

BREAST VOLUME AND MILK PRODUCTION DURING EXTENDED LACTATION IN WOMEN

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SUMMARY

Quantitative measurements were made of relative breast volume and milk production from 1 month of lactation until 3 months after weaning, and the storage capacity of the breasts was calculated. The increase in breast tissue volume from before conception until 1 month of lactation was maintained for the first 6 months of lactation (means \pm s.e.m.) (190.3 ± 13.1 ml, number of breasts, $n_b = 46$). During this period of exclusive breast-feeding, 24 h milk production from each breast remained relatively constant (453.6 ± 20.1 g, $n_b = 48$), and storage capacity was 209.9 ± 11.0 ml ($n_b = 46$). After 6 months, breast volume, milk production and storage capacity all decreased. There was a relationship between 24 h milk production and the storage capacity of the breasts, and these both appeared to be responding to infant demand for milk. At 15 months of lactation, the 24 h milk production of each breast was substantial (208.0 ± 56.7 g, $n_b = 6$), even though the breasts had returned to preconception size. This was associated with an apparent increased efficiency of the breast (milk production per unit breast tissue) after 6 months, which may have been due to redistribution of tissues within the breast. The possible causes of the decrease in breast volume are discussed.

INTRODUCTION

The computerized breast measurement (CBM) system (Daly *et al.* 1992), which measures breast volume, has been developed further to allow us to make quantitative longitudinal measurements of breast volume from before conception, throughout pregnancy, until 1 month of lactation (Cox *et al.* 1999). That study demonstrated the wide variability between women in the amount and pattern of breast growth during pregnancy. While Neifert *et al.* (1990) found an association between minimal prenatal breast enlargement and insufficient lactation up to 21 days after birth, Cox *et al.* (1999) found no such relationship. The relationship between breast size and milk production beyond 1 month of lactation has not previously been studied.

Milk production is relatively constant over the first 6 months of lactation (Dewey & Lönnerdal, 1983; Hartmann *et al.* 1995; Cox *et al.* 1996). From 6 to 16 months milk production declines even if mothers are not deliberately weaning their infants (Neville *et al.* 1991). Furthermore, milk production during the first 6 months of lactation is not determined by the concentration of prolactin in the blood (Cox *et al.* 1996). It has been demonstrated that milk production 4–10 months after birth responds to the demand for milk by infants (Daly *et al.* 1993b) but factors controlling milk production beyond this time have not been established.

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As lactation proceeds, mothers are aware of changes in the size of their breasts, but these changes have never been quantified, and their physiological significance has not been determined. We aimed to measure the changes in breast volume from 1 month of lactation until weaning; to determine whether the breast returned to its original pre-conception volume; and to determine whether milk production throughout lactation was limited by either the size or storage capacity of the breast.

METHODS

Subjects

The study was approved by the Committee for Human Rights at The University of Western Australia. The subjects were the same as described in Cox *et al.* (1999). Briefly, the eight subjects who had been studied throughout pregnancy fully breast-fed their infants for at least 6 months. All the infants were introduced to solid foods between 6 and 12 months of age, and complete weaning occurred between 18 and 24 months for subjects 1, 2, 3, 4, 7 (left breast) and 8. We measured their breast volume and 24 h milk production at 1, 2, 4, and 6 months of lactation, at 3-monthly intervals until weaning, and where possible 3 months after weaning. Subject 1 was unavailable for measurements after 9 months of lactation but returned for measurement after weaning. Subject 3 was only able to have 24 h milk production measured at 6 weeks, and breast volume and 24 h milk production at 6 and 12 months of lactation and after weaning. The final measurement of breast volume in subjects 5, 6 and 7 (right breast) was made between 30 and 33 months after birth. Their infants were still going to the breast between twice a day and once every two days, but the amount of milk taken from each breast was less than 70 g per day.

Milk production

Milk production from each breast was measured over a period of at least 24 h by test weighing the mother (Arthur *et al.* 1987). Milk samples (≤ 1 ml) were collected from each breast by either manual breast pump or hand expression into 5 ml polypropylene plastic vials, immediately before and after each breast feed. Samples were frozen as soon as possible and kept at -15°C until analysis. Although subject 2 successfully breast-fed her infant, no milk samples were collected due to her inability to express milk.

Breast volume

Relative breast volume (RBV) (the volume of breast tissue and chest wall contained within an arbitrarily but repeatably drawn black line encompassing all the breast tissue) was measured in the Department of Biochemistry using the CBM system (Cox *et al.* 1999) during each 24 h period of measurement of milk production. Between one and four measurements were made on each breast over a period of up to 5 h. Milk samples (≤ 1 ml) were collected from each breast each time a breast volume measurement was made, and stored as above.

Subject body weight and infant growth

Subjects were weighed in the Department of Biochemistry before conception and during each occasion on which breast volume was measured. Subjects provided records of the birth weight of their infants as measured by midwives attending the birth, and weights of their infants up to 6 months of age as measured by the Community Health Nurse at each subject's Child Health Centre.

Esterified fatty acid measurement

Total esterified fatty acids (EFA) were measured in all the milk samples by the method of Stern & Shapiro (1953) as modified by Atwood & Hartmann (1992). As 98 % of lipid in human milk is present as triacylglycerols (Jensen, 1989), the EFA concentration of the samples was used as a measure of the fat content of the milk assuming an average molecular weight for milk triacylglycerols of 843.1 Da (Daly *et al.* 1993a).

