ARTICLE

Effect of Preterm Birth and Antenatal Corticosteroid Treatment on Lactogenesis II in Women

Jennifer J. Henderson, PhD, MPH[a], Peter E. Hartmann, PhD[b], John P. Newnham, MD[a], Karen Simmer, PhD[a]

[a]School of Women's and Infants' Health and [b]School of Biomedical, Biomolecular and Chemical Sciences, University of Western Australia, Crawley, Western Australia, Australia

The authors have indicated they have no financial relationships relevant to this article to disclose.

ABSTRACT

OBJECTIVE. The onset of copious milk secretion after birth is known as lactogenesis II. The objective of this study was to investigate the effect of preterm birth and antenatal corticosteroids on the timing of lactogenesis II after birth.

METHODS. Women who had received antenatal betamethasone treatment and were expressing for a preterm infant whose gestational age was <34 weeks ($N = 50$) were included. On days 1 to 10 postpartum, participants measured the volume of milk expressed in 24-hour periods and collected milk samples. Lactose and citrate levels were analyzed in the milk.

RESULTS. The gestational age at delivery was 31 weeks (range: 24.2–33.7). Milk volume was recorded by 46 women on 320 expression days and was positively associated with gestational age. Gestational age modified the effect of interval between betamethasone administration and delivery on milk volume. At gestational age 28 to 34 weeks, women who delivered 0 to 2 days after betamethasone treatment obtained significantly greater volumes than women who delivered 3 to 9 days after treatment. Milk samples ($N = 324$) were collected by 42 mothers. Mean ± SD lactose and citrate levels were 156.800 ± 36.217 and 3.458 ± 1.442 mM, respectively. There was a significant positive effect of gestational age on milk lactose levels but not citrate levels. Betamethasone treatment did not alter lactose or citrate levels in milk.

CONCLUSIONS. Delivery at extremely preterm gestational ages caused a significant delay in the onset of lactogenesis II. The volume of milk was reduced further when antenatal corticosteroids were administered between 28 and 34 weeks' gestation and delivery occurred 3 to 9 days later. In view of the advantages of mothers' own milk, additional support with lactation is recommended for mothers of preterm infants, particularly those who have been treated with corticosteroids before the delivery.

www.pediatrics.org/cgi/doi/10.1542/peds.2007-1107

doi:10.1542/peds.2007-1107

Key Words
lactation, initiation of lactation, breast milk, preterm birth, milk production, lactose, antenatal corticosteroids

Accepted for publication May 29, 2007

Address correspondence to Jennifer J. Henderson, PhD, MPH, University of Western Australia, School of Women's and Infants' Health, M550, King Edward Memorial Hospital, 347 Bagot Rd, Subiaco, Western Australia 6008, Australia. E-mail: jhenderson@meddent.uwa.edu.au

MOTHERS' OWN MILK is the optimum form of nutrition for preterm infants when fortified and has been shown to prevent or reduce a wide range of morbidities that are associated with preterm birth.[1] Despite the advantages of breast milk, fewer mothers of preterm infants initiate lactation and the duration of breastfeeding is shorter compared with mothers of healthy term infants.[2–6]

The initiation of copious milk secretion is known as lactogenesis II[7] and is accompanied by increased synthesis by mammary lactocytes of a number of milk components, including lactose and citrate.[8,9] Once lactation is established, paracellular pathways between lactocytes close, leading to rapid increases in the concentration of lactose and citrate in milk.

Lactogenesis II occurs ~30 to 48 hours after birth in mothers of term infants.[10,11] A delay in the onset of lactogenesis II has an adverse effect on the success of subsequent lactation.[12–15] Mothers of preterm infants are more likely to have problems at this stage, as a result of stress, maternal illness, operative delivery, or antenatal pharmacologic therapies. There nevertheless is a paucity of research about the impact of preterm delivery on lactogenesis II, despite evidence showing problems with milk supply in many mothers of preterm infants during the early postnatal weeks.[16,17] Lactogenesis may also be influenced by the previous administration of antenatal corticosteroids, given to enhance fetal maturation.

We showed previously that, in sheep, the administration of antenatal betamethasone causes a precocious initiation of lactation during pregnancy and disrupts lactogenesis II after parturition (unpublished observations, 2003). The effect of antenatal corticosteroid treatment on lactogenesis II in women has not been examined to date.

In this study, we investigated the effect of both preterm birth and antenatal corticosteroid treatment on the timing of lactogenesis II after birth. Lactogenesis II was determined by differences in expressed milk volume and also by the measurement of the concentration in milk of the biochemical markers lactose and citrate.

METHODS

Study Population

Women who presented to King Edward Memorial Hospital for Women (Subiaco, Western Australia) and were at risk for delivery before 34 weeks' gestational age were eligible for inclusion. The women were recruited within 24 hours of receiving their first dose of corticosteroids. Exclusion criteria were age younger than 18 years, no intention to breastfeed, likely poor perinatal outcome, and multiple pregnancy of triplets or greater order. All women received a single course of 2 intramuscular injections of 11.4 mg of betamethasone 24 hours apart (Celestone chronodose; Schering Plough, North Ryde, NSW, Australia) per institutional policy unless delivery occurred before the second dose could be given. Women who delivered before 34 weeks' gestational age and whose infants had not commenced suckling at the breast were included in this analysis. Institutional ethics approval was granted by the University of Western Australia and the Women's and Children's Health Service ethics committees.

