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Relation of Plasma Oxytocin and Prolactin Concentrations to Milk Production in Mothers of Preterm Infants: Influence of Stress*

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ABSTRACT

Responses of oxytocin and PRL to mechanical breast pumping and the influence of physiological indicators of stress were measured at 2, 4, and 6 weeks postpartum to determine potential causes of inadequate milk production in 18 women with prematurely delivered, nonnursing (<1500 g) infants. Median milk production was similar to that reported in breastfeeding mothers, but a third of mothers were producing less than half as much by week 6. Plasma oxytocin was similar to that previously reported for breastfeeding mothers. The oxytocin area under the curve (AUC) for breast-pumping sessions (70 min) was correlated at each occasion (r = 0.37, 0.58, and 0.55, respectively) with milk yield. Unlike reports of PRL levels in breastfeeding women, PRL AUC declined between weeks 2 and 6 weeks postpartum ($P = 0.03$); significant increases in plasma PRL occurred in response to pumping at 2 and 4 weeks, but not at 6 weeks. Salivary amylase, a measure of α-adrenergic activity, was highly negatively correlated on each occasion with PRL AUC (r = −0.58, −0.68, and −0.86, respectively), but not with oxytocin. Salivary cortisol was negatively correlated to a lesser degree. We hypothesize that deficiencies in preterm lactation are mediated in part upon stress-induced suppression of PRL secretion through an adrenergic mechanism. (*J Clin Endocrinol Metab* **85:** 3661–3668, 2000)

FOR INFANTS LESS than 34 weeks gestation at birth, poor coordination of the suck/swallow pattern, poor neurobehavioral control, and an immature oral motor musculature exist (1). Thus, mothers desiring to breastfeed a premature infant may need to develop their milk supply through manual or mechanical milk expression techniques. In addition, many of these same women are asked to provide breast milk for their infants while the infants develop in the nursery.

Two major endocrine factors, PRL and oxytocin, play important roles in the initiation and control of lactation. PRL is essential for lactogenesis, the initiation of lactation, but its role in the maintenance of lactation is less clear (2, 3). The correlation between PRL levels and milk production is generally very low (4–6). Nevertheless, bromoergocriptine, which suppresses PRL secretion at the level of the pituitary, is capable of completely suppressing milk secretion even in established lactation (7, 8), and when complete emptying of the breast is assured, there is evidence for a relationship between PRL levels and milk production (9).

Unlike PRL, oxytocin release generally occurs as a conditioned response; release occurs in most women before the tactile stimulus of suckling (5, 10) and can be stimulated by various sensory inputs, such as seeing the baby or hearing it cry. Oxytocin is important for milk ejection, although whether it is essential for milk let-down in the human has been debated (11). Nevertheless, it clearly facilitates emptying of the breast and thereby the continuation of milk secretion (10, 12). Administration of oxytocin has been shown to enhance milk production in mothers delivering prematurely (13), so oxytocin responses in mothers of the present study may be an indication of the success or failure of lactation.

Most studies of PRL responses to infant suckling as well as the association between PRL and milk production have been conducted in women after normal term deliveries (6, 9, 14–19). Some data are available on the response to mechanical breast stimulation (6, 17), but there is little information on the evaluation of mechanical pumping devices in mothers of preterm infants. Studies of oxytocin have primarily focused upon the response to infant suckling (5, 11, 18) and low milk yield with inadequate emptying of the breast (6, 10, 12, 13). Because premature delivery is associated with a high degree of psychological distress (20, 21), we recorded self-reports of stress and assessed physiological responses to stress for comparison with measures of milk production and hormone levels.

The purpose of this study was 2- fold: 1) to determine the relationship between milk production and PRL and oxytocin levels with time during mechanical breast stimulation, and 2) to examine the relationship between milk production, PRL, and oxytocin levels and measures of stress. Both were studied in women with prematurely delivered infants.

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Subjects and Methods

Subjects

Subjects were limited to mothers able to read, speak, and write English or Spanish, with access to a telephone, who were nonsmokers, who had no thyroid or other endocrine disorders, who intended to lactate a minimum of 2 months, and who were willing to transport their milk to the hospital at least weekly. Additionally, only mothers delivering an infant(s) of 1500 g or less and 30 weeks gestation or less at birth were eligible for study inclusion. Subjects were given the telephone numbers of the research nurse and principal investigator and were encouraged to phone with questions or concerns. Subjects signed a consent form, which had been approved by the appropriate institutional review boards. A total of 39 subjects were enrolled and participated in measurements of milk production. Eighteen subjects consented to have blood drawn for oxytocin and PRL assays; 10 collected all samples as outlined in the protocol.

Among the 39 subjects, ages ranged from 19–40, with a mean of 29.7 yr. Education in years ranged from 12–18, with a mean of 14.6. Infant birth weight was 1058 ± 253 g (SD), and gestational age was 27.2 ± 1.5 weeks (SD). Thirty-three (84.6%) of the women were married; 29 (76.5%) were white. Thirty (76.9%) had no prior breastfeeding experience. Twenty-one (53.8%) of the mothers had a vaginal birth. The 18 subjects who enrolled in the blood-sampling protocol did not differ significantly from these averages.