'Empty' breast volume

RBV is a measure of the volume of breast tissue and chest wall in front of the plane defined by the black ellipse. During lactation this RBV also includes the volume of milk contained in the breast at the time of

measurement. Daly *et al.* (1993a) have described an equation which relates the fat content of the milk to the degree of fullness of the breast. Using this equation and the measured fat content of each sample, the degree of fullness of the breast at the particular time the sample was collected can be calculated (Cox *et al.* 1996). From the changes in degree of fullness from before to after each feed and the volumes of milk removed from the breast at each feed, the amount of milk remaining in the breast at each time can be calculated. The RBV measured by the CBM system can then be corrected for the amount of milk in the breast to give the 'empty' breast volume (EBV) (Cox *et al.* 1999). While the breast may never be actually empty, this is the minimum volume of the breast for that day and it can be used to represent the volume of breast tissue (glandular and fatty). The breast volumes quoted in this paper will be expressed as the difference between the calculated 'empty' breast volume and the pre-conception breast volume and are termed proliferated breast volumes (PBV).

Thus, measurements of breast volume can be represented as follows:

$$\begin{aligned} \text{RBV} &= \text{volume of breast tissue} + \text{volume of chest wall} + \text{volume of milk in the breast;} \\ \text{EBV} &= \text{RBV} - \text{calculated volume of milk in the breast;} \\ \text{PBV} &= \text{EBV} - \text{pre-conception RBV.} \end{aligned}$$

That is, PBV represents the difference in the volume of breast tissue between the current measurement and the measurement made before conception.

Storage capacity

The storage capacity of the breast is the amount of milk calculated to be in the breast when it is full. Since no milk samples were collected from subject 2 we were unable to calculate either her EBV (and therefore PBV) or storage capacity, and her data are not included in this paper.

Statistics

Repeated measures analysis of variance was used to test significance of differences between times (SuperAnova, Abacus Concepts, Inc., Berkeley, CA, USA, 1987). Correlations and Student's paired *t* tests were performed using the StatView SE + graphics software package (Abacus Concepts). Results are presented as means \pm S.E.M. unless otherwise stated. *P* values greater than 0.05 are regarded as not significant. The number of subjects is represented by *N* and the number of breasts by n_b .

RESULTS

Milk production and feeding frequency

The 24 h milk production of each breast of each subject is shown in Fig. 1 and the mean 24 h milk production of all breasts in Fig. 2. For the first 6 months of lactation the 24 h milk production from each breast remained relatively constant, averaging 453.6 ± 20.1 g ($n_b = 48$) (Fig. 2). At 9 months of lactation the 24 h milk production from each breast (369.3 ± 47.7 g, $n_b = 12$) was significantly different from that at 1, 2 and 4 months (459.3 ± 48.2 g, $P = 0.029$, $n_b = 12$; 450.0 ± 40.4 g, $P = 0.040$, $n_b = 12$ and 449.7 ± 34.5 g, $P = 0.034$, $n_b = 10$, respectively), but not different from that at 6 months (425.7 ± 39.4 g, $n = 12_b$) or from the mean for each mother for 1–6 months. Between 9 and 12 months of lactation there was a significant decrease in 24 h milk production to 260.3 ± 55.8 g ($P = 0.029$, $n_b = 10$), with a further decrease to 109.2 ± 47.7 g ($P = 0.006$, $n_b = 6$) for subjects 5, 6 and 7 who were breast-feeding at 18 months. At 24 months each breast of these mothers was producing 57.4 ± 41.7 g ($n_b = 5$). Apart from subject 7, there were no consistent bilateral differences in the milk production of the breasts of the subjects.

The mean total daily breast milk intake of the infants over the first 6 months ranged widely from 574 ± 60 g for subject 4 to 1181 ± 94 g for subject 1.

During the first 12 months, each breast was suckled between 3 and 8 times per day, with mean values of 6.4 ± 0.3 ($n_b = 12$), 5.2 ± 0.3 ($n_b = 12$) and 5.1 ± 0.3 ($n_b = 10$) times per day at 1, 6 and 12 months, respectively. From 15 to 24 months each breast was suckled between 2

