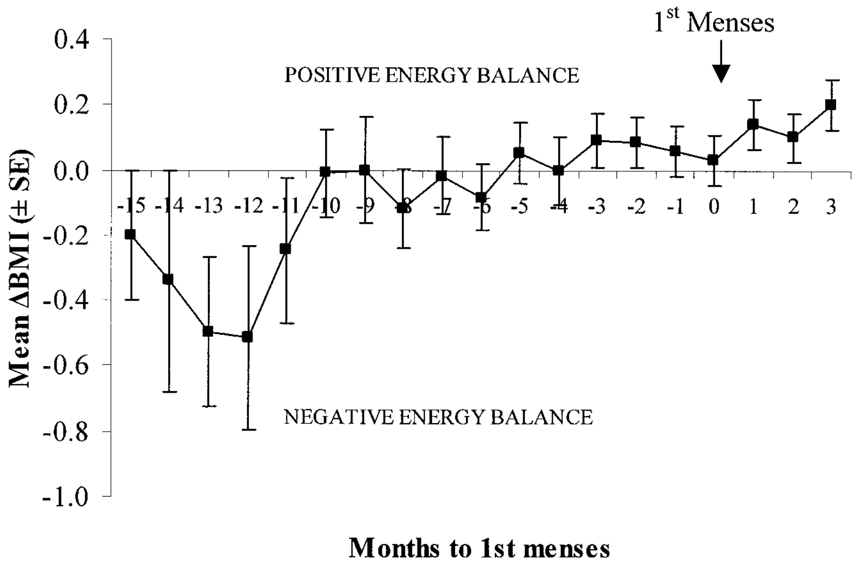



**Fig. 6.** Changes in energy balance (as estimated using the ΔBMI index) in breast-feeding women as they approach their first postpartum menses (time 0).

to test a hypothesis – the relative metabolic load hypothesis – that does not consider breast-feeding behaviour and maternal nutrition as competing determinants but rather integrates both as part of the more encompassing concept of *maternal energetics*.

To test the metabolic load hypothesis, a group of well-nourished, intensively breast-feeding Toba women was selected. This population was characterized by high fertility (6·8 children/post-reproductive woman) and median inter-birth intervals of 29 months. All women participating in this study had a normal pregnancy weight gain and remained well nourished during the postpartum period. A detailed analysis of nutritional status during the first 18 month postpartum has been published elsewhere (Valeggia & Ellison, 2003). Monthly average indices of nutritional status (i.e. BMI, percentage body fat, skinfold thickness and upper arm circumference) indicated that most breast-feeding Toba women had considerable energy reserves during the postpartum period. These indices are similar to those found in more affluent societies (Lewis *et al.*, 1991; Dewey *et al.*, 1993; Heinig *et al.*, 1994; Cohen *et al.*, 1995; Taylor *et al.*, 1999). The situation in Namqom probably reflects the combination of high-caloric intake/low energy expenditure that characterizes this population, which represents an example of the nutritional transition many Amerindian populations are undergoing (Popkin, 2001, 2002). In fewer than 50 years, the Toba who settled in peri-urban villages changed their low-fat, high-protein diet typical of their foraging lifestyle for a high-fat, low-protein one. In addition, this study's behavioural observations indicated that their energy expenditure is very low, with women spending most of their wake time sitting.

Lifestyle changes might also explain changes in fertility patterns observed in this population. Age at first birth and inter-birth intervals are declining whereas number