Milk Volume Measurement

Women were encouraged to express a minimum of 6 times per day including once overnight by using a commercial electric breast pump (either Medela Lactina or Medela Symphony; Medela AG, Baar, Switzerland). At each expression, women were asked to continue pumping until all milk was removed. After birth, women used a single electric breast pump and graduated to double pumping when they were confident using the pump.

Using digital scales that were accurate to 0.1 g (Tanita Digital Scale; Model 1212, Tanita Corp, Tokyo, Japan), participants measured the weight of milk obtained at every expression on days 1 to 10 postpartum. The day of delivery was counted as day 1. Milk volume (mL) was estimated to equal milk weight without adjustment for density. When the volume was not recorded for 1 expression session within a 24-hour period, the average volume for the other sessions in the day was used for the missing volume. When >1 volume was missing, the remaining data for that day were excluded.

Lactose Levels in Milk

Mixed milk samples (≤1 mL) were collected daily into 5-mL polypropylene vials and were stored at −20°C for analysis. Lactose levels in milk were measured using a modification of methods previously described.[7,8] In brief, duplicate aliquots of diluted, defatted milk and standards were hydrolyzed with β-galactosidase (Roche Diagnostics, Mannheim, Germany), and the resulting glucose was measured. After incubation with a glucose reagent that contained 9.6 U/mL glucose oxidase and 2.5 U/mL peroxidase (Sigma, St Louis, MO) and 500 μg/mL reduced azinodiethylbenzthiazoline sulfonate (Boehringer Mannheim, North Ryde, NSW, Australia) in a phosphate buffer, the absorbance was measured at 405 nm with a microtiter plate spectrometer (Titertek Multiskan MCC/340; Flow Laboratories, McLean, VA). Intra-assay and interassay coefficients of variation were 5% and 2%, respectively, and the detection limit was 3.02 mM. Recovery ± SD of a known amount of lactose added to milk samples was 101.9 ± 3.5%.

Citrate Levels in Milk

Citrate levels in milk were measured using the method of Arthur et al.[8] Defatted milk samples were deproteinized using perchloric acid and neutralized with a potassium hydroxide solution. Duplicates of deproteinized samples and diluted standards were incubated for 10 minutes with a reagent that contained 0.34 mM NADH and 1200 U/mg malate dehydrogenase (Roche Diagnostics) and the absorbance was measured at 340 nm. The decrease in absorbance was then measured after incubation for an additional 10 minutes with the second reagent that contained citrate lyase 120 U/350 mg (Roche Diagnostics). Intra-assay and interassay coefficients of variation were 3.8% and 15%, respectively. The detection limit was 0.78 mM. The recovery of a known amount of citrate added to deproteinized milk samples was 103% ± 10%.

Statistical Analysis

The primary end points were 24-hour expressed milk volume on days 1 to 10 postpartum as well as levels of lactose and citrate in milk. The principal independent variables were gestational age at delivery and time interval between antenatal betamethasone treatment and delivery.

For ease of interpretation, gestational age at delivery is depicted in the figures in 2 groups: <28 weeks (n = 13) and 28 to 33 weeks (n = 37). Although the cutoff between groups of 28 weeks was arbitrarily assigned, it also allowed sufficient numbers in each group and reflected the general distinction, made by many authors, between very preterm and extremely preterm infants.[18] Analysis of variance for repeated measures was used, adjusted for day postpartum and gestational age at delivery as a continuous variable where appropriate. Milk volume was log transformed to obtain normality. Lactose and citrate data, which were normally distributed, were weighted for women who gave >1 sample per day. Significance levels were set at .01 for testing interactions (to attain an overall .05 significance level for all contrasts) and at .05 for testing of main effects. With a sample size of 50, a repeated-measures design could achieve almost 100% power to test for a reduction in

TABLE 1 **Maternal Characteristics ($N = 50$)**

Characteristic	Value
Age, mean (SD), y	32.2 (5.4)
Completed secondary school, *n* (%)[a]	26 (54.2)
Marital status: married or de facto, *n* (%)[a]	45 (93.8)
Intended breastfeeding duration, *n* (%)[a]	
Short-term only	1 (2.1)
≤6 mo	20 (41.7)
>6 mo	27 (56.3)
Previous breastfeeding experience, *n* (%)	29 (58.0)
Smoking, *n* (%)[a]	
During pregnancy	14 (29.2)
In postnatal period	9 (18.8)
Primiparous	20 (40.0)
Twin pregnancy, *n* (%)	7 (14.0)
Obstetric complications, *n* (%)	
Preterm labor	40 (80.0)
PPROM	24 (48.0)
Chorioamnionitis	6 (12.0)
Antepartum hemorrhage	13 (26.0)
Gestational diabetes/type 1 diabetes	5 (10.0)
Mode of delivery, *n* (%)	
Vaginal	24 (48.0)
Cesarean section	26 (52.0)
Gestation at first betamethasone treatment, median (range), wk	29.4 (23.7–33.1)

PPROM indicates prolonged preterm rupture of membranes.
[a] $N = 48$; demographic data were missing for 2 women.