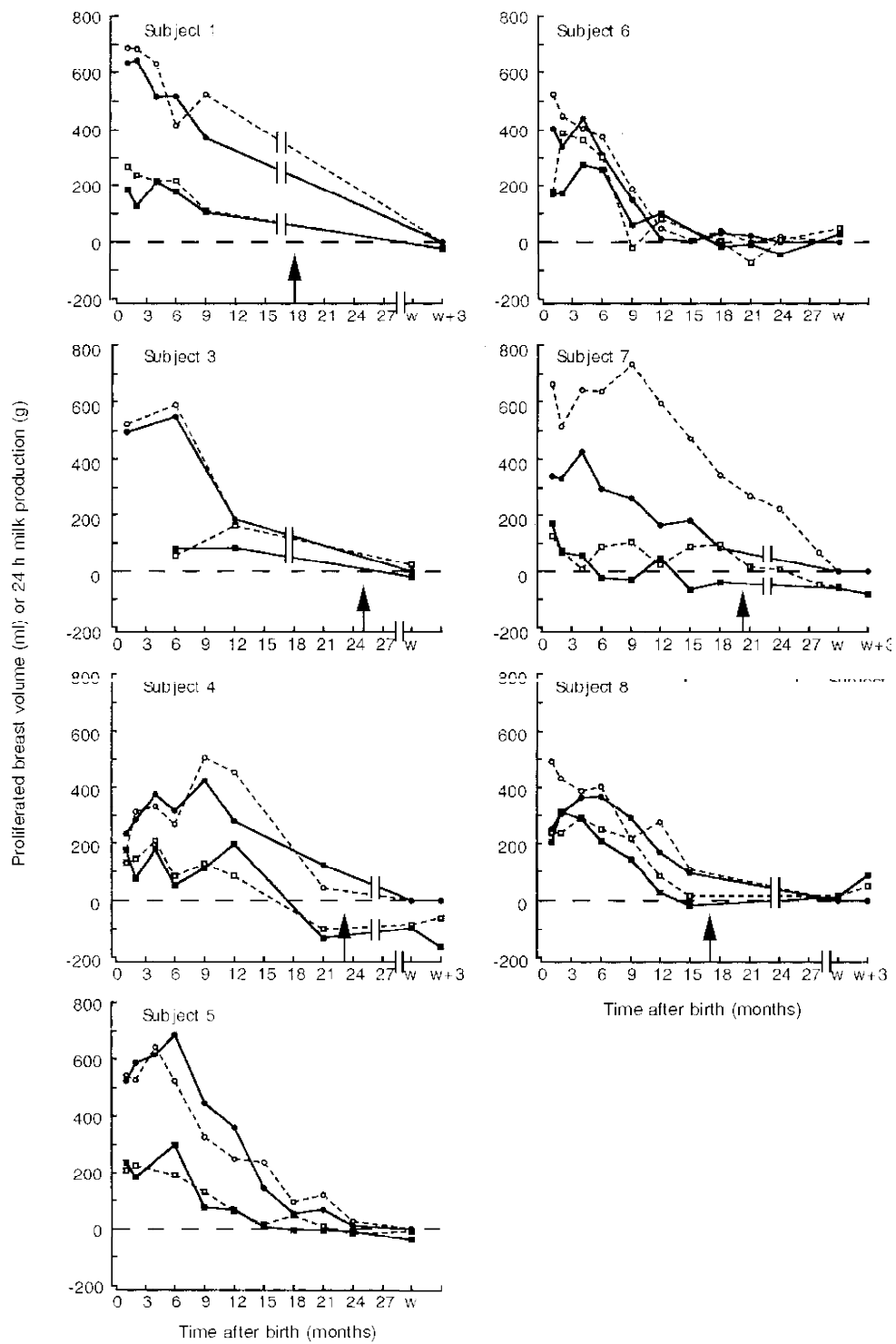


Fig. 1. For legend see facing page.

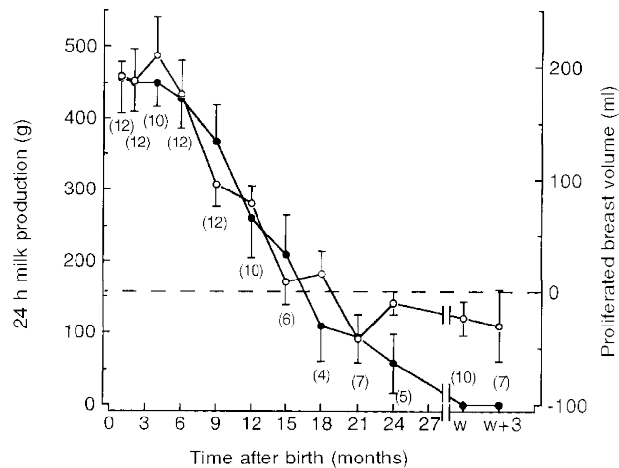


Fig. 2. 24 h milk production (●) and proliferated breast volume (○) of left and right breasts of 6 subjects from 1 month of lactation until 3 months after weaning. w is the time of weaning, w + 3 is 3 months after weaning. Values are means with S.E.M. represented by vertical bars. Number of observations for each point is shown in parentheses. Zero proliferated breast volume is the volume of the breast before conception.

and 6 times per day, with mean values of 3.3 ± 0.3 ($n_b = 8$) and 2.8 ± 0.2 ($n_b = 5$) times per day at 15 and 24 months, respectively. There was no change in the mean amount of milk consumed per feed from each breast during the first 9 months, ranging from 73.8 ± 8.9 g ($n_b = 12$) at 1 month to 83.8 ± 7.2 g ($n_b = 12$) at 6 months. This decreased to 50.4 ± 9.8 g ($n_b = 10$) and 53.1 ± 17.1 g ($n_b = 8$) at 12 months and 15 months, respectively, then decreased further to 34.0 ± 11.5 g ($n_b = 6$), 25.3 ± 8.5 g ($n_b = 7$) and 26.5 ± 21.3 g ($n_b = 5$) at 18, 21 and 24 months, respectively.

Breast volume

The PBV of each breast of each subject is shown in Fig. 1 and the mean of all breasts in Fig. 2. For the first 6 months of lactation PBV remained relatively constant, averaging 190.3 ± 13.1 ml ($n_b = 46$) (Fig. 2). At 9 months of lactation the PBV (95.8 ± 19.8 ml) was significantly different from that at 6 months ($P = 0.003$, $n_b = 12$). The PBV at 12 months (78.8 ± 15.6 ml, $n_b = 10$) was not different from that at 9 months. By 15 months PBV had decreased ($P = 0.035$, $n_b = 6$) and was not significantly different from preconception volume. There was no further change in PBV until weaning. There was no difference in PBV between measurements made both at weaning and 3 months later (subjects 4, 7 (left breast) and 8). The final RBV averaged 17.8 ± 13.8 ml less than pre-conception RBV ($n_b = 14$), but this difference was not significant. Apart from subject 7, there were no consistent bilateral differences in PBV of the subjects.