Procedures

Mothers who met study criteria were approached within 48 h after delivery by a registered nurse hired for the purpose of this study. The research nurse at each tertiary care center who recruited subjects as well as other nurses who weighed milk samples and performed the venipuncture were trained by the principal investigator with respect to study objectives, protocols, and procedures. The research nurses were knowledgeable about lactation and breast pumps, and had received training from a Medela representative with respect to the Lactina model 016 breast pump (Medela, Inc., McHenry, IL). In addition to the Medela Lactina breast pump, mothers received a stopwatch, a carrying case, four cooler packs, sterile collection milk bags, and other necessary supplies for recording of data. Participants received verbal and written instructions on the study protocol for milk collection, storage, and transport of milk. Subjects viewed a videotape by Medela on breast pump assembly and then assembled the equipment in the presence of the research nurse.

Milk pumping instrumentation

The breast-pumping regimens employed either the Medela single or double collection kit attached to the Medela 016 Lactina breast pump. The single collection kit allows suction to be applied to the breasts sequentially, whereas the double collection kit allows suction to be applied to both breasts simultaneously. The pumps feature varying breast flange sizes, user-controlled vacuum, and piston action. The adjustable suction range is approximately 110–240 mm Hg, and the pump is preset to apply suction about 42–48 times/min.

Pumping protocol

Mothers were requested to follow the written pumping protocol which consisted of five times per day during hospitalization and eight times per day after hospital discharge through day 42 postpartum and to record in the logbook the date and start and end times of each pumping. Subjects using the single pump were instructed to pump for a minimum of 5 min, then switch to the other side; this was done twice, so that each breast was stimulated a minimum of 10 min total time. They were to switch sides when the milk stopped spraying or dripping regularly. Mothers were to begin by using warm compresses (a washcloth/towel dipped in warm water), using both hands to lift their breasts gently off the rib cage from the side under their arm and then in the cleavage from the front. Then they were to massage their breasts just before using the breast pump. In addition, all mothers were to look at a picture of their baby or inhale the baby's scent from a blanket or piece of clothing the baby had worn. These instructions were designed to aid in letting down the milk. Subjects using the double pumping device were instructed to pump a minimum of 10 min and stop when the spraying or dripping of milk ceased.

Subjects brought their milk to the hospital almost daily. Each bag of mother's expressed milk was weighed on an electronic, digital scale to the nearest 0.1 g (2.8 g was subtracted for the bag and tie) and recorded in the logbook by the research nurse. On the day before the scheduled venipuncture, the principal investigator telephoned each subject to remind her to meet the designated research nurse in the neonatal intensive care unit at 0830 h on the following day.

Sample collection procedures

At 1030 h on study days 7, 14, 21, 28, 35, and 42 postpartum, subjects collected a saliva sample for analysis of cortisol and α-amylase at home. Saliva was collected by chewing for 60 s on a 1-in. cube of sponge. The sponge was then removed from the mouth by the subject and placed in a disposable plastic beaker, and the saliva was expressed into a vial by manually squeezing the beaker. The vials were stoppered and placed in the home freezer until brought to the hospital in insulated carrying packs provided. At the hospital the saliva was stored at −20 C until brought to the laboratory on dry ice. Storage in the laboratory was at −20 C.

On weeks 2, 4, and 6 after delivery, the subjects arrived at the hospital at 0830 h for collection of multiple blood samples during breast pumping. Subjects were requested to fast after 0830 h on the scheduled day until completion of the procedure and not to use the breast pump 2–3 h before venipuncture. At 0900 h, the research nurse inserted a heparin lock into an antecubital vein. After a 50-min waiting period to allow for any stress-induced changes in PRL and oxytocin to recede (22), the iv line was flushed with saline and connected to an infusion/withdrawal syringe pump. At 0950 h, blood was withdrawn by use of an exfusion pump at the rate of 0.5 mL/min for a total of 70 min. The collection syringe was surrounded by an ice pack. After each 10-min withdrawal period, blood was removed and transferred to a tube, which was stoppered and placed on ice. The subjects began the pumping regimen at 1000 h and continued for 10 or 20 min depending upon which collection kit they used. At 1030 h, a sample of saliva was collected. At 1100 h, the iv catheter was removed. The blood samples were then brought to the laboratory, where they were centrifuged at 4 C; the plasma was withdrawn and stored in sealed vials at −70 C. Samples from outside Northwestern University Medical Center were shipped on dry ice to the assay laboratory. Procedures for collection of samples for oxytocin assay were designed after consultation with Dr. Laird Wilson, University of Illinois College of Medicine.

Psychological assessment

The Multiple Affect Adjective Checklist–Revised (MAACL-R) (23) was self-administered by the subjects weekly at 1030 h. The completed forms were brought to the clinic with the milk. Standard scores were calculated according to the procedures described in the manual (23).