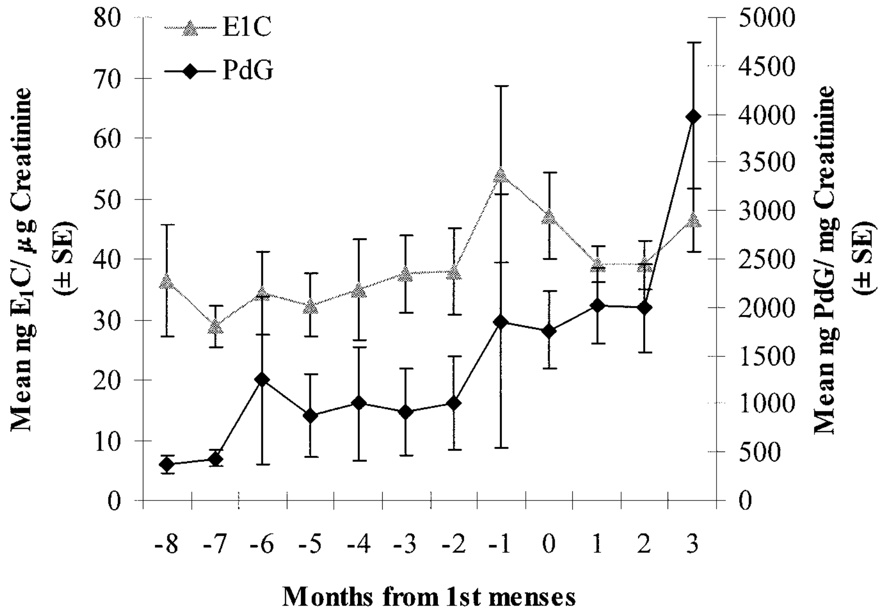



**Fig. 7.** Changes in levels of urinary metabolites of oestrogen ($E_1C$) and progesterone (PdG) in breast-feeding women as they approach their first postpartum menses (time 0). Hormone concentrations are expressed in micrograms or nanograms of hormone per milligrams of creatinine.

of live children seems to be increasing for younger cohorts. There are no data available on age at menarche or on the nutritional status of these women in the past. However, an interaction among several biocultural factors could be hypothesized to underlie these changes. For example, interviews with elders (data not published) indicated that, 50 years ago, women entered marital unions in their early twenties and abortion and infanticide were common in young, unmarried women (Vitar, 1999). In addition, a postpartum sexual taboo prescribed abstinence until the child was able to walk (Tola, 1998). Nowadays, many of these traditional cultural rules have been relaxed. Adolescents meet at school and start living together when the woman is as young as 15 years old. Abortion and infanticide are prohibited by their newly acquired Christian religion and the postpartum period of sexual abstinence is often shortened or altogether ignored by young couples. These cultural changes and the general improvement in health and nutrition are associated with the demographic, epidemiological and nutritional transition that the Toba are currently experiencing.

Still, certain behavioural/cultural patterns remain deeply rooted. Toba mothers breast-feed their infants intensively. Most infants are exclusively breast-fed until almost the sixth month when supplements are gradually introduced. Only one woman in this sample weaned her infant completely before the study was completed. Although individual variation in nursing style is considerable, breast-feeding bouts do not follow a scheduled pattern. As a whole, Toba mothers tend to nurse their infants more intensively in the afternoon than in the morning. Although none of the study participants worked outside their home, household chores (washing clothes, cooking, house cleaning) tend to be done during the morning leaving the afternoon for more socially oriented activities.

Nursing frequency and duration in this population are comparable to those found in other non-Western societies for which breast-feeding has been characterized as 'on demand' (Konner & Worthman, 1980; Rich, 1984; Worthman *et al.*, 1993; Vitzthum, 1994). In contrast to observations in other populations in which older mothers invest more in their offspring, younger Toba mothers nursed their infants more frequently than their adult counterparts. Toba adolescent mothers tend to live with their extended family group, do not have the same responsibilities as adult mothers and, in general, enjoy more free time during the day. Because of this lighter activity pattern, it seems likely that they can afford to breast-feed their infants more frequently than adult mothers.

Contrary to the predictions that follow from the nursing intensity hypothesis, Toba women experienced a relatively *short* period of lactational amenorrhoea and a *high* life-time fertility. Despite intensive and prolonged nursing, these women, on average, resumed menses at 10 months postpartum. These results are consistent with those found in intensively breast-feeding, well-nourished women in other non-Western populations (Rich, 1984; Worthman *et al.*, 1993) and in more affluent societies (Lewis *et al.*, 1991; Heinig *et al.*, 1994; Taylor *et al.*, 1999). They contrast with the long periods of lactational amenorrhoea documented for intensively breast-feeding populations who are also under nutritional stress (Konner & Worthman, 1980; Wood, 1985).