TABLE 2 **Volume, Frequency, and Duration of Milk Expressions Per Day ($N = 320$ Expression Days From 46 Women)**

Day Postpartum	Total Daily Volume, Median (Range), mL	Frequency of Expressions, Median (Range), *n*/d	Duration of Expressions, Median (Range), min
1	5.5 (0.0–105.0)	3 (1–6)	45 (0–100)
2	19.0 (0.0–213.1)	5 (1–9)	121 (20–234)
3	76.5 (0.0–455.7)	5 (1–10)	110 (20–280)
4	229.5 (25.6–736.0)	6 (4–8)	140 (62–280)
5	322.9 (56.0–760.1)	5 (3–8)	144 (70–245)
6	442.5 (50.0–860.2)	6 (3–8)	140 (50–345)
7	540.0 (133.0–1607.8)	6 (2–8)	125 (68–265)
8	459.0 (112.0–1104.8)	6 (4–8)	125 (60–340)
9	571.4 (133.0–1164.2)	6 (4–8)	145 (58–225)
10	531.0 (135.0–1141.0)	5 (3–8)	130 (60–275)

mean concentration of lactose in milk of 25%, assuming an interaction between treatment and postnatal day. SAS 8.2 (SAS Institute, Cary, NC) was used for all statistical analysis.

RESULTS

A total of 100 women were recruited. Forty-four women were excluded from this analysis because they delivered at ≥34 weeks' gestational age. An additional 6 women failed to provide either milk volume measurements or milk samples, leaving a sample size of 50 women. Maternal and obstetric characteristics are presented in Table 1. More than 56% of participants intended to breastfeed for >6 months, and 40% were primiparous. The median (range) gestational age at delivery was 31 weeks (24.2 to 33.7 weeks), and birth weight was 1465 g (640 to 2580 g). Seventy percent of infants were male, and all infants were admitted to the level 3 intensive care nursery (median [range] length of stay: 4 days [0–113 days]) and/or the level 2 special care nursery (median [range] length of stay: 31 days [0–62 days]).

24-Hour Milk Volume

A total of 46 women recorded the volume of milk obtained on 320 expression days. Volume of milk expressed, frequency, and duration of expressing for each postpartum day are shown in Table 2. There was a wide range of volumes obtained by individual women on each day. Overall, milk volume increased from day 1 to day 7 postpartum. After adjustment for day postpartum, volumes also increased significantly with advancing gestational age at delivery ($P = .017$). Figure 1 shows the difference in daily milk volume between women who delivered between 28 and 33 weeks' gestational age and those who delivered <28 weeks' gestational age. Significant increases in milk volume from day 1 postpartum occurred on day 3 in women whose delivered between 28 and 33 weeks but not until day 4 postpartum in women who delivered before 28 weeks.

Milk volume (24 hour) was significantly associated with frequency of expression ($P < .001$). Women who expressed ≥6 times per day obtained significantly greater milk volumes than women who expressed less frequently. There was no effect of gestational age on frequency of expressing ($P = .650$).

After adjustment for postnatal day, there was a trend toward a significant interaction between gestational age at delivery and betamethasone treatment interval ($P = .088$; Fig 2). Overall, there was a trend for women who delivered in the first 2 days after treatment ($n = 20$) to obtain greater volumes than women who delivered 3 to 9 days after treatment ($n = 16$; $P = .086$). Women who delivered >10 days after betamethasone treatment ($n = 12$) obtained daily volumes that were not significantly different from the other treatment-delivery intervals (compared with women who delivered 0–2 days after treatment, $P = .732$; compared with women who delivered 3–9 days after treatment, $P = .446$).

When delivery occurred before 28 weeks' gestation, there was no difference in milk volume at different intervals between betamethasone treatment and delivery, although there were small numbers in each treatment interval group at earlier gestational ages (Fig 2A). In contrast, when delivery occurred at more advanced gestational ages (between 28 and 33 weeks), there were differences between treatment interval groups (Fig 2B). Women who delivered 3 to 9 days after treatment obtained significantly reduced volumes compared with women who delivered 0 to 2 days after treatment.

The mother's intended duration of breastfeeding was significantly associated with 24-hour milk volume ($P = .002$). Women who intended to breastfeed for >6 months expressed significantly greater volumes of milk on the first 3 days postpartum than women who intended to breastfeed for shorter durations. This effect

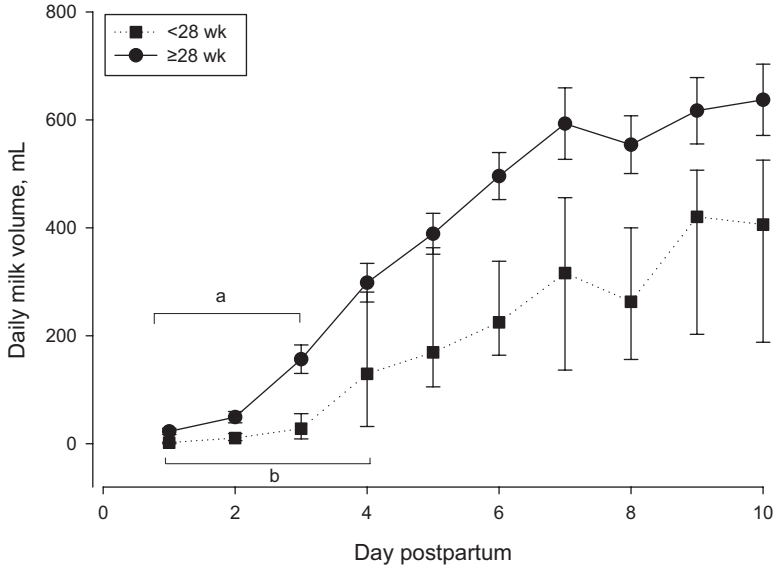

FIGURE 1
Relationship between volume of milk expressed per day and gestational age at delivery. Symbols represent median (interquartile range) milk volume expressed per day when gestational age at delivery was <28 weeks (black squares; $n = 13$) and ≥28 weeks (black circles; $n = 37$). Significantly lower volumes were obtained at earlier gestational ages ($P = .017$). [a] At ≥28 weeks' gestation, significant increases in volume were found between days 1 and 3 postpartum. [b] At <28 weeks' gestation, significant increases in volume were found between days 1 and 4 postpartum.