Hormone assays

Oxytocin was assayed as described by McNeilly *et al.* (5) and in the information provided by Advance ChemTech, Inc. (Louisville, KY). After dilution of the 1.0-mL plasma sample with 3 mL 0.1% trifluoroacetic acid, the precipitated proteins were removed by centrifugation. Oxytocin was extracted from the supernatant liquid on Sep-Pak C_{18} cartridges (Millipore Corp., Bedford, MA); the cartridges were washed with 0.1% trifluoroacetic acid and eluted with 60% acetonitrile in 0.1% trifluoroacetic acid. The solvent was evaporated from the eluted oxytocin under a stream of air at 50 C, the residue was reconstituted in 0.01 mol/L phosphate buffer, pH 7.4, and samples were analyzed by a double antibody method. Antiserum, ^{125}I-labeled oxytocin, and the reference material were obtained from Advanced ChemTech, Inc. The antiserum used cross-reacts with arginine vasopressin, α-atrial natriuretic peptide, CRH, GHRH, GnRH, somatostatin, TRH, and vasoactive intestinal polypeptide less than 0.01%. The minimal detectable concentration is 0.4 pg/tube. The intraassay coefficient of variation (CV) for these assays was 8.3%, and the interassay CV was 10.2%.

PRL was assayed by a direct, two-site immunoradiometric assay without extraction using materials supplied by Diagnostics Systems Laboratories, Inc. (Webster, TX). The antiserum cross-reacts less than

0.1% with hCG, human GH, TSH, LH, FSH, and insulin. The minimal detectable concentration according to the provider is 0.1 ng/mL. The intraassay CV for these assays was 8.0%. The interassay CV was 11.5%.

Salivary cortisol was measured as described previously (24) without extraction. The antiserum cross-reacts 17.4% with 11-deoxycortisol, 5.4% with corticosterone, and less than 0.2% with all other steroids tested. The minimal detectable concentration is 68 pmol/L. The intraassay CV for these samples was 11.6%. The interassay CV was 14.2%.

Salivary α-amylase was measured by its ability to hydrolyze the substrate, 4,6-ethylidene (α-D-maltoheptaside)$_7$-p-nitrophenyl, as described previously (24) using reagents obtained from Sigma (St. Louis, MO). The sensitivity of the assay was 24 U/mL. The interassay CV was 8.5%.

Data analysis

This was a longitudinal study with a repeated measures design for determining the pattern of response of oxytocin and PRL during 6 weeks of mechanical breast stimulation and for ascertaining the relationship of these hormones and two stress measures to milk production.

Data were analyzed using Pearson's correlation procedure, simple regression, backward stepwise multiple regression, and ANOVA with repeated measures and Student's *t* tests. Differences were considered significant at $P \leq 0.05$.

Results

Consistency among measures

Correlations within subjects across time were determined for the hormones and milk volume measures. The area under the curve (AUC) for plasma oxytocin and PRL concentrations over 70 min of sampling was measured on weeks 2, 4, and 6. The correlations among adjacent measurements for each hormone are shown in Table 1. Over 65% of the variance in both oxytocin and PRL in week 4 could be predicted from the measurements made in week 2, but the values in week 6 were less well predicted from week 4 values. Milk volume and salivary cortisol and α-amylase were measured weekly. The average amount of milk produced each week was plotted for single and double pumping procedures (Fig. 1). As shown in Table 2, the correlation between adjacent weeks in milk volume was very high. Salivary cortisol and α-amylase concentrations, measured to assess physiological stress in the subjects, were less predictable from time to time within women (Table 2).

Milk production

Milk production in the first week of breast pumping was not related to gestational age at delivery ($r = -0.03$), and production increased significantly ($P < 0.001$) across the 6-week period by repeated measures ANOVA (Fig. 1). As the variance between subjects was 27.6 times as great as the within-subject variance, the apparent difference between the two types of pumping procedures (a between-subject variable) was not significant ($P = 0.17$).

The distribution of women across ranges of milk produced is shown in Fig. 2 for the fourth week of the study. The distribution is extremely broad with no mode. In the sixth week of the study, 8 of the 32 women remaining in the study were producing 1600 mL milk/week or less; these had a mean pumping frequency of 30.6 compared to 42.3 for those producing 1600 mL/week or more ($P = 0.02$).

TABLE 1. Correlations across time within hormone measures

Hormone	Week 2 *vs.* 4	Week 4 *vs.* 6
Oxytocin	0.81	0.61
Prolactin	0.82	0.59

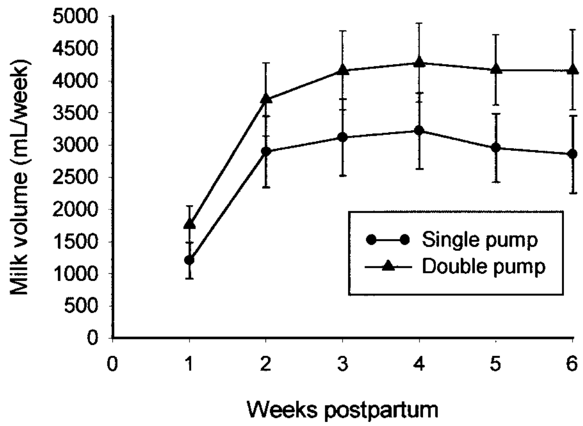

FIG. 1. Mean milk volume per week for single and double pumping procedures for the first 6 weeks postpartum in women with premature infants. The difference between pumping procedures was not significant by repeated measures ANOVA.