In this population, as in many others (McNeilly, 2001), none of the various estimates of nutritional status was correlated with the duration of lactational amenorrhoea. However, a key concept in the formulation of the relative metabolic load hypothesis is the distinction between static vs dynamic maternal energetics. Previous analyses of determinants of lactational amenorrhoea evaluated *static* measures maternal energetics, such as BMI and body weight, taken at certain times during the postpartum period. This study's data analysis differed from these traditional approaches by considering nutritional status as a time-dependent variable. The energetic budget of the breast-feeding mother varies with time, as a consequence of many factors such as differences in calorie intake, physical activity, lactation demands from a growing or supplemented infant and maternal metabolic efficiency. The data indicated that, when individual changes in measures of nutritional status (i.e. $\Delta$BMI) are aligned by date of resumption of menses, the first postpartum menses occurs after a period of positive energy balance. That is, independently of the previous body weight or BMI, for a given woman, time of resumption is correlated with a period of sustained weight gain. In this sample, this period was correlated, albeit mildly, with the introduction of supplements to breast milk, which represents a reduction in the energy demands of lactation as well as with subtle changes in activity patterns (less time walking, more time sitting).

In sum, these results seem to support the relative metabolic load hypothesis. Differences in the duration of lactational amenorrhoea among the Toba can be explained, at least partly, by differences in individual metabolic budgets. Compared with other populations in which nursing is equally intensive but resumption of postpartum menses takes longer (for a review see Ellison, 1995), Toba women can 'afford' to channel metabolic energy towards reproduction earlier. It is important to emphasize that these data do not undervalue the importance of nursing intensity in the dynamics of postpartum fertility. When the breast-feeding woman's energetic budget is limited, e.g. under malnutrition or under high energetic output, nursing intensity can be the most important single determinant of the relative cost of lactation for that woman (Valeggia & Ellison, 2001).

This view of the determinants of postpartum fertility has been also supported by changes in maternal insulin metabolism, a detailed analysis of which has been published elsewhere (Ellison & Valeggia, 2002, 2003). Briefly, Toba women experience a transient insulin resistance phase that coincides with the resumption of ovarian activity. Because insulin directly synergizes with gonadotropins in stimulating ovarian steroidogenesis, the high levels of insulin during the insulin-resistant phase in lactating women may play a role in stimulating the resumption of ovarian activity. The dynamics of insulin sensitivity during lactation, by reflecting maternal energy metabolism, may aid in synchronizing the resumption of ovarian function with a reduction in the energy demands of milk production. This could serve as a mechanism for adjusting the duration of lactational amenorrhoea to the relative metabolic load of lactation.

## Acknowledgments

The authors thank the Toba community of Namqom for their generosity and patience. They are indebted to Claudia Fernández and their skilled research assistant team for a superb job. Dr R. Stevens and Dr C. Mirassou provided continuous support in the field. Eduardo Fernández-Duque and two anonymous reviewers are thanked for their comments and suggestions on an earlier version of this manuscript. This work was funded by the Nestlé Foundation, the National Institute of Infant Health and Development (USA), the Wenner-Gren Foundation, the National Council for Scientific and Technological Research of Argentina (CONICET) and the David Rockefeller Center for Latin American Studies at Harvard University.

## References

**Braunstein, J. & Miller, E.** (1999) Ethnohistorical introduction. In Miller, E. (ed.) *Peoples of the Gran Chaco*. Bergin & Garvey, Westport, CT.

**Brüning, J. C., Gautam, D., Burks, D. J., Gillette, J., Schubert, M., Orban, P. C., Klein, R., Krone, W., Muller-Wieland, D. & Kahn, C.** (2000) Role of brain insulin receptor in control of body weight and reproduction. *Science* **289**, 2122–2125.

**Cohen, R. J., Haddix, K., Hurtado, E. & Dewey, K.** (1995) Maternal activity budgets, feasibility of exclusive breastfeeding for six months among urban women in Honduras. *Social Science and Medicine* **41**(1), 527–536.

**Dewey, K. G.** (1996) Does maternal supplementation shorten the duration of lactational amenorrhea? *American Journal of Clinical Nutrition* **64**, 377–378.