was unrelated to gestational age at delivery. There was no relationship between intended duration of breastfeeding and frequency of expressing ($P = .780$). Expressed milk volume was not associated with any other maternal, antenatal, intrapartum, or neonatal factor, including maternal age, smoking, parity, obstetric complications, cesarean section delivery, or birth weight.

Lactose Levels in Milk

A total of 42 mothers provided 324 samples for analysis for lactose and citrate concentration. Mean ± SD lactose levels were 156.800 ± 36.217 mM. There was a significant positive effect of postnatal day ($P < .001$) and gestational age on milk lactose levels ($P < .001$; Fig 3). Milk lactose levels were reduced at early gestational ages and increased with increasing gestational age at delivery. There was a significant effect of frequency of breast expression on milk lactose levels ($P = .010$) with higher milk lactose levels occurring in women who expressed 6 to 8 times a day compared with those who expressed less frequently. No other maternal, obstetric, or neonatal factor had any significant association with milk lactose levels, including intended duration of breastfeeding or the infant's taking any breastfeeds. After adjustment for postpartum day, the interval between antenatal betamethasone treatment and delivery was not associated with milk lactose levels ($P = .857$).

Citrate Levels in Milk

Mean ± SD citrate levels were 3.458 ± 1.442 mM. Milk citrate levels were significantly associated with postpartum day ($P = .003$), but the effect of gestational age at birth on milk citrate levels was not significant ($P = .082$; Fig 4).

Milk citrate levels were significantly associated with frequency of expression ($P = .010$). Women who expressed ≤5 times per day had lower levels of citrate in their milk. Primiparae had significantly higher milk citrate levels than women who had >1 child ($P = .004$). Women who delivered by nonelective cesarean section also had significantly higher milk citrate levels than other modes of delivery ($P = .022$). No other maternal, obstetric, or neonatal factor had any significant association with milk citrate levels, including intended duration of breastfeeding, the infant's taking any breastfeeds, or betamethasone treatment ($P = .312$).

Relationship Between Milk Volume and Milk Lactose and Citrate Levels

Milk volume was significantly associated with both lactose ($P < .001$) and citrate ($P < .001$) levels in milk. Increasing volume was associated with both increasing lactose and increasing citrate levels. This positive association persisted at different gestational ages at delivery.

DISCUSSION

This study is the first of which we are aware to investigate the effects of antenatal corticosteroids on lactogenesis II in women who deliver preterm. These findings suggest that antenatal corticosteroids may have an impact on lactogenesis II, but this depends on gestational age at delivery and the time interval between glucocorticoid treatment and delivery.

At more advanced preterm gestational ages, expressed milk volumes were reduced in women who delivered between 3 and 9 days after being treated with betamethasone compared with women who delivered soon after betamethasone treatment. There was no difference in women who delivered at extremely preterm gestational ages, suggesting either insufficient power as a result of low numbers or that lactocytes may not be sufficiently developed at this early gestational age for synthetic corticosteroids to have a significant lactogenic effect. The significant association with antenatal betamethasone treatment did not persist when either milk lactose or citrate levels were used as markers of lactogenesis II.