TABLE 2. Correlations within milk volume and stress hormones between adjacent weekly measurements

Measure	Median	Range
Milk volume	0.96	0.90–0.98
Cortisol	0.62	0.56–0.92
α-Amylase	0.44	0.31–0.65

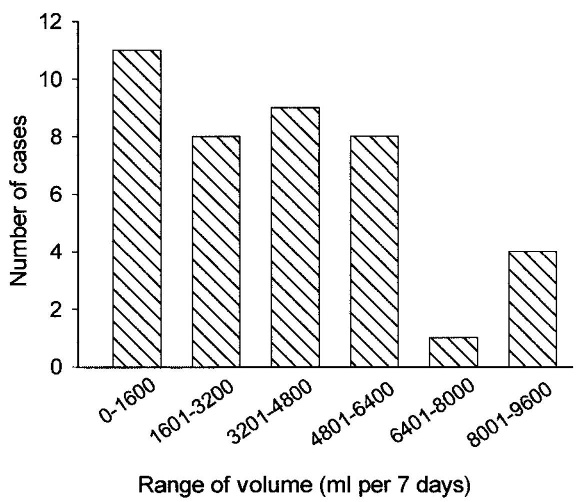

FIG. 2. Milk production in the fourth week postpartum. Distribution of subjects within 1600-mL increments. The median is within the normal range for breastfeeding women with term deliveries, but there is a high incidence of low producers.

Plasma oxytocin levels

Oxytocin AUC did not change significantly with weeks of lactation (2, 4, and 6 weeks after delivery) when analyzed by repeated measures ANOVA. Ten subjects completed all 3 sampling periods for this analysis. The mean values from all 18 subjects during the 7 sampling intervals at each session are shown in Fig. 3. Values are plotted at the midpoint of each of the 7 continuous 10-min blood withdrawal periods. The

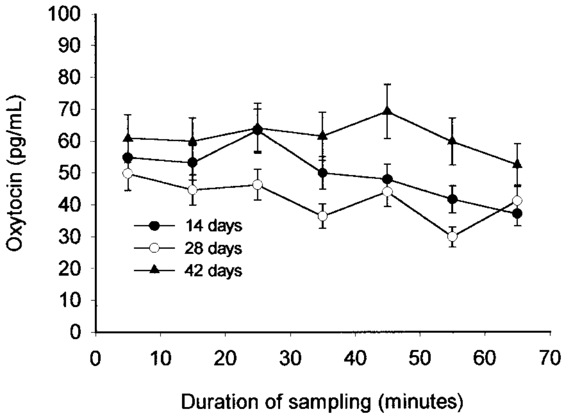

FIG. 3. Plasma oxytocin levels during breast pumping at 14, 28, and 42 days postpartum (all 18 subjects). Breast pumping occurred from 10–30 min (single breast pump) or 10–20 min (double breast pump). Mean plasma concentrations are plotted at the midpoint of each of the 7 continuous 10-min blood withdrawal periods. Oxytocin AUC, based upon the same number of subjects (n = 10) at each time point, did not differ by day of lactation by repeated measures ANOVA. At 14 and 28 days, but not at 42 days, the negative slope of the response was significant.

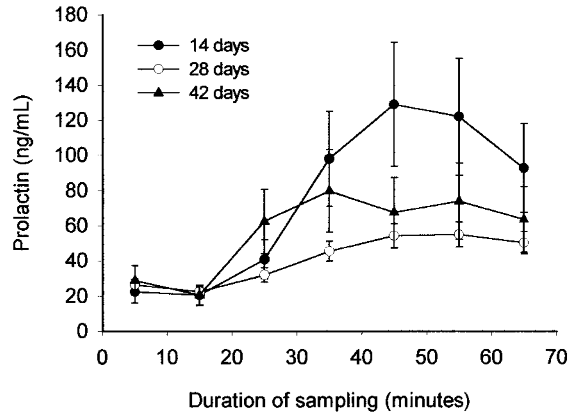

FIG. 4. Plasma PRL levels during breast pumping at 14, 28, and 42 days postpartum (all 18 subjects). Breast pumping occurred from 10–30 min (single breast pump) or 10–20 min (double breast pump). Mean plasma concentrations are plotted at the midpoint of each of the 7 continuous 10-min blood withdrawal periods. PRL AUC, based upon the same number of subjects (n = 10) at each time point, decreased significantly with time postpartum.

high levels at the initiation of breast pumping provide evidence that oxytocin release has become a conditioned response by day 14. There was a significant decline in plasma oxytocin during the 70-min sessions in each of the first 2 sessions, but not in the third (P = 0.011, 0.042, and 0.840, respectively). The between-subject variance was 3.4-fold greater than the within-subject variance for oxytocin, indicating a relatively large intersubject variation.