**Dewey, K. G., Heinig, M. J. & Nommsen-Rivers, L. A.** (1993) Maternal weight-loss patterns during prolonged lactation. *American Journal of Clinical Nutrition* **58**, 162–166.

**Diaz, S.** (1989) Determinants of lactational amenorrhea. *International Journal of Gynecology and Obstetrics* **1**, 83–89.

**Diaz, S., Cardenas, H., Zepeda, A., Brandeis, A., Schiappacasse, V., Miranda, P., Seron-Ferre, M. & Croxatto, H. B.** (1995) Luteinizing hormone pulsatile release and the length of lactational amenorrhoea. *Human Reproduction* **10**(8), 1957–1961.

**Ellison, P. T.** (1994) Advances in human reproductive ecology. *Annual Review of Anthropology* **23**, 225–275.

**Ellison, P. T.** (1995) Breastfeeding, fertility, and maternal condition. In Stuart-Macadam, P. & Dettwyler, K. A. (eds) *Breastfeeding: Biocultural Perspectives.* Aldine de Gruyter, New York, pp. 305–345.

**Ellison, P. T., Panter-Brick, C., Lipson, S. F. & O'Rourke, M. R.** (1993) The ecological context of human ovarian function. *Human Reproduction* **8**(12), 2248–2258.

**Ellison, P. T. & Valeggia, C. R.** (2002) Postpartum changes in urinary C-peptide levels in relation to resumption of menses in lactating Toba women of northern Argentina. *American Journal of Human Biology* **14**(1), 115.

**Ellison, P. T. & Valeggia, C.** (2003) Postpartum changes in urinary C-peptide levels in relation to resumption of menses in lactating Toba women of northern Argentina. *Fertility and Sterilit* **80**(4), 880–881.

**Fink, A. E., Fink, G., Wilson, H., Bennie, J., Carroll, S. & Dick, H.** (1992) Lactation, nutrition and fertility and the secretion of prolactin and gonadotrophins in Mopan Mayan women. *Journal of Biosocial Science* **24**, 35–52.

**Glasier, A. F., McNeilly, A. S. & Baird, D. T.** (1986) Induction of ovarian activity by pulsatile infusion of LHRH in women with lactational amenorrhea. *Clinical Endocrinology* **24**, 243–252.

**Goldman, N., Westhoff, C. F. & Paul, L. E.** (1987) Variations in natural fertility: the effect of lactation and other determinants. *Population Studies* **41**, 127–146.

**Gordillo, G.** (1995) *La subordinación y sus mediaciones, Dinámica cazadora-recolectora, relaciones de producción, capital comercial y estado entre los Tobas del oeste de Formosa*. Producción doméstica y capital, estudios desde la antropología económica. H. Trinchero. Biblos, Buenos Aires.

**Heinig, M. J., Nommsen-Rivers, L. A., Peerson, J. M. & Dewey, K. G.** (1994) Factors related to duration of postpartum amenorrhoea among USA women with prolonged lactation. *Journal of Biosocial Science* **26**, 517–527.

**Howie, P. W. & McNeilly, A. S.** (1982) Effect of breastfeeding patterns on human birth intervals. *Journal of Reproduction and Fertility* **65**, 545–557.

**Huffman, S. L., Chowdhury, A., Allen, H. & Nahar, L.** (1987) Suckling patterns and post-partum amenorrhoea in Bangladesh. *Journal of Biosocial Science* **19**, 171–179.

**Jones, R. E.** (1989) Breastfeeding and postpartum amenorrhea in Indonesia. *Journal of Biosocial Science* **21**, 83–100.

**Karsten, R.** (1967) *The Toba Indians of the Bolivian Gran Chaco*. Anthropological Publications, Oosterhout NB.

**Konner, M. J. & Worthman, C.** (1980) Nursing frequency, gonadal function, and birth spacing among !Kung hunter-gatherers. *Science* **207**, 788–791.

**Labbok, M. H. & Krasovec, K.** (1990) Toward consistency in breastfeeding definitions. *Studies in Family Planning* **21**(4), 226–230.

