

Prolactin concentrations in serum and milk of mothers with and without insulin-dependent diabetes mellitus¹⁻⁴

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ABSTRACT Diabetes may affect the secretion of prolactin, the principal lactogenic hormone. Because adequate amounts are critical to the establishment of lactation, we assessed the prolactin status of 33 women with insulin-dependent diabetes mellitus (IDDM), 33 women without diabetes, and 11 reference women participating in a study of lactation from 2 to 84 d postpartum. Circulating concentrations of serum prolactin declined temporally for all women and did not differ significantly among any of the groups. During the first postnatal week, milk immunoreactive prolactin concentrations were lower for women with IDDM than for control and reference women and the inverse relationship between lactose and milk prolactin, which was significant at day 2 postpartum for reference women, was delayed until day 14 postpartum for women with IDDM. Early breast-feeding activity, increased breast-feeding frequency, and good glycemic control enhance prolactin secretion and should be promoted during lactation in women with IDDM. *Am J Clin Nutr* 1993;58:49-53.

KEY WORDS Prolactin, lactation, diabetes, human milk

Introduction

Streptozotocin-induced diabetic rats display a reduced response of the lactational hormone prolactin to known stimulators of prolactin secretion—estradiol and thyrotropin-releasing hormone—with a concomitant reduction in pituitary prolactin content. The lactotrophic cells of the pituitary exhibit cellular atrophy in rats not treated with insulin. Thus, both prolactin reserves and the prolactin response to provocative stimuli appear to be reduced in diabetic rats (1). Prolactin, insulin, and hydrocortisone are the minimal hormonal requirements for normal lactation to occur (2), thereby stimulating gene expression of casein, α -lactalbumin, and lactose (3). Milk secretion and the synthesis of lactose, casein, and lipid were depressed in tissue cultures of mammary glands from rats rendered diabetic with alloxan (4).

However, in humans there are no studies to date that indicate whether prolactin secretion is compromised in women with insulin-dependent diabetes mellitus (IDDM). During pregnancy, normoglycemia has been associated with a hormonal profile of increased prolactin secretion that corresponds to that of non-diabetic pregnancies (5) whereas hyperglycemia has been associated with lower than normal values for serum prolactin (6) or less of an increase in prolactin concentrations over the course of the pregnancy (7). The importance of glycemic control in

maintaining adequate amounts of prolactin during lactation, however, has not been previously explored.

Frequency of breast-feeding may also be a critical determinant in establishing lactation for women with IDDM (8). Breast-feeding is the most potent and specific physiologic stimulus for prolactin release. Aono et al (9) found that milk yield from days 2 to 6 postpartum was dependent on the degree of prolactin response to adequate infant suckling. In the study of lactation in women with IDDM conducted by our group, Ferris et al (10) reported that women with IDDM breast-fed less frequently than did comparison groups of control or reference women.

Our research group has evaluated composition and adequacy of breast milk collected from lactating mothers with IDDM compared with women without diabetes with similar delivery methods and lactation experiences and with reference women free of delivery complications (11). Milk from women with IDDM had significantly less lactose and higher nitrogen at 2-3 d postpartum than did milk from control and/or reference women. Also, infants of mothers with IDDM had significantly less milk intake (7-14 d postpartum) than did infants of control or reference women, but they grew normally. Whether these dissimilarities in milk composition and infant milk intake are related to aberrations in pituitary hormone secretion for women with IDDM has not been investigated to date. Considering the essential role that the pituitary hormone prolactin plays in the initiation and maintenance of lactation (12), we investigated the effect of the endocrine disease diabetes on the secretion of immunoreactive prolactin.

Methods

Study design

To assess the amount of prolactin secreted during the first 3 mo of milk production in women with IDDM, serum and milk

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immunoreactive prolactin concentrations were determined in 33 women with IDDM, 33 women without diabetes, and 11 reference subjects at days 3, 14, and 42 postpartum. In addition, milk prolactin was also measured at days 2, 7, and 84 postpartum. The relationship of serum and milk prolactin to milk composition and infant milk intake was evaluated and the effect of glycemic control and determinants of lactation behavior on prolactin concentrations assessed.

Subjects

A complete description of the subjects and recruitment procedures for the longitudinal study of lactation in women with IDDM is presented by Ferris et al. (10). In this study, for comparisons based on disease severity, mothers with IDDM were divided into two subclasses (13). Twenty-one (64%) were in White's classes B and C, which indicated that they had minimal diabetic complications. The majority of this subgroup, 17 women with IDDM, had developed diabetes between the ages of 10 and 19 y and the remainder, 4 women with IDDM, when aged > 20 y. Twelve women with IDDM had early onset of diabetes with complications and were grouped into a second subset that included White's classes D, R, and RF.