Relation of oxytocin to milk volume

The AUC of plasma oxytocin was correlated with milk volume within each of the periods studied (r = 0.37, 0.58, and 0.55 in weeks 2, 4, and 6, respectively). However, by regression analysis, oxytocin AUC was significantly related to milk volume only in week 6 (P = 0.043).

There was no significant difference in plasma oxytocin concentrations between pumping procedures in the second and fourth weeks; the differences between single and double pumping procedures averaged 0.1 ± 3.1 and 4.0 ± 4.0 pg/mL, respectively. The patterns across the 70-min blood sampling period were similar despite the fact that the single pumping procedure was twice as long as the double pumping procedure. At week 6, however, the double pumping procedure resulted in more than twice as much oxytocin across the 70-min sampling period (by t test of differences, $P < 0.001$). The difference was 38.0 ± 9.2 pg/mL.

Plasma PRL levels

PRL AUC declined significantly with weeks of lactation by repeated measures ANOVA (P = 0.033). The mean AUC values for the second, fourth, and sixth weeks were 3894, 3064, and 2454 ng·min/mL, respectively, for the 70-min period among the 9 subjects who completed all 3 sampling periods. The mean plasma PRL concentrations from all 18 subjects during the 7 sampling intervals at each session are shown in Fig. 4. Values are plotted at the midpoint of each of the 7 continuous 10-min blood withdrawal periods. There was a significant increase in plasma PRL during the 70-min sessions by repeated measures ANOVA in each of the first 2 sessions, but not in the third ($P < 0.001$, 0.001, and 0.100, respectively). The between-subject variance in PRL was only 1.75-fold greater than the within-subject variance. PRL AUC was negatively correlated with the oxytocin AUC at 2, 4, and 6 weeks; the correlations were −0.41, −0.70, and −0.34, respectively.

Relation of PRL to milk volume

There were no correlations between PRL and milk volume within any of the sampling periods (r = −0.23, −0.44, and 0.05 in weeks 2, 4, and 6, respectively). Including PRL AUC in the regression analysis with oxytocin AUC increased the significance of the relationship in week 6 (P = 0.023); however, by stepwise regression the contribution of PRL AUC did not reach significance. Thus, the decrease in milk volume in one third of the subjects was not related contemporaneously to the decline in PRL.

The pattern of PRL concentrations during the 70-min sampling period was also compared between the single and double pumping procedures. The patterns within types of pumps were similar at 2, 4, and 6 weeks, but between pumps there was a significant difference; the mean PRL concentration for the single pump was 43 ng/mL, and that for the double pump was 73 ng/mL (by t test of differences, P = 0.002). The difference was only evident in blood samples drawn after 20 min (Table 3).

Psychological assessment of distress

Using the values for the normal population from the manual for the MAACL-R (Table 20 in Ref. 23), the mothers, tested weekly for the first 5 weeks, had anxiety subscores that were consistently significantly above the norm (mean standard score of 64.2 ± 21.0 SD compared to 52.1 for the norm; $P < 0.01$). The anxiety subscores by weeks were significantly different by ANOVA with repeated measures (P = 0.006),

TABLE 3. Average differences in PRL concentrations (double minus single pumping procedures) 2, 4, and 6 weeks postpartum

Interval (min)	Mean (ng/mL) ± SE for interval
0–10	−18.5 ± 11.5
11–20	0.4 ± 2.4
21–30	32.8 ± 13.2
31–40	50.9 ± 15.4
41–50	62.7 ± 34.4
51–60	40.2 ± 13.2
61–70	44.3 ± 18.6

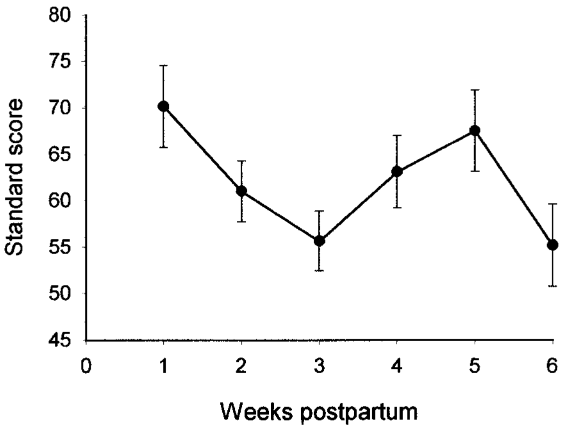

FIG. 5. Anxiety subscores as assessed by self-administered MAACL-R weekly throughout the study. Overall, there was a highly significant elevation in anxiety during the study, with a highly significant cubic trend with time. The standard score among a comparable control group was 52.1 (23).