**Labbok, M. H., Perez, A., Valdes, V., Sevilla, F., Wade, K., Laukaran, V. H., Cooney, K. A., Coly, S., Sanders, C. & Queenan, J. T.** (1994) The Lactational Amenorrhea Method (LAM): a postpartum introductory family planning method with policy and program implications. *Advances in Contraception* **10**, 93–109.

**Lasley, B. L. & Shideler, S. E.** (1994) Methods for evaluating reproductive health of women. *Occupational Medicine: State of the Art Reviews* **9**(3), 423–433.

**Lewis, P. R., Brown, J. B., Renfree, M. B. & Short, R. V.** (1991) The resumption of ovulation and menstruation in a well-nourished population of women breastfeeding for an extended period of time. *Fertility and Sterility* **33**(3), 259–536.

**Lukaski, H. & Bolonchuk, W.** (1987) Theory and validation of tetrapolar bioelectrical impedance method to assess human body composition. In Ellis, K., Yasamura, S. & Morgan, W. (eds) *Proceedings of an International Symposium of In Vivo Body Composition Studies*. The Institute of Medicine and Physics, London, pp. 410–414.

**Lunn, P. G., Austin, S., Prentice, A. M. & Whitehead, R. G.** (1980) Influence of maternal diet on plasma-prolactin levels during lactation. *Lancet* **22**, 623–625.

**Lunn, P. G., Austin, S., Prentice, A. M. & Whitehead, R. G.** (1984) The effect of improved nutrition on plasma prolactin concentrations and postpartum infertility in lactating Gambian women. *American Journal of Clinical Nutrition* **39**, 227–235.

**McNeilly, A. S.** (1993) Lactational amenorrhea. *Neuroendocrinology II* **22**(1), 59–73.

**McNeilly, A. S.** (2001) Lactational control of reproduction. *Reproductive Fertility and Development* **13**, 583–590.

**Martin, P. R. & Bateson, P.** (1993) *Measuring Behaviour: An Introductory Guide.* Cambridge University Press, Cambridge.

**Mendoza, M. & Wright, P. G.** (1989) Sociocultural and economic elements of the adaptation systems of the Argentine Toba: the Nacilamolek and Taksek cases of Formosa Province. In Shennan, S. J. (ed.) *Archeological Approaches to Cultural Identity*. Unwin Human Ltd, MA, USA, pp. 243–257.

**Miller, E. S.** (1980) *Harmony and Dissonance in Argentine Toba Society.* Human Relations Area Files, Inc., New Haven, CT.

**Miller, E. S.** (ed.) (1999) *Peoples of the Gran Chaco. Native Peoples of the Americas*. Bergin & Garvey, Westport, CT.

**Olson B. R., Scott, D. C., Wetsel, W. C., Elliot, S. J., Tomic, M., Stojilkovic, S., Nieman, L. K. & Wray, S.** (1995) Effects of insulin-like growth factors I and II and insulin on the immortalized hypothalamic GTI-7 cell line. *Neuroendocrionlogy* **62**(2), 155–165.

**Peng, Y-K., Hight-Laukaran, V., Peterson, A. E. & Perez-Escamilla, R.** (1998) Maternal nutritional status is inversely associated with lactational amenorrhea in Sub-Saharan Africa: results from Demographic and Health Surveys II and III. *Journal of Nutrition* **128**(10), 1672–1680.

**Peterson, A. E., Perez-Escamilla, R., Labbok, M. H., Hight-Laukaran, V., von Hertzen, H. & Van Look, P.** (2000) Multicenter study of the lactational amenorrhea method (LAM) III, effectiveness, duration, and satisfaction with reduced client–provider contact. *Contraception* **62**, 221–230.

**Popkin, B. M.** (2001) The nutrition transition and obesity in the developing world. *Journal of Nutrition* **131**, 871–873S.

**Popkin, B. M.** (2002) An overview on the nutrition transition and its health implications: the Bellagio meeting. *Public Health and Nutrition* **5**(1A), 93–103.

**Programa NacyDef** (2000) *Informe interno sobre estadísticas vitales de la Provincia de Formosa para el año 2000*. Derpartamento de Vigilancia Epidemológica, Ministerio de Desarrollo Humano, Formosa.