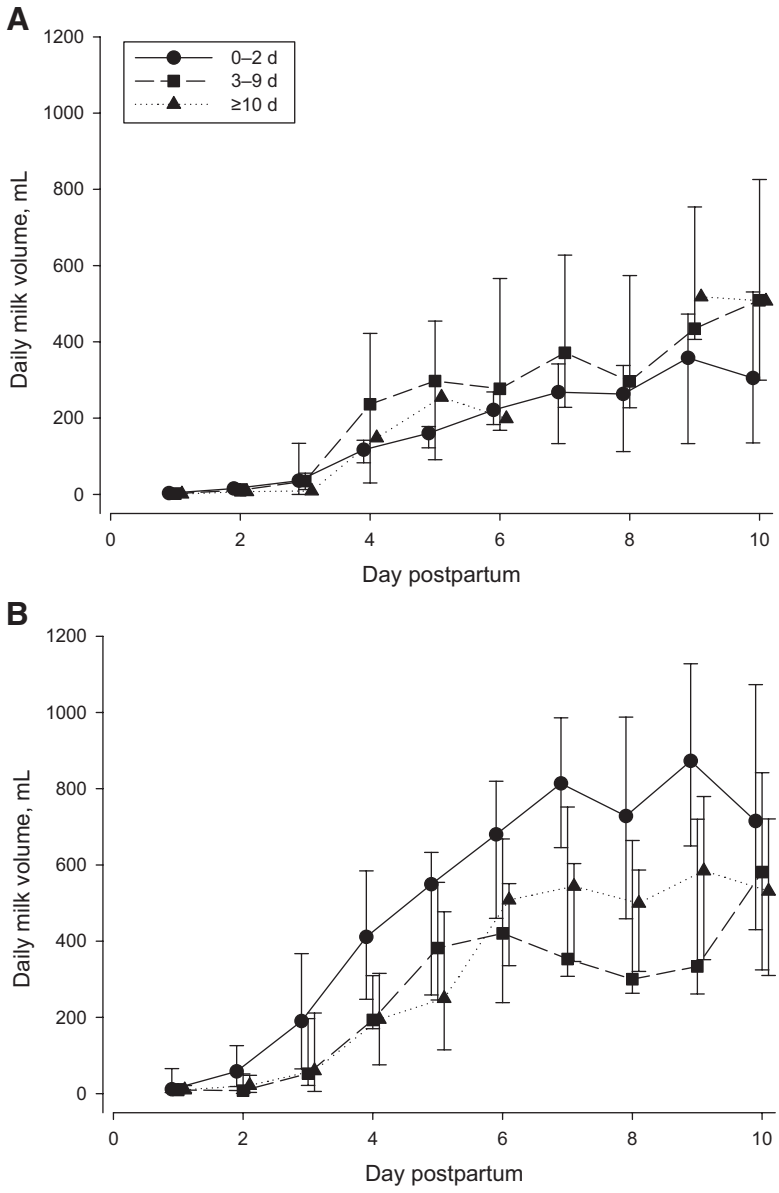

FIGURE 2

Relationship between volume of milk expressed per day and interval between betamethasone treatment and delivery. Symbols represent median (interquartile range) milk volume expressed per day when delivery occurred 0 to 2 days (black circles), 3 to 9 days (black squares), and ≥10 days (black triangles) after betamethasone treatment. Gestational age at birth modified the effect of betamethasone treatment-delivery interval on milk volume ($P = .088$). A, At <28 weeks' gestational age, there was no difference in milk volume between different intervals between betamethasone treatment and delivery. B, At ≥28 weeks' gestational age, women who delivered 3 to 9 days after betamethasone treatment expressed the least milk volume, and women who delivered 0 to 2 days after betamethasone treatment expressed the greatest volume.

These findings were less conclusive than our findings in sheep that antenatal betamethasone caused a profound effect on postpartum milk production (unpublished observations, 2003). In that sheep study, we found that antenatal betamethasone treatment caused premature withdrawal of progesterone in the presence of high levels of the lactogenic hormones prolactin and synthetic corticosteroids, leading to premature lactogenesis II during pregnancy. Subsequent lactation after term delivery was profoundly reduced; however, sheep are known to be very sensitive to antenatal maternal corticosteroid administration[19] in comparison with women. It remains to be shown that similar processes occur in women, although this study suggests that antenatal corticosteroid treatment may be responsible for a delayed onset of lactogenesis II after preterm delivery in women who do not deliver within 2 days of treatment.

We speculate that this lactogenic effect of betamethasone treatment in women may only occur in individuals who are more sensitive than others. The dosage of betamethasone used was in accordance with standard practice, based on the original research by Liggins et al.[20] Differences in response were unrelated to the number of doses received by individual women. Apart from gestational age at delivery, no other maternal or obstetric factor modified the relationship between betamethasone treatment and lactogenesis II. These factors included maternal age, parity, previous breastfeeding, twins, antenatal complications, mode of delivery, birth weight, and neonatal complications. Delayed lactogenesis II was not observed in women who delivered >10 days after betamethasone treatment, suggesting that betamethasone-induced changes in lactation resolved with time and that when delivery was later

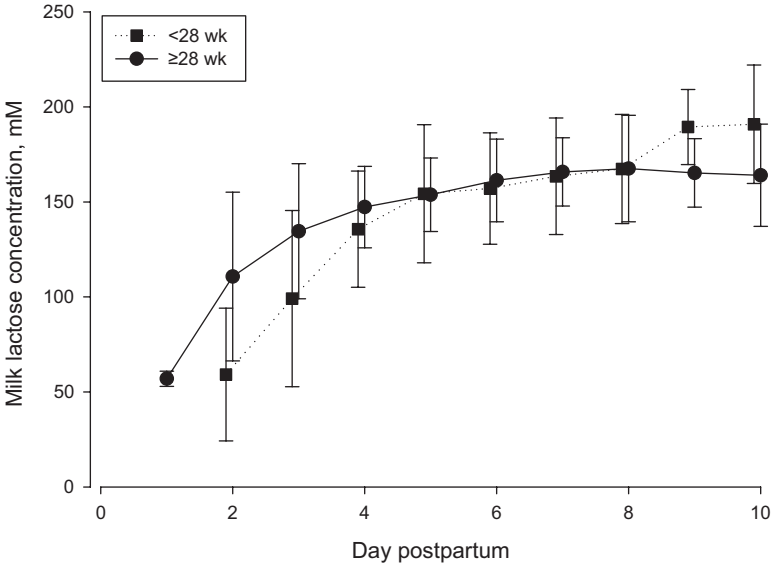

FIGURE 3
Relationship between milk lactose concentration and gestational age at delivery. Symbols represent mean ± SD of milk lactose concentration (mM) stratified according to gestational age at delivery (<28 weeks [black squares; $n = 13$] and ≥28 weeks [black circles; $n = 37$]). For the first 3 days, there was significantly lower concentration of lactose in women who delivered earlier than at 28 weeks' gestational age than those who delivered at ≥28 weeks ($P < .001$).

than 10 days after treatment, lactogenesis II occurred normally.