There was a significant relationship between 24 h milk production and PBV when these measurements over the lactation period were incorporated ($r^2 = 0.428$, $P = 0.0001$, $n_b = 105$)

Fig. 1. 24 h milk production (circles) and proliferated breast volume (squares) of 7 subjects from 1 month of lactation until 3 months after weaning. Open symbols represent the right breast, closed symbols represent the left breast. Arrow indicates time of weaning. w is the time of weaning, w + 3 is 3 months after weaning. For subject 7 the infant was weaned only from the left breast. Zero proliferated breast volume is the volume of the breast before conception.

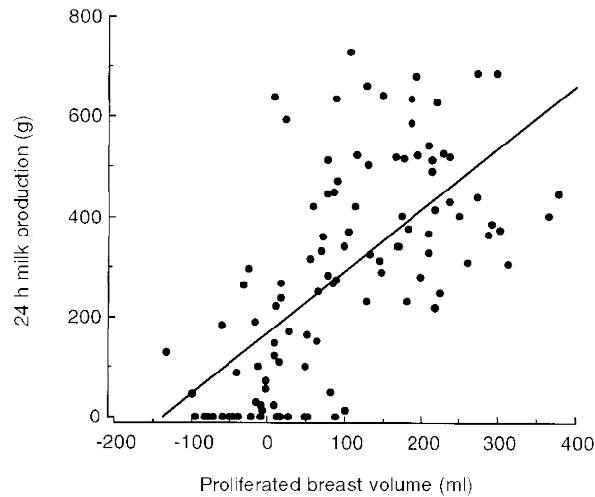


Fig. 3. Relationship between 24 h milk production and proliferated breast volume for left and right breasts of 6 subjects from 1 month of lactation until 3 months after weaning.

(Fig. 3) and for each subject analysed individually (ranging from $r^2 = 0.505$, $P = 0.0002$, $n_b = 22$, in subject 7, to $r^2 = 0.892$, $P = 0.0001$, $n_b = 20$, in subject 5) (Fig. 4). However, when each measurement time was analysed individually, there was no relationship between these parameters.

As a measure of the 'efficiency' of the breast tissue, the ratio of the mean 24 h milk production to the mean PBV at each measurement time was calculated. This showed only a small variation during the first 6 months (2.16 – 2.43 g ml $^{-1}$), then increased to 3.85 g ml $^{-1}$ at 9 months and 3.30 g ml $^{-1}$ at 12 months.

Storage capacity

The change in storage capacity of the breasts is shown in Fig. 5. The average storage capacity at 1 month (179.9 ± 20.2 ml, $n_b = 12$) increased to 231.9 ± 27.3 ml at 4 months ($P = 0.016$, $n_b = 10$). This was followed by a decrease from 6 to 9 months (234.6 ± 17.5 to 174.5 ± 11.7 ml, $P = 0.005$, $n_b = 12$). Apart from subject 7, there were no consistent bilateral differences in the storage capacities of the breasts of the subjects.

There was a strong relationship between storage capacity and 24 h milk production when these measurements for individual breasts over the lactation period were incorporated ($r^2 = 0.588$, $P = 0.0001$, $n_b = 83$) (Fig. 6 and Table 1); and between the sum of the storage capacity of left and right breasts and sum of the 24 h milk production from left and right breasts when these measurements over the lactation period were incorporated ($r^2 = 0.712$, $P = 0.0001$, $N = 43$). There were significant relationships between these parameters at 1 month ($r^2 = 0.739$, $P = 0.0282$, $N = 6$), 2 months ($r^2 = 0.729$, $P = 0.0304$, $N = 6$) and 12 months ($r^2 = 0.827$, $P = 0.0119$, $N = 5$), but not at 4, 6, 9 or 15 months.

There was a relationship between storage capacity and PBV when these measurements over the lactation period were incorporated ($r^2 = 0.147$, $P = 0.0004$, $n_b = 82$). However, when each measurement time was analysed individually, there was no relationship between these parameters.