Control women with no history of diabetes were compared with women with IDDM based on a set of criteria that were thought to impact on lactational performance, eg, similar methods of infant delivery, same sex of infants, same prior lactation experiences, and similar lengths of gestation. Normal, healthy mothers of full-term newborns who were exclusive breast-feeders and delivered vaginally served as a reference group. All subjects signed an informed consent form and the study had human subjects' approval at the University of Connecticut and at all participating hospitals (Memorial Hospital, Worcester, MA; Hartford Hospital, Hartford, CT; and Yale New Haven Hospital, New Haven, CT).

Procedures

At each visit postprandial samples of capillary blood and breast milk were obtained 80 min after breakfast. Because time from breakfast was well-controlled, food-intake effects on prolactin secretion (14) were minimized and variations in prolactin concentrations due to circadian rhythm (15) were similar for all subjects. Capillary blood glucose was measured immediately before breast pumping and 30 min after pumping with a glucometer (Glucoscan 2000 Reflectance Meter; Lifescan Inc, Mountain View, CA). In addition, blood was collected by venipuncture before pumping and serum glucose was determined on days 3, 14, and 42 postpartum; hemoglobin A_{1c} (HbA_{1c}), on day 3 and 42 postpartum. Prepartum HbA_{1c} values were obtained from medical records.

Breast-milk samples were collected by using an Engell Electric Pump (Engell Inc, Cary, IL) as described by Ferris and Jensen (16). Complete pumping was required to obtain a representative lipid sample. In an effort to control time from the last breast-feeding, mothers were asked to feed their infants by breakfast time and to delay the next feeding until after our visit. Therefore, at least 90 min to 2 h had elapsed since the last breast-feeding to allow circulating prolactin concentrations to reach or approach basal concentrations (17).

Sample analysis

To date, serum prolactin has been measured by radioimmunoassay in most studies reported in the literature. A com-

mercial immunoenzymetric assay specific for human serum prolactin was developed by Hybritech (Tandem-E Prolactin; Hybritech, San Diego), which uses mouse IgG1 monoclonal antibodies. The assay was evaluated by Clark and Price (18) and found to be fast, precise, and accurate; measurements were comparable with those obtained by radioimmunoassay. The initial 45% of serum samples in this study were analyzed by the Clinical Laboratory at Hartford Hospital by using this commercial assay system and the remaining samples were analyzed in our laboratory by using the same method. No differences were found between samples analyzed by both laboratories; thus, data from both sites were combined for analyses.

Serum and milk samples were stored frozen at -70°C and defrosted at room temperature. All samples were assayed in duplicate within the same trial. The interassay and intraassay CVs for serum prolactin were 6.9% and 6.0%, respectively. Recovery of known amounts of prolactin added to serum was $88.5 \pm 7.8\%$. The serum reference mean ($\pm$ SD) in our laboratory was $9.95 \pm 9.02 \mu\text{g/L}$ (range 2.8–29.5) for a group of 10 healthy nonpregnant, nonlactating premenopausal women. Normal nonlactating serum values for women reported in the literature range from 1 to $25 \mu\text{g/L}$ ($8.00 \pm 4.96 \mu\text{g/L}$) (19).

In this study, the Hybritech Tandem-E immunoassay for serum was used to determine milk prolactin concentrations as well. The method for analyzing milk prolactin reported in the literature has historically been by radioimmunoassay (20). For the enzyme assay, the within-run precision for milk was 5.7%; between-run precision was 9.8%. Recovery of known amounts of serum prolactin added to milk was 95% and when milk was diluted serially and each serial dilution analyzed for prolactin, there was a mean recovery of 108%. Although some researchers have defatted milk before analyzing prolactin by radioimmunoassay (21, 22), others have found defatting unnecessary (8, 23–25). No statistical differences in the prolactin content of human milk defatted by centrifugation compared with whole milk were observed by us in a preliminary study. Thus, undiluted whole-milk samples were analyzed throughout with the Hybritech immunoassay.

Of the 231 projected determinations of serum prolactin (77 subjects $\times$ 3 visits = 231), 168 actual measurements were made, which included 67 for women with IDDM and 69 control and 32 reference evaluations. Twenty-one women with IDDM, 26 control subjects, and all reference women breast-fed through day 84 postpartum (10). Subject dropout was the main reason for missing serum prolactin values. Of the projected 924 milk prolactin determinations, 548 (59%) were available for analysis, which included 208 for women with IDDM and 226 control and 114 reference values. Nineteen percent of the projected milk samples were missing because of insufficient sample, usually at days 2 or 3 postpartum, and 17% because of subject dropout.