Gestational Age

Gestational age at delivery strongly predicted the timing of onset of lactogenesis II when measured either by change in milk volume or by increase in levels of milk components, suggesting delays at early gestational ages. These findings compare adversely with studies of mothers of term infants that show that the onset of lactogenesis II generally occurs within 2 days of the birth[9,10] but are consistent with other studies of mothers of preterm infants.[17]

Women who delivered at extremely preterm gestational ages continued to express significantly lower volumes at day 10 postpartum, suggesting a prolonged effect of extreme prematurity on milk production. Low milk production may have a significant impact on their ability to maintain a sufficient supply for older infants and may lead to early unplanned cessation of breastfeeding. In mothers of preterm infants, milk output in the first week postpartum significantly predicts milk production for at least 6 weeks postpartum.[16] Furthermore, studies of mothers' breastfeeding their term infants have found that low milk production in the first week postpartum has adverse implications for breastfeeding duration.[14,15,21]

Influence of Other Factors on Lactogenesis II

Frequency of expressing strongly predicted both the volume of milk expressed and the concentration of lactose and citrate in milk. This confirms previous studies that found increased milk volume with increasing frequency[22–25] and supports current guidelines that encourage

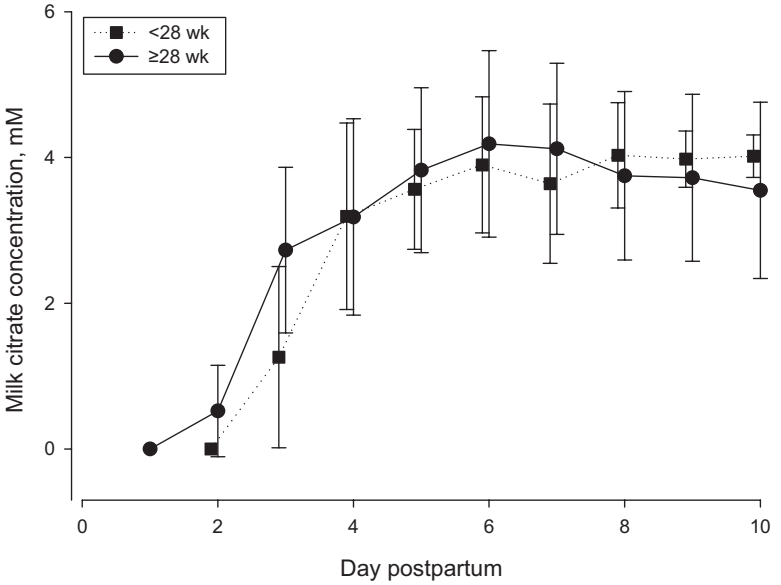

FIGURE 4
Relationship between milk citrate concentration and gestational age at delivery. Symbols represent mean ± SD of milk citrate concentration (mM) stratified according to gestational age at delivery (<28 weeks [black squares] and ≥28 week' [black circles]). Women who delivered at earlier gestational ages tended to have lower citrate concentrations, but this tendency was not significant ($P = .082$).

women to express at least 6 times per day to establish and maintain supply.

No other maternal demographic, obstetric, labor and delivery, or neonatal factor was found to affect either milk volume or milk lactose concentration. Cesarean section deliveries, experienced by 50% of study participants, were not found to delay the onset of lactogenesis II, contrasting with studies of mothers of term infants.[26,27]

No delay was found in women with diabetes, contradicting findings from studies of women who had diabetes and delivered at term[8,28]; however, low numbers of women with diabetes suggests insufficient power to explore associations with diabetes in this study. Other studies have found that primiparous mothers at term are more likely to perceive delayed onset of lactogenesis II.[26] This study found no effect of parity on lactogenesis II in mothers of preterm infants; neither was smoking found to lead to reduced supply as opposed to findings of an American study,[24] despite a large proportion (30%) of the cohort's admitting to having smoked during the pregnancy.

Effectiveness of Measures of Lactogenesis II

In this cohort of women who were solely expressing for all of their infants' requirements, it was possible to estimate accurately the exact timing of lactogenesis II by measurement of volume of milk expressed. Rapid increases in measured volume indicated the onset of copious milk secretion in these women.[29] This approach is limited only by the frequency of expressing of individual women and the ability of pumping to empty effectively the breast at each expression.[22–25]

In studies of breastfeeding mothers, in which milk production cannot be so reliably determined, the concentration of biochemical markers in milk is often used as a secondary indicator of milk volume.[8–10,17] This study has shown that lactose and citrate levels in milk are effective markers of lactogenesis II in mothers of preterm infants. In contrast to milk volume, however, significant associations were not shown between these markers and antenatal corticosteroid treatment. We speculate that this may be attributable to individual variation in the concentration of milk constituents.