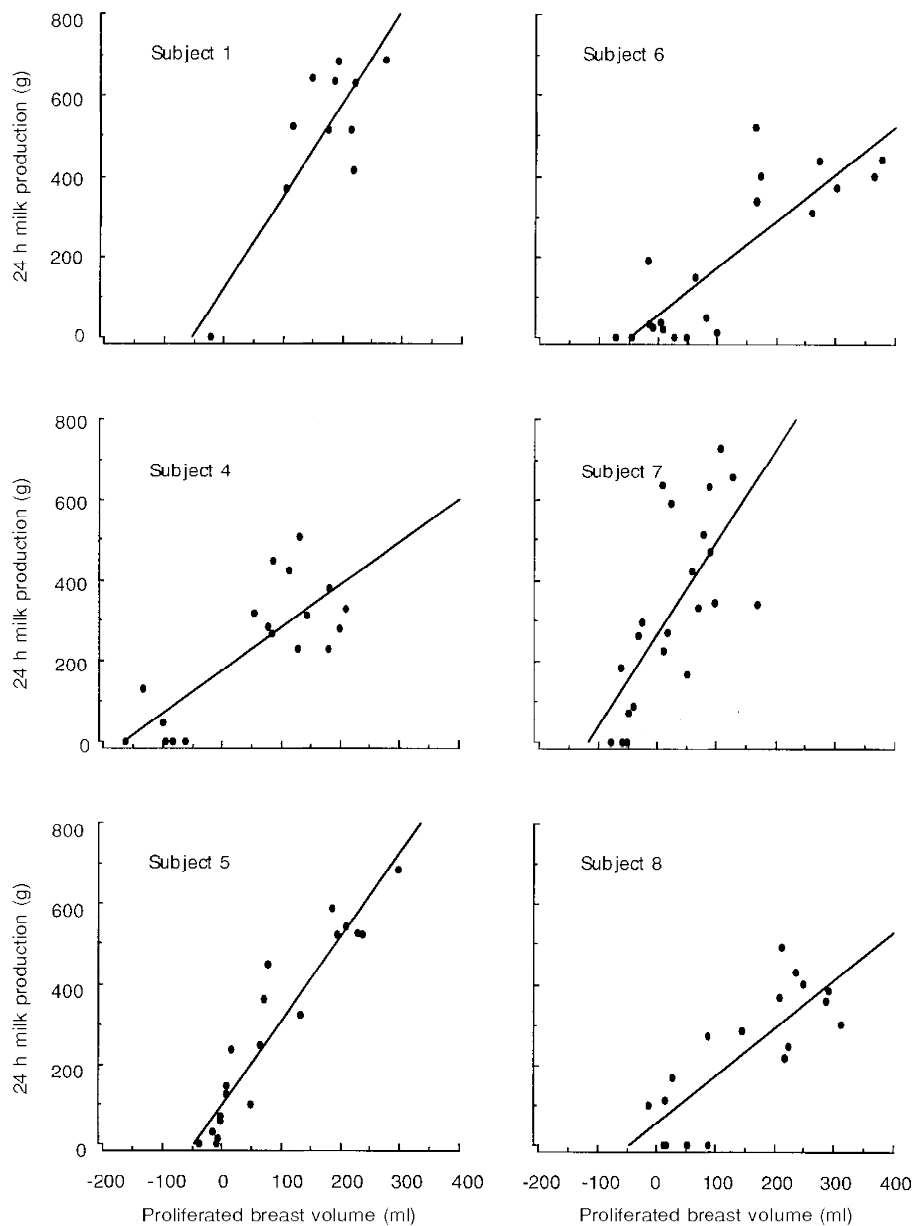


Fig. 4. Relationship between 24 h milk production and proliferated breast volume for left and right breasts of 6 subjects (individual plots) from 1 month of lactation until 3 months after weaning.

At 1 month of lactation there was no relationship between the number of feeds per day and the storage capacity of the breast. While there was a decrease in the number of feeds per day as lactation progressed, the number of feeds per day was not dependent on the storage capacity either throughout lactation or during the first 6 months when milk production was relatively constant.

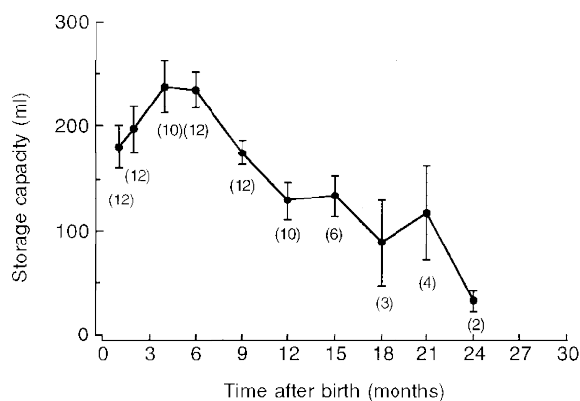


Fig. 5. Storage capacity of left and right breasts of 6 subjects from 1 month until 24 months of lactation. Values are means with S.E.M. represented by vertical bars. Number of observations for each point is shown in parentheses.

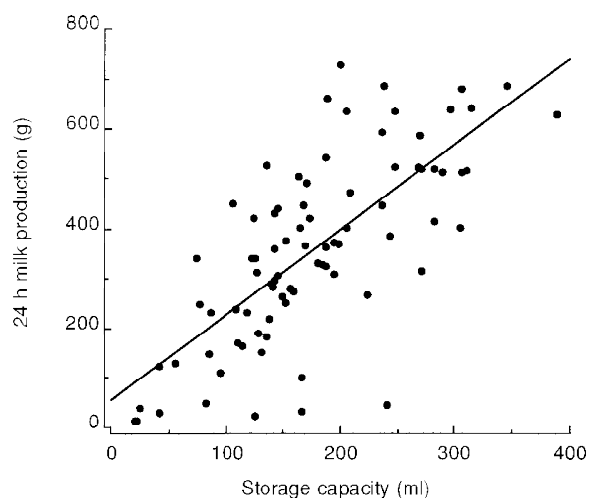


Fig. 6. Relationship between the 24 h milk production and the storage capacity for left and right breasts of 6 subjects from 1 month until 24 months of lactation.

Subject body weight and infant growth

Subject body weight was significantly higher at 1 month of lactation (67.8 ± 4.2 kg) than before conception (64.7 ± 3.4 kg, $P = 0.035$, $N = 6$). During lactation, two subjects lost weight, two subjects gained weight, while the weight of the remaining two fluctuated. Overall, there was a decrease in weight between 6 and 9 months and, for the four subjects still feeding, a further decrease between 9 and 21 months (Fig. 7). The final body weight of the mothers showed a wide range (67.5 ± 4.8 kg) which was not significantly different from before conception (Student's paired t test, $P = 0.170$, $N = 6$).