with a significant cubic relationship (P = 0.001; data are shown in Fig. 5). Depression subscores were also significantly elevated, but only in the first 2 weeks, 66.7 ± 28.5 and 64.3 ± 26.16 SD compared to a value of 50.4 for the norm ($P <$ 0.01). Thereafter, the depression subscores were not significantly different from the norm. The depression subscores by weeks were significantly different by ANOVA with repeated measures ($P < 0.001$), with a significant linear relationship ($P = 0.002$; data are shown in Fig. 6). Hostility subscores were not different from the norm at any time (data not shown). The three subscores of the MAACL-R were not significantly correlated at any of the weekly periods studied with either salivary α-amylase or cortisol, nor were any of the subscores correlated with milk volume. However, the patterns with time in the anxiety and depression subscores were similar to the physiological measures, in that all measures decreased during the first 3 weeks postpartum.

Pattern of salivary α-amylase and cortisol levels during the study

Salivary α-amylase levels, a measure of sympathetic adrenergic activity, decreased with experience with the pumping procedure (Fig. 7). The polynomial test of order-1 (linear) was significant ($P = 0.045$). Between-subject variance was not significantly greater than within-subject variance.

Cortisol levels also decreased with experience with the pumping procedure (Fig. 8). The polynomial test of order-2 (quadratic) was significant (P = 0.028), indicating a significant curvilinear change with time. Between-subject variance was not significantly greater than within-subject variance.

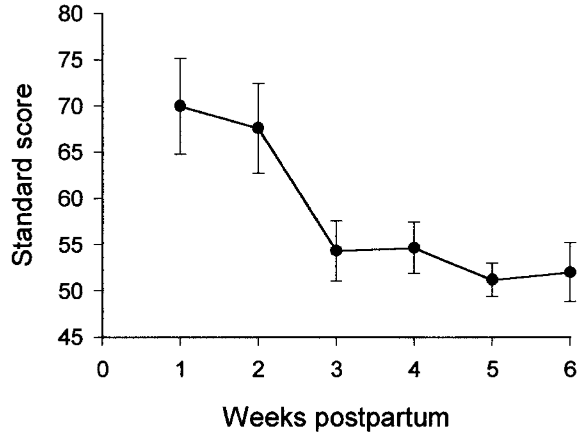

FIG. 6. Depression subscores as assessed by self-administered MAACL-R weekly throughout the study. There was a highly significant elevation in the depression subscores, but only in the first 2 weeks. The standard score among a comparable control group was 50.4 (23).

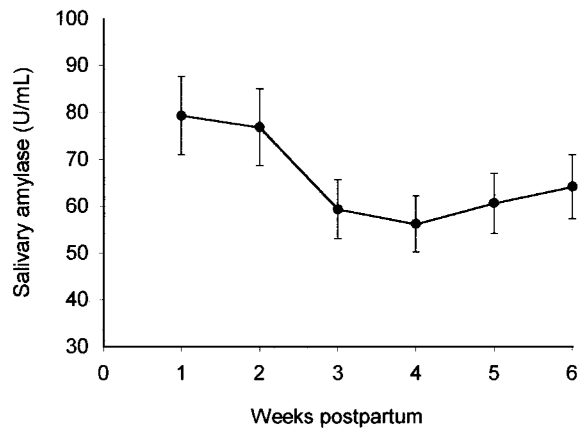

FIG. 7. Salivary α-amylase measured weekly throughout the study (all subjects). There was a significant linear decrease in α-amylase with duration postpartum. The mean level of salivary α-amylase in a group of 10 nonpregnant, nonlactating control women was 50.8 ± 8.2 (±SD) U/mL.

Relation of α-amylase and cortisol to PRL and oxytocin levels

Salivary α-amylase concentrations were highly negatively correlated with plasma PRL AUC; the correlations within the 2-, 4-, and 6-week intervals were −0.58, −0.68, and −0.86, respectively. Thus, this measure of stress is associated with suppression of PRL levels. Nevertheless, neither α-amylase nor PRL levels were associated with milk production under conditions of this study. Statistically, salivary α-amylase concentrations were not consistently related to oxytocin AUC across all three time intervals, but were positively correlated within the 6-week observations (r = 0.61).

Salivary cortisol was negatively correlated with plasma PRL AUC also, but less consistently. The correlations for 2, 4, and 6 weeks were −0.04, −0.44, and −0.69, respectively.