Milk Production and Biochemical Markers of Lactogenesis II

This study found that daily milk production rose to a median of 323 mL on day 5 and 530 mL by day 10 postpartum, although there was an extremely wide range on all days. Few studies have reported milk volume in mothers of preterm infants during the first postpartum week, but other studies of production in mothers of preterm infants within the first month have found similar ranges. Hill et al[23] found milk yields of 433 mL/d at week 2 postpartum, and a separate study that compared frequency of pumping in weeks 2 to 5 found that high-frequency pumping yielded a mean of 632 mL/day compared with 319 mL per day with low-frequency pumping.[25] Nonsmokers in another study were producing 639 ± 344 mL/day at 4 weeks.[24]

It is less easy to measure milk production in mothers whose infants are feeding at the breast. Most studies have weighed either the infant or the mother before and after feeds. This method is subject to error because it measures only the infant's capacity and does not account for women who produce more than the infant's requirements, a common event in the first postpartum week.

Neville et al[15] found a mean milk transfer of ~498 mL/day at 5 days postpartum. Other authors have found milk yield of mothers of term infants at 3 days postpartum to be 408 mL/day[30] and at 6 days postpartum to be 556 mL/day[8] up to as much as 1.2 kg/day in the first month.[31] These reported volumes exceed those found in this study, confirming that milk volumes in the first week to 10 days postpartum are reduced in mothers of preterm infants compared with mothers of term infants.

Lactose and citrate levels in milk in this study were within the ranges of those in published studies. Cregan et al[17] found day 5 means ± SD of lactose (147 ± 10 mM) and citrate (4.3 ± 0.7 mM) levels with a wide range in mothers of preterm infants. Rapid increases in lactose and citrate levels signifying lactogenesis II occurred between 1 and 3 days after delivery in this study, although this was delayed by at least 1 day in mothers of extremely preterm infants. This contrasts with published findings for mothers of term infants, for whom increases usually occurred between days 1 and 2 postpartum,[8,30] although Neville et al[15] found lactose and citrate levels in mothers of term infants to be similar to findings in this study at 160 mM and 4.0 mM, respectively.

A limitation of the study design was the exclusion of volume data from women who were already giving some breastfeeds during the time interval when lactogenesis II was expected to occur ($n = 6$). This was because of the difficulty of estimating total intake during breastfeeds, although levels of milk markers were assessed in these women. It is possible that having the infant feeding directly on the breast may stimulate earlier lactogenesis II than what occurs when milk removal is obtained only by pumping the breast; however, lactose and citrate levels were not different between mothers who were giving some breastfeeds and those who were expressing only. Moreover, lactogenesis II has been shown to occur always after birth regardless of whether milk is removed.[32,33]

This study is limited by being an observational study only; however, in the absence of an ability to conduct a randomized trial, the prospective recruitment of participants and collection of data in this study minimized most potential bias in the findings. Although the sample size was determined a priori to be sufficient to find a significant effect, there were small numbers in some betamethasone-delivery intervals in the earlier gestational ages. Additional investigation with larger sample size is suggested, perhaps weighted to recruit women at extremely early gestational ages to determine the strength of any effect.

CONCLUSIONS

Gestational age at delivery was strongly associated with timing of the onset of lactogenesis II: women who de-

livered at extremely preterm gestational ages demonstrated a significant delay in onset of lactation. This study is the first to show a potential small effect of a single course of antenatal corticosteroids on lactogenesis II.

In view of the significant advantages of mothers' own milk for the growth and development of the preterm neonate, additional research into the effects on lactation of both preterm birth and antenatal corticosteroids is indicated.

Concern has been raised about the adverse effects of multiple courses of corticosteroids on fetal growth and development.[34–36] Because the effects of multiple courses of corticosteroids on lactation are unknown, these findings suggest that lactation outcomes should be included in the randomized, controlled trials that are under way to investigate repeated courses of corticosteroids.[36]

ACKNOWLEDGMENTS

This work was supported in part by grants from Medela AG, Switzerland. Dr Henderson was supported by a grant from the National Health and Medical Research Council (Australia).

We thank D. Doherty, PhD, for statistical advice and L. Mitoulas, PhD, and C. T. Lai for advice on the biochemical analysis. We particularly thank the participating mothers, without whom this study would not have been possible, for generous contributions at a time when they were undergoing an extremely stressful experience.