Table 1. *Correlations of 24 h milk production and storage capacity of individual breasts at different measurement times throughout lactation*

Time (months)	r^2	P	n
1	0.655	0.0014	12
2	0.666	0.0001	12
4	0.491	0.0241	10
6	0.343	0.0453	12
9	0.500	0.0102	12
12	0.662	0.0042	10
15	0.458	n.s.	6
1-24	0.588	0.0001	83

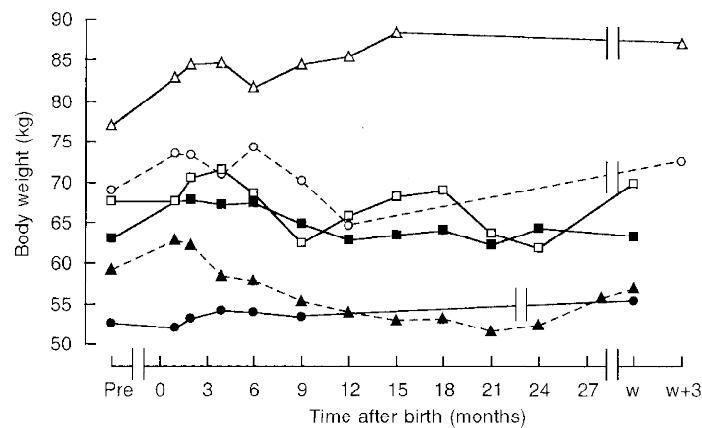


Fig. 7. Body weight of subjects before conception (Pre) and throughout lactation. w is the time of weaning, w + 3 is 3 months after weaning. Subject 1 (●), 4 (○), 5 (■), 6 (□), 7 (▲), 8 (△).

Infants weighed between 2790 g (infant of subject 7 who was delivered at 34 weeks gestation due to placenta praevia) and 4930 g at birth, which is between approximately the 20th and > 97th centiles on the NCHS growth curves for children (US Department of Health, Education and Welfare, 1977). During the first month the weight gain of the infants ranged from 25.7 to 81.8 g per day. At 2 months, the smallest infant was on the 35th centile, while the others were between the 50th and 90th centiles. The mean weight gain for each infant over the first 6 months ranged between 16.1 and 29.5 g per day. At 6 months the infants were between the 20th and just over the 75th centiles. At 12 months all infants ranged between the 3rd and the 50th centiles, and between 19 and 26 months, when two of the infants were weaned and the remaining infants were partially breast-fed, they still ranged between the 3rd and 50th centiles. There was no relationship between the infant weight gain during the first month and PBV (average of left and right for each mother) at 1 month. However, there was a relationship between the mean weight gain of each infant over the first 6 months and its mean daily breast milk intake ($r^2 = 0.827$, $P = 0.045$, $N = 6$) (Fig. 8).

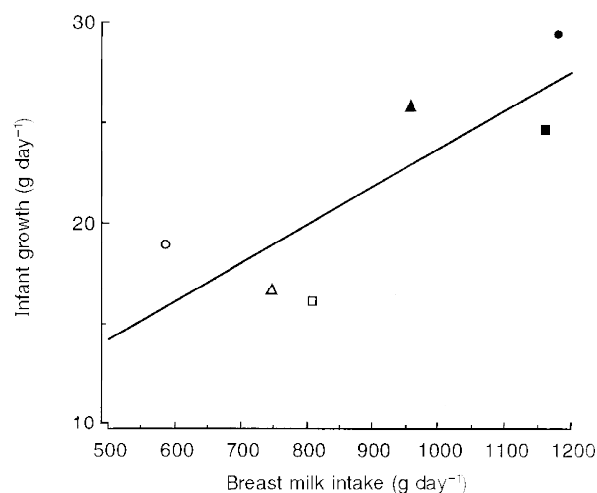


Fig. 8. Relationship between the mean daily growth rate of infants and their mean daily breastmilk intake for the first 6 months of lactation. Subject 1 (●), 4 (○) 5 (■), 6 (□), 7 (▲), 8 (△).

DISCUSSION

Dewey *et al.* (1986) using data pooled from four studies found that 24 h milk production of multiparous women between 6 and 18 weeks of lactation was 755 ± 177 g (mean \pm s.d.). These milk production values were determined by weighing the infants without correction for evaporative water losses, which would lead to an underestimation by 10% (Arthur *et al.* 1987). Therefore, normal milk production would be 830 ± 195 g (mean \pm s.d.) giving a normal range of 440–1220 g (mean \pm 2 s.d.). Based on these figures, the milk production of subject 4 at 1 month (413 g) was lower than normal, and subject 1 at 1 and 2 months (1324 and 1325 g, respectively) and subject 5 at 4 months (1259 g) were higher than normal, while the remainder in the present study were within the normal range. The present data (24 h milk production for 1–6 months of lactation of 905.3 ± 245.3 g, mean \pm s.d.) are also consistent with our previous data (Hartmann *et al.* 1995; Cox *et al.* 1996) for 24 h milk production of 798 ± 216 g and 837 ± 190 g (mean \pm s.d.), respectively, between 1 and 6 months of lactation. The decline in milk production from 6 to 15 months is similar to the data of Neville *et al.* (1991) for mothers who were not deliberately weaning their infants.