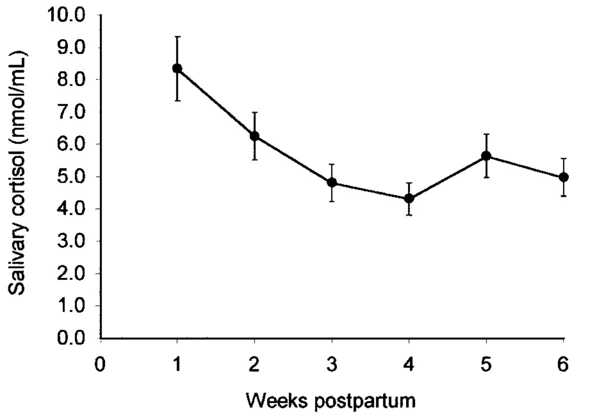

FIG. 8. Salivary cortisol levels measured weekly throughout the study (all subjects). The polynomial test of order was significant for a quadratic decrease with duration postpartum. The mean level of salivary cortisol in a group of 10 nonpregnant, nonlactating control women was 7.3 ± 2.8 (±SD) nmol/L.

There was no correlation between salivary cortisol and oxytocin AUC.

Relation of infant prognosis to PRL and stress measures

The relative health of the infants was assessed by the length of the hospital stay. There were no statistically significant correlations between the length of hospital stay and milk production, self-assessment of stress, or physiological measures of stress in this small study.

Discussion

Milk production

The milk production among the women in the present study was similar to that reported for women after term deliveries. Milk production in this study for the first 7 days averaged between 8–564 g/day for the women in the study. Bohnet and Kato (4) reported a range of 101–267 g/day for the first 7 days in 35 mothers after term deliveries. Using mechanical pumping devices, Zinaman *et al.* (17) found, assuming six feedings per day, a range of 450-1050 g/day 28–42 days after term deliveries. In the present study the milk production for the period from 29–35 days postpartum had an average range of 4–1358 g/day, with a mean of 473 g/day. There is no evidence that the amount of milk was uniformly affected by the premature delivery, and there was no correlation in this study between gestational age and milk production during the first week of lactation. Nevertheless, almost one third of the mothers were producing less than 250 mL milk/day by week 4. At week 6, the women with lower yields of milk were pumping significantly less frequently than those with higher yields (≥1600 mL/week). Whether the lower frequency of breast pumping is the cause rather than the effect of less milk production cannot be determined at this time, but previous studies have suggested that maintenance of lactation is dependent upon suckling frequency (6, 15). No differences between the single and double pumping procedures were detected. This confirms earlier reports in which a double and a single pumping system were compared after term (17) and (25) preterm deliveries.

Oxytocin response

In the present study plasma oxytocin levels during the first 10 min of sampling, before pumping, averaged 55.1 ± 5.6 (±SD) pg/mL. At 30–40 min, the mean value was 52.5 ± 7.9 pg/mL. It is evident that the protocol employed as preparation for pumping was effective in elevating oxytocin before physical pumping began, as may have been expected based on previous work (5). The levels achieved are similar to the maximal levels reported by other investigators (5, 11). Therefore, there is no evidence that the women with preterm deliveries using the Medela pump were deficient in oxytocin. Also, there was no evidence for a decline in oxytocin levels from 2–6 weeks of lactation. This is consistent with the study by Johnston and Amico (18), in which the response was found to be undiminished even after 6 months of lactation. The range of concentrations was great among individuals, with some as low as 2 pg/mL. However, unlike the report by Lucas *et al.* (11) all of the 15 subjects studied produced a response to the pumping regimen.

Relation of oxytocin to milk volume

There was a positive correlation between oxytocin and milk volume in all three postpartum periods studied, although the regression was significant only in the sixth week. Ruis *et al.* (13) demonstrated a beneficial effect of oxytocin administration on milk yield in women after preterm deliveries; the volume of milk, obtained by use of a breast pump, was increased 4-fold over control values in women who received 3 U oxytocin four times a day just before pumping. Thus, at least in the mothers who delivered preterm infants, oxytocin appears to be a limiting factor in milk production.

In established lactation there is some evidence that oxytocin may not be required for breast emptying in the human (11). However, other investigations have not corroborated this finding (5, 18).

PRL levels in lactation

During pregnancy, PRL levels rise from approximately 10 to 150–200 ng/mL at term (26). A significant rise continues between the 24th and 36th weeks of gestation (4). Thus, the mothers of preterm infants may have baseline PRL levels that are significantly lower than those in mothers of term babies. It could be argued that this is a reason for the lower milk production in women with premature delivery. However, PRL levels in the present study varied widely among individual women and were not correlated with the amount of milk produced. The PRL AUC (determined from the same subjects at each time point) declined significantly from the second to the sixth week. This is in contrast to studies of breastfeeding mothers of term infants in whom no significant decrease in PRL response to suckling occurred within the first 40 days (14, 15). The increase in PRL in response to the pumping stimulus, although highly significant in weeks 2 and 4, had become nonsignificant in week 6. The lack of response in week 6 is also inconsistent with responses in breastfeeding mothers of term infants (14, 15). This may represent a declining responsiveness that results in inadequate milk production in mothers of some preterm infants.