Statistical analysis

SAS was used to perform all statistical analyses for this project (26). Mean values are reported as least-squares means and SEMs. Differences in the dependent variables serum prolactin and milk prolactin for the three groups (IDDM, control, and reference women) and time effects were analyzed by using a quasiexperimental repeated-measures (split-plot) analysis of variance (ANCOVA). Differences in serum and milk prolactin between the two subgroups of women with IDDM (based on White's classifica-

TABLE 1

Serum prolactin concentrations in subjects with insulin-dependent diabetes mellitus (IDDM) and in control and reference women*

Days postpartum	IDDM	Control	Reference
	$\mu\text{g/L}$		
3	142.7 $\pm$ 9.9 [30] ^a	182.5 $\pm$ 9.9 [30] ^a	200.2 $\pm$ 16.1 [11] ^a
14	94.0 $\pm$ 12.4 [23] ^b	127.2 $\pm$ 13.4 [21] ^b	116.8 $\pm$ 17.2 [21] ^b
42	76.6 $\pm$ 16.5 [14] ^b	79.2 $\pm$ 14.6 [18] ^c	116.3 $\pm$ 16.1 [11] ^b

* Least-square mean $\pm$ SEM; *n* in brackets. Serum prolactin concentrations for all groups decreased from 3 to 42 d postpartum. No significant group differences existed at any time. Values with different lettered superscripts on each vertical column are statistically different, $P < 0.05$.

tion) were determined by unpaired *t* tests or by ANOVA for group and time effects.

The relationships between the two variables, serum prolactin and milk prolactin, and infant breast milk intake (10), milk macronutrient content (10), determinants of lactational behavior, and measures of glycemic control were evaluated for statistical significance by Pearson correlation coefficients. Regression analysis was used to determine whether prolactin status predicted infant breast milk intake evaluated by 24-h test weighing. For milk prolactin, time from the breast-feeding was used as a covariate to adjust the multifactorial analysis. Appropriate post hoc tests indicated group and time significance. Milk prolactin from left and right breast milk samples were compared by paired *t* tests.

Results

Serum prolactin

Serum prolactin concentrations for all groups decreased from 3 to 42 d postpartum with the largest decrease from 3 to 14 d postpartum (Table 1). No significant group differences existed at any time measured. The range for serum prolactin was 8.7–357 $\mu\text{g/L}$. At 42 d postpartum, women with more severe diabetes (White's class D or greater) had a significantly lower serum prolactin concentration (LSM $\pm$ SEM 42.3 $\pm$ 26.7 $\mu\text{g/L}$, $n = 6$) than did women with less severe diabetes (White's class B or C) (123.7 $\pm$ 69.9 $\mu\text{g/L}$, $n = 8$; $P < 0.05$). No differences due to severity of diabetes were found at days 3 or 14 postpartum for serum prolactin or at any time for milk prolactin.

For women with IDDM, serum prolactin was associated with some determinants of lactation behavior: earlier breast-feeding ($r = -0.42$, $P < 0.05$) and more frequent feedings in the first 12 h after delivery ($r = 0.58$, $P < 0.005$). Associations with components of milk composition included a positive correlation of serum prolactin with milk energy ($r = 0.62$, $P < 0.005$) at day 14 postpartum and milk lipid concentrations ($r = 0.84$, $P < 0.05$; $r = 0.58$, $P < 0.05$) at days 3 and 14 postpartum for women with IDDM. Serum prolactin did not predict infant milk intake by regression analysis for any of the groups.

Milk prolactin

Milk prolactin concentrations did not differ significantly between breasts; thus, values for both right and left breasts are presented throughout. Milk prolactin concentrations for all

groups decreased significantly from day 3 to 84 ($F = 114.2$, $df = 5$, $P < 0.0001$) (Table 2). At day 2, milk prolactin values for women with IDDM and control women were significantly lower than for reference women, and on day 3 milk prolactin values for women with IDDM were significantly lower than both control and reference women. By day 7, values for women with IDDM were lower than control values but not lower than reference values. After day 7, milk prolactin concentrations did not differ among any of the groups.