REFERENCES

1. Gartner L, Morton J, Lawrence R, et al. Breastfeeding and the use of human milk. *Pediatrics.* 2005;115:496–506
2. Meier PP, Brown LP, Hurst NM. Breastfeeding the preterm infant. In: Riordan J, Auerbach KG, eds. *Breastfeeding and Human Lactation*. 2nd ed. Sudbury, MA: Jones and Bartlett; 1998: 449–481
3. Flacking R, Nyqvist KH, Ewald U, Wallin L. Long-term duration of breastfeeding in Swedish low birth weight infants. *J Hum Lact.* 2003;19:157–165
4. Furman L, Minich NM, Hack M. Breastfeeding of very low birth weight infants. *J Hum Lact.* 1998;14:29–34
5. Killersreiter B, Grimmer I, Buhrer C, Dudenhausen JW, Obladen M. Early cessation of breast milk feeding in very low birthweight infants. *Early Hum Dev.* 2001;60:193–205
6. Yip E, Lee J, Sheehy Y. Breast-feeding in neonatal intensive care. *J Paediatr Child Health.* 1996;32:296–298
7. Kuhn NJ, Lowenstein JM. Lactogenesis II in the rat. *J Biochem.* 1967;105:995–1002
8. Arthur PG, Smith M, Hartmann PE. Milk lactose, citrate, and glucose as markers of lactogenesis in normal and diabetic women. *J Pediatr Gastroenterol Nutr.* 1989;9:488–496
9. Neville MC, Allen JC, Archer PC, et al. Studies in human lactation: milk volume and nutrient composition during weaning and lactogenesis. *Am J Clin Nutr.* 1991;54:81–92
10. Kulski JK, Smith M, Hartmann PE. Perinatal concentrations of progesterone, lactose and a-lactalbumin in the mammary secretion of women. *J Endocrinol.* 1977;74:509–510
11. Neville MC, Morton J. Physiology and endocrine changes underlying human lactogenesis II. *J Nutr.* 2001;131: 3005S–3008S
12. Chapman DJ, Pérez-Escamilla R. Identification of risk factors for delayed onset of lactation. *J Am Diet Assoc.* 1999;99: 450–454
13. Sievers E, Haase S, Oldigs H-D, Schaub J. The impact of peripartum factors on the onset and duration of lactation. *Biol Neonate.* 2003;83:246–252
14. Houston MJ, Howie PW, McNeilly AS. Factors affecting the duration of breast feeding: 1. Measurement of breast milk intake in the first week of life. *Early Hum Dev.* 1983;8:49–54
15. Neville MC, Keller RP, Seacat J, et al. Studies in human lactation: milk volumes in lactating women during the onset of lactation and full lactation. *Am J Clin Nutr.* 1988;48: 1375–1386
16. Hill PD, Aldag JC, Chatterton RT, Zinaman MJ. Comparison of milk output between mothers of preterm and term infants: the first 6 weeks after birth. *J Hum Lact.* 2005;21:22–30
17. Cregan MD, De Mello TR, Kershaw D, McDougall K, Hartmann PE. Initiation of lactation in women after preterm delivery. *Acta Obstet Gynecol Scand.* 2002;81:870–877
18. Field D, Petersen S, Clarke M, Draper ES. Extreme prematurity in the UK and Denmark: population differences in viability. *Arch Dis Child Fetal Neonatal Ed.* 2002;87:F172–F175
19. Jobe AH, Newnham JP, Moss TJ, Ikegami M. Differential effects of maternal betamethasone and cortisol on lung maturation and growth in fetal sheep. *Am J Obstet Gynecol.* 2003;188: 22–28
20. Liggins GC, Howie RN. A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. *Pediatrics.* 1972;50: 515–525
21. Chapman DJ, Pérez-Escamilla R. Does delayed perception of the onset of lactation shorten breastfeeding duration? *J Hum Lact.* 1999;15:107–111
22. de Carvalho M, Anderson DM, Giangreco A, Pittard WB. Frequency of milk expression and milk production by mothers of nonnursing premature neonates. *Am J Dis Child.* 1985;139: 483–485
23. Hill PD, Aldag JC, Chatterton RT. Effects of pumping style on milk production in mothers of non-nursing preterm infants. *J Hum Lact.* 1999;15:209–215
24. Hopkinson JM, Schanler RJ, Garza C. Milk production by mothers of premature infants. *Pediatrics.* 1988;81:815–820
25. Hill PD, Aldag JC, Chatterton RT. Initiation and frequency of pumping and milk production in mothers of non-nursing preterm infants. *J Hum Lact.* 2001;17:9–13
26. Dewey KG, Nommsen-Rivers LA, Heinig MJ, Cohen RJ. Risk factors for suboptimal infant breastfeeding behavior, delayed onset of lactation, and excess neonatal weight loss. *Pediatrics.* 2003;112:607–619
27. Evans KC, Evans RG, Royal R, Esterman AJ, James SL. Effect of caesarean section on breast milk transfer to the normal term newborn over the first week of life. *Arch Dis Child Fetal Neonatal Ed.* 2003;88:F380–F382
28. Neubauer SH, Ferris AM, Chase CG, et al. Delayed lactogenesis in women with insulin-dependent diabetes mellitus. *Am J Clin Nutr.* 1993;58:54–60
29. Hartmann PE. Changes in the composition and yield of the mammary secretion of cows during the initiation of lactation. *J Endocrinol.* 1973;59:231–247
30. Saint L, Smith M, Hartmann PE. The yield and nutrient content of colostrum and milk of women from giving birth to 1 month post-partum. *Br J Nutr.* 1984;52:87–95
31. Hartmann PE. Lactation and reproduction in Western Australian women. *J Reprod Med.* 1987;32:543–547
32. Neville MC, Morton J, Umemura S. Lactogenesis: the transition from pregnancy to lactation. *Pediatr Clin North Am.* 2001;48: 35–52

33. Kulski JK, Hartmann PE. Changes in human milk composition during the initiation of lactation. *Aust J Exp Biol Med Sci.* 1981; 59:101–114
34. French NP, Hagan R, Evans SF, Godfrey M, Newnham JP. Repeated antenatal corticosteroids: size at birth and subsequent development. *Am J Obstet Gynecol.* 1999;180:114–121
35. Moss TJ, Sloboda DM, Gurrin LC, Harding R, Challis JR, Newnham JP. Programming effects in sheep of prenatal growth restriction and glucocorticoid exposure. *Am J Physiol Regul Integr Comp Physiol.* 2001;281:R960–R970
36. National Institutes of Health Consensus Development Panel. Antenatal corticosteroids revisited: repeat courses—National Institutes of Health Consensus Development Conference Statement. *Obstet Gynecol.* 2001;98:144–150