Strength of stimulus and PRL

The basal (unstimulated) levels of PRL in the present study were considerably lower at 2, 4, and 6 weeks [25.8 ± 3.2 (±SD) ng/mL] compared to those reported in studies of lactation after term delivery. The range of basal concentrations in 5 studies representing 135 subjects was 36–105, with a mean of 64.2 ± 28.3 ng/mL (assuming 1.0 mg = 30 IU for studies reporting international units) (6, 9, 15–17). However, the peak levels were in the same range as in the studies of women with term deliveries (54–129 ng/mL) despite the fact that in the present study the peak values were diminished by the continuous blood withdrawal procedure. The strength of the stimulus is clearly important for stimulation of PRL secretion. Zinaman *et al.* (17) found that the pump with the gentlest action produced significantly less PRL than the other pumps tested. In the present study the double pump gave a higher serum PRL concentration, particularly after 20 min of sampling (*i.e.* after the pumping has ceased), similar to the findings of Zinaman *et al.* (17) in the term lactation condition.

PRL and milk volume

PRL AUC was unrelated to milk volume. This has been the experience of some investigators after term deliveries as well (4, 6, 15), although Aono *et al.* (9) were able to distinguish a relationship between PRL and milk production when they used a breast pump to empty the breast after the mothers had suckled their babies. Even though the relationship between PRL and milk production has been difficult to demonstrate from measurements taken concurrently, there can be no doubt about the necessity of PRL for lactogenesis. Suppression of PRL secretion by bromocriptine in the puerperium leads to cessation of milk production (27).

Psychological measures of distress

The experience and consequences of premature delivery are very stressful. Women with very low birth weight (VLBW) infants were found in a recent study to have significantly (n = 123; $P = 0.003$) more psychological distress, as measured by standardized, normative, self-report measures, than mothers of term infants (20). At 2 yr postpartum, mothers of low risk VLBW infants did not differ from term mothers, whereas mothers of high risk infants continued to report psychological distress. By 3 yr, mothers of high risk VLBW children did not differ from mothers of term children in distress symptoms. Another study, using the same psychological instrument as that used in the present study, the Multiple Affect Adjective Checklist-Revised, also found significant differences in both anxiety (n = 47; $P = 0.05$) and depression (n = 47; $P = 0.01$), but not in hostility, in mothers of premature infants when comparing responses in the women at the time of discharge with those 9 months later (21). Data from the present study are consistent with the earlier reports.

Physiological measures of stress

The catecholamines are particularly known for responses to acute stress. However, psychological stress of the sort that these subjects have endured has been shown to result in chronically elevated plasma levels of epinephrine and norepinephrine as well (28).

The α-amylase concentration in saliva has been shown to correlate with plasma norepinephrine and to increase in response to a variety of stressors (24, 29). Indeed, the salivary glands have been shown to respond to β-adrenergic agonists with an increase in secretion of α-amylase without an increase in salivary flow (30). In the present study salivary α-amylase negatively correlated with PRL AUC at 2, 4, and 6 weeks. Thus, increased stress measured by this criterion is associated with suppression of PRL. Salivary cortisol concentrations were also negatively correlated with PRL AUC in the fourth and sixth weeks postpartum, but not as significantly as α-amylase. This is not surprising, as the levels of salivary cortisol in women with preterm deliveries were not significantly higher than those in nonlactating control women at any time during the period of observation.

Usually, one associates a stress response, including psychological stress, with an increase in PRL (31, 32). However, this process may be reversed under conditions in which PRL levels are normally elevated in both humans and rats (33, 34). Such appears to be the case under the conditions of the present study. This finding was not hypothesized at the outset of the study and should be examined further for confirmation.

Neither salivary α-amylase nor cortisol had a consistent association with oxytocin levels in the present study. Pain or fright has been shown to inhibit milk let-down in animal species through an adrenergic mechanism (35), and in the human there is evidence that distractions can decrease milk yield, apparently through suppression of oxytocin release (10). Nevertheless, in other studies emotional stress has been shown to increase oxytocin secretion (36). If both types of observations are applicable, they would appear to have cancelled each other out in our study.

Relation between psychological and physiological measures of stress

Although all of the psychological and physiological measures of stress decreased with time after delivery, within subjects there were no significant correlations between any of the psychological measures and salivary α-amylase or cortisol when measured at any of the six weekly periods of assessment. It appears that the psychological and physiological variables are measuring different responses or the cumulative effect of the psychological distress is not exhibited similarly among subjects. Alternatively, it may be that the time for the physiological expression of psychological distress is different among the subjects. Even in the acute stress associated with skydiving, highly significant increases in MAACL-R anxiety subscores were obtained before any increase in plasma or salivary cortisol or salivary α-amylase was observed (24).

Conclusion

The most significant relationship found in this study was the relationship between salivary α-amylase and PRL. Although no close correlation exists between PRL and milk production, PRL is unquestionably essential for the maintenance of lactation. After inhibition of PRL secretion by administration of bromocriptine, milk secretion ceases after 3–4 days (27). Indeed, suppression of PRL secretion by any means is likely to decrease milk production. Therefore, because of the high negative correlation between salivary α-amylase and plasma PRL, we hypothesize that the stress associated with preterm deliveries may result in inadequate lactation through an adrenergic mechanism.

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