It is immediately apparent from Fig. 1 that there was a small variation in PBV between subjects at 1 month (coefficient of variation (c.v.) 15.1%), compared with the large variation in 24 h milk production (c.v. 48.2%). This confirms that 24 h milk production at 1 month is not dependent on the amount of breast growth from before conception until 1 month of lactation (Cox *et al.* 1999). Since milk production is unchanged for the first 6 months of lactation, we conclude that breast growth during pregnancy and the first month of lactation had no effect on milk production while infants were being fully breast-fed. Even though there was a significant correlation between 24 h milk production and PBV when these measurements over the lactation period were incorporated (Figs 3 and 4), there was no relationship between these parameters when each measurement time was analysed individually. This implies that the 24 h milk production is not dependent on PBV, but rather both are decreasing with time.

Between 6 and 9 months of lactation there was a significant decrease in PBV, but no change in 24 h milk production. This resulted in an increase in the ratio of the mean 24 h milk production to the mean PBV at 9 months and implies an increase in efficiency of the breast tissue. This may be due either to an increase in efficiency of the secretory cells during this time, or to mobilization of fat tissue from the breast. Five of the subjects lost weight between 6 and 9 months, so fat tissue may have been lost from the breast along with overall fat loss. However, PBV was not related to body weight changes at other times. It is possible that mobilization of fat from the breast may occur separately from overall loss in body weight, since Hartmann *et al.* (1995) have shown a redistribution of fat stores during lactation.

The decrease in PBV after 6 months may be due to a combination of both mobilization of fat from the breast and loss of mammary cells. Programmed cell death is part of the normal repertoire of the lactating and involuting mammary gland (Quarrie *et al.* 1996). It occurs in rodents and ruminants (Wilde *et al.* 1997) and appears to be under both endocrine and local regulation. In rats, a deficiency of prolactin and growth hormone causes apoptosis in the mammary gland, which is reversible by prolactin treatment (Travers *et al.* 1996). In women, Hennart *et al.* (1981) found that prolactin concentrations in blood samples collected before breast feeds decreased gradually from 1–3 months to 12–15 months, then dropped sharply at 15–18 months to concentrations not different from non-pregnant non-lactating women. Our data showed that the breast volume returned to pre-conception size on average at 15 months, consistent with apoptosis occurring as a result of decreased blood prolactin concentrations.

Mammary apoptosis also appears to be associated with frequency of milk removal. Wilde *et al.* (1997) suggested that frequent milking of goats may inhibit mammary apoptosis. Consequently, a decline in feeding frequency would facilitate mammary apoptosis. The decrease we measured in PBV between 12 and 15 months when the frequency decreased from 5.1 to 3.3 feeds per breast per day is consistent with this hypothesis. In addition to removing inhibition of apoptosis, decline in the frequency of breast-feeds may lead to milk stasis, which has been shown to induce apoptosis in the mammary gland of the rat (Quarrie *et al.* 1996).

Whether the decline in breast volume is due to mobilization of fat from the breast, apoptosis caused by endocrine and local factors, or a combination of these effects, we have found that even when the breast volume had returned to a minimum at 15 months, the combined 24 h milk production from both breasts of the mothers was still between 95 and 315 g. Hennart *et al.* (1981) also found 24 h milk production of between 150 and 300 g from 15–18 months until 27–30 months. This suggests that while proliferation of breast tissue is necessary for sufficient milk production to sustain an infant, the breast does not need to be enlarged above pre-conception size to continue significant milk production. Further studies are needed to identify the proportions of different tissues in the lactating and quiescent breast.

The lack of correlation between storage capacity of the breast and PBV, when each measurement time was analysed individually, implies that the storage capacity is not dependent on PBV, but rather both are decreasing with time.

Our finding of a relationship between the sum of the storage capacity of left and right breasts and the sum of the 24 h milk production from left and right breasts, when these measurements over the lactation period were incorporated, is in contrast to Daly *et al.* (1993b) who found no relationship. However, the subjects in that study were between 4 and 10 months of lactation, and we also found no significant relationship at 4, 6 or 9 months. Treating left and right breasts individually, storage capacity was strongly related to the 24 h milk production both when these measurements over the lactation period were incorporated, and at each measurement time between 1 and 12 months. Analysis of the data of Daly *et al.*

(1993b) also shows a relationship between the storage capacity of each breast and the 24 h milk production from that breast ($r^2 = 0.348$, $P = 0.0039$, $n_b = 22$).

More than 20% of the stored milk remained in each breast after at least two feeds during all 24 h measurement periods. That is, the infant demand for milk was usually satisfied before the breast was empty of available milk. Therefore, the storage capacity was not limiting the infant's intake, but rather responding to the degree of emptying. The only exception was the left breast of subject 7 which was consistently emptied at 1, 2 and 4 months. It is of interest that the left breast of this subject always produced less milk than the right, and that the infant she was feeding during the current study and her previous infant both weaned themselves from this breast before the right breast.

Current growth charts are based on data from a sample of predominantly formula-fed infants. The high growth rate of the infants we measured over the first 2 months was consistent with the finding of Dewey *et al.* (1995) that breast-fed infants grow more rapidly in the first 2 months compared with current growth charts. The lower growth rate between 2 and 12 months was also consistent with the finding of Dewey *et al.* (1995) that breast-fed infants grow less rapidly from 3 to 12 months when compared with current growth charts. The relationship between the average growth rate over the first 6 months and the average daily breast milk intake suggests that milk production of the mother is either limiting growth rate or responding to infant appetite. Since all but one of the breasts was not consistently emptied, it would appear that each mother had the potential to produce more milk. Furthermore milk production, as well as storage capacity, was responding to the infant demand for milk.