**Rahman, M., Mascie-Taylor, C. & Rosetta, L.** (2002) The duration of lactational amenorrhea in urban Bangladesh women. *Journal of Biosocial Science* **34**, 75–89.

**Retheford, R. D., Choe, M. K., Thapa, S. & Gubhaju, B. B.** (1989) To what extent does breastfeeding explain birth-interval effects on early childhood mortality? *Demography* **2**6, 439–450.

**Rich, J.** (1984) Patterns of breast feeding and lactational infertility: a comparison of the Yolngu with the !Kung San and La Leche League. Unpublished Thesis, Department of Anthropology, Harvard University, Cambridge, USA.

**Taussky, H. H.** (1954) A microcolorimetric determination of creatinine in urine by the Jaffe reaction. *Journal of Biological Chemistry* **208**, 853–861.

**Tay, C. C. K., Glasier, A. F. & McNeilly, A. S.** (1996) Twenty-four hour patterns of prolactin secretion during lactation and the relationship to suckling and the resumption of fertility in breast-feeding women. *Human Reproduction* **11**(5), 950–955.

**Taylor, W., Vázquez-Geffroy, M., Samuels, S. J. & Taylor, D. M.** (1999) Continuously recorded suckling behaviour and its effects on lactational amenorrhoea. *Journal of Biosocial Science* **31**, 289–310.

**Tola, F.** (1998) A restricción sexual en la lactancia y la 'lucha entre hermanos' en un grupo Toba de Formosa. *Anales de la Sociedad Científica Argentina* **228**(2), 27–38.

**Valeggia, C. & Ellison, P. T.** (1998) Nursing patterns, maternal energetics, and postpartum fertility among Tobas of Formosa, Argentina. *American Journal of Physical Anthropology* **26** (supplement), 54.

**Valeggia, C. & Ellison, P. T.** (2001) Lactation, energetics, and postpartum fecundity. In Ellison, P. T. (ed.) *Reproductive Ecology and Human Evolution.* Aldine de Gruyter, New York.

**Valeggia, C. & Ellison, P. T.** (2003) Impact of breastfeeding on anthropometric changes in peri-urban Toba women (Argentina). *American Journal of Human Biology* **15**,1–8

**Vitar, B.** (1999) *Prácticas abortivas entre las indí genas chaqueñas en el siglo XVII. Ethnohistorial CD-ROM. CM Cóceres*. Equipo NAyA (Noticias de Antropología y Arqueología), Buenos Aires.

**Vitzthum, V. J.** (1989) Nursing behaviour and its relation to duration of post-partum amenorrhoea in an Andean community. *Journal of Biosocial Science* **21**, 145–160.

**Vitzthum, V. J.** (1994) Comparative study of breastfeeding structure and its relation to human reproductive ecology. *Yearbook of Physical Anthropology* **37**, 307–349.

**WHO Task Force** (1998a) The World Health Organization multinational study of breastfeeding and lactational amenorrhea: I. Description of infant feeding patterns and the return of menses. *Fertility and Sterility* **70**, 448–460.

**WHO Task Force** (1998b) The World Health Organization multinational study of breastfeeding and lactational amenorrhoea: II. Factors associated with the length of amenorrhoea. *Fertility and Sterility* **70**, 461–471.

**Wood, J. W.** (1994) *Dynamics of Human Reproduction: Biology, Biometry, Demography*. Aldine de Gruyter, New York.

**Wood, J. W., Lai, D., Johnson, P., Campbell, K. L. & Maslar, I.** (1985) Lactation and birth spacing in Highland New Guinea. *Journal of Biosocial Science* **9** (supplement), 159–169.

**Worthman, C. M., Jenkins, C. L., Stallings, J. F. & Lai, D.** (1993) Attenuation of nursing-related ovarian suppression and high fertility in well-nourished, intensively breast-feeding Amele women of Lowland Papua New Guinea. *Journal of Biosocial Science* **25**, 425–443.

**Wu, X., Sallinen, K., Anttila, L., Makinen, M., Luo, C., Pollanen, P. & Erkkola, R.** (2000) Expression of insulin-receptor substrate-1 and -2 in ovaries from women with insulin resistance and from controls. *Fertility and Sterility* **73**(3), 564–572.