In conclusion, we have found that milk production, breast volume and storage capacity all declined after 6 months of lactation. However, significant milk production persisted after the breast had returned to pre-conception volume at 15 months of lactation. Milk production was not dependent on PBV at any stage of lactation. Milk production was closely related to the storage capacity of the breast, and both were apparently responding to the infant demand for milk mediated by the degree of emptying of the breast. Therefore, our data are consistent with the premise that milk production responds to infant demand throughout lactation.

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REFERENCES

- ARTHUR, P. G., HARTMANN, P. E. & SMITH, M. (1987). Measurement of milk intake of breast-fed infants. *Journal of Pediatric Gastroenterology and Nutrition* **6**, 758–763.
- ATWOOD, C. S. & HARTMANN, P. E. (1992). Collection of fore and hind milk from the sow and the changes in milk composition during suckling. *Journal of Dairy Research* **59**, 287–298.
- COX, D. B., KENT, J. C., CASEY, T. M., OWENS, R. A. & HARTMANN, P. E. (1999). Breast growth and the urinary excretion of lactose during human pregnancy and early lactation: endocrine relationships. *Experimental Physiology* **84**, 421–434.
- COX, D. B., OWENS, R. A. & HARTMANN, P. E. (1996). Blood and milk prolactin and the rate of milk synthesis in women. *Experimental Physiology* **81**, 1007–1020.
- DALY, S. E. J., DI ROSSO, A., OWENS, R. A. & HARTMANN, P. E. (1993a). Degree of breast emptying explains changes in the fat content, but not fatty acid composition, of human milk. *Experimental Physiology* **78**, 741–755.

- DALY, S. E. J., KENT, J. C., HUYNH, D. Q., OWENS, R. A., ALEXANDER, B. F., NG, K. C. & HARTMANN, P. E. (1992). The determination of short-term breast volume changes and the rate of synthesis of human milk using computerized breast measurement. *Experimental Physiology* **77**, 79–87.
- DALY, S. E. J., OWENS, R. A. & HARTMANN, P. E. (1993b). The short-term synthesis and infant-regulated removal of milk in lactating women. *Experimental Physiology* **78**, 209–220.
- DEWEY, K.G., FINLEY, D.A., STRODE, M.A. & LÖNNERDAL, B. (1986). Relationship of maternal age to breast milk volume and composition. In *Human Lactation 2: Maternal and Environmental Factors*, ed. HAMOSH, M. & GOLDMAN, A.S., pp. 263–273. Plenum Press, New York, London.
- DEWEY, K.G. & LÖNNERDAL, B. (1983). Milk and nutrient intake of breast-fed infants from 1 to 6 months: relation to growth and fatness. *Journal of Pediatric Gastroenterology and Nutrition* **2**, 497–506.
- DEWEY, K. G., PEERSON, J. M., BROWN, K. H., KREBS, N. F., MICHAELSEN, K. F., PERSSON, L. A., SALMENPERA, L., WHITEHEAD, R. G., YEUNG, D. L. & THE WORLD HEALTH ORGANIZATION WORKING GROUP ON INFANT GROWTH (1995). Growth of breast-fed infants deviates from current reference data: a pooled analysis of US, Canadian, and European data sets. *Pediatrics* **96**, 495–503.
- HARTMANN, P. E., SHERRIFF, J. & KENT, J. (1995). Maternal nutrition and the regulation of milk synthesis. *Proceedings of the Nutrition Society* **54**, 379–389.
- HENNART, P., DELOGNE-DESNOECK, H. VIS & ROBYN, C. (1981). Serum levels of prolactin and milk production in women during a lactation period of thirty months. *Clinical Endocrinology* **14**, 349–353.
- JENSEN, R. G. (1989). *The Lipids of Human Milk*. CRC Press, Boca Raton, FL, USA.
- NEIFERT, M., DEMARZO, S., SEACAT, J., YOUNG, D., LEFF, M. & ORLEANS, M. (1990). The influence of breast surgery, breast appearance, and pregnancy-induced breast changes on lactation sufficiency as measured by infant weight gain. *Birth* **17**, 31–38.
- NEVILLE, M. C., ALLEN, J. C., ARCHER, P. C., CASEY, C. E., SEACAT, J., KELLER, R. P., LUTES, V. J. R. & NEIFERT, M. (1991). Studies in human lactation: milk volume and nutrient composition during weaning and lactogenesis. *American Journal of Clinical Nutrition* **54**, 81–92.
- QUARRIE, L. H., ADDEY, C. V. P. & WILDE, C. J. (1996). Programmed cell death during mammary involution induced by weaning, litter removal and milk stasis. *Journal of Cell Physiology* **168**, 559–569.
- STERN, I. & SHAPIRO, B. (1953). A rapid and simple method for the determination of esterified fatty acids and for total fatty acids in blood. *Journal of Clinical Pathology* **17**, 184–187.
- TRAVERS, M. T., BARBER, M. C., TONNER, E., QUARRIE, L., WILDE, C. J. & FLINT, D. J. (1996). The role of prolactin and growth hormone in the regulation of casein gene expression and mammary cell survival – relationships to milk synthesis and secretion. *Endocrinology* **137**, 1530–1539.
- US DEPARTMENT OF HEALTH, EDUCATION AND WELFARE (1977). NCHS growth curves for children, birth–18 years. DHEW publication (PHS) 78–1650.
- WILDE, C. J., QUARRIE, L. H., TONNER, E., FLINT, D. J. & PEAKER, M. (1997). Mammary apoptosis. *Livestock Production Science* **50**, 29–37.