Sixty-four percent of the variance in milk prolactin measurements for mothers with IDDM at day 2 postpartum was explained by elevated postprandial capillary glucose. Prepartum insulin and first-trimester HbA_{1c} explained 25% and 49% of the variance in milk prolactin at day 3 postpartum, respectively. For mothers with IDDM, lower milk prolactin values were associated with higher postprandial capillary glucose concentrations ($r = -0.81$, $P < 0.05$) at day 2 postpartum, higher pre-delivery dosages of insulin ($r = -0.54$, $P < 0.05$) at day 3 postpartum, higher first-trimester HbA_{1c} ($r = -0.75$, $P < 0.05$) at day 3 postpartum, and higher total morning dosages of insulin ($r = -0.52$, $P < 0.05$) at day 14 postpartum.

For women with IDDM, determinants of lactation behavior associated with milk prolactin included higher milk prolactin values at day 3 postpartum correlated with a greater number of breast-feedings in the first 12 h ($r = 0.74$, $P < 0.005$), a shorter time interval to first breast-feeding ($r = -0.54$, $P < 0.05$), and an earlier time of perceived onset of milk production ($r = -0.69$, $P < 0.05$). In addition, more frequent breast-feeding in the past 24 h was associated with greater milk prolactin concentrations at days 2 ($r = 0.84$, $P < 0.05$), 7 ($r = 0.38$, $P < 0.05$), and 84 ($r = 0.50$, $P < 0.05$) postpartum.

Milk energy and lipid were positively correlated with milk prolactin at days 3 and 7 postpartum. For women with IDDM at day 42 postpartum, higher milk prolactin values were associated with higher concentrations of milk energy ($r = 0.62$, $P < 0.005$) and lipid ($r = 0.45$, $P < 0.05$) and lower milk glucose ($r = -0.45$, $P < 0.05$) concentrations. For control women, higher milk prolactin values were associated with higher concentrations of milk lipid at day 42 postpartum ($r = 0.50$, $P < 0.05$). Milk lactose was negatively correlated with milk prolactin at day 2 postpartum ($r = -0.83$, $P < 0.05$) for reference women and at days 14 ($r = -0.43$, $P < 0.05$) and 42 ($r = -0.49$, $P < 0.05$) for

TABLE 2

Milk prolactin concentrations in subjects with insulin-dependent diabetes mellitus (IDDM) and in control and reference women*

Days postpartum	IDDM	Control	Reference
	$\mu\text{g/L}$		
2	66.3 $\pm$ 4.1 [6] ^a	66.7 $\pm$ 3.1 [11] ^a	91.9 $\pm$ 3.6 [7] ^b
3	65.8 $\pm$ 2.4 [17] ^c	75.2 $\pm$ 2.0 [23] ^d	76.5 $\pm$ 3.1 [9] ^d
7	62.0 $\pm$ 1.8 [29] ^c	67.6 $\pm$ 1.7 [22] ^d	63.3 $\pm$ 2.8 [11] ^{cd}
14	48.7 $\pm$ 2.0 [24]	51.8 $\pm$ 2.1 [22]	52.6 $\pm$ 2.8 [11]
42	39.7 $\pm$ 2.2 [21]	41.2 $\pm$ 2.1 [16]	41.9 $\pm$ 2.9 [11]
84	33.8 $\pm$ 2.4 [17]	35.2 $\pm$ 2.5 [16]	35.6 $\pm$ 2.8 [11]

* Least-square mean $\pm$ SEM; *n* in brackets. Values with different lettered superscripts on each horizontal row are statistically different: ^a $P < 0.0001$; ^{cd} $P < 0.05$.

women with IDDM. Whereas serum prolactin did not predict infant milk intake by regression analysis, milk prolactin was a predictor of infant breast milk intake (g/24 h) for all groups combined ($P < 0.0001$).

When the multifactorial model was adjusted for covariates of milk prolactin, capillary glucose, serum glucose, time from last breast-feeding, and breast-feeding frequency in the last 24 h, women with IDDM still differed from reference women at day 2 postpartum but the differences between women with IDDM and control women were no longer evident at day 7 postpartum. When breast-feeding frequency was used as a covariate, the milk prolactin concentration ($65.6 \pm 3.1 \mu\text{g/L}$) of women with IDDM was still lower than control ($73.1 \pm 2.6 \mu\text{g/L}$) and reference ($76.7 \pm 4.2 \mu\text{g/L}$) values at 3 days postpartum; however, differences between women with IDDM and control women were not significant.

Discussion

The considerable subject-to-subject variation for serum prolactin that existed within all groups tended to obscure any differences among groups. The temporal decline in serum prolactin for all groups was greater from days 3 to 14 than from days 42 to 84. Apparently, circulating prolactin concentrations decreased significantly as lactation began but then diminished slowly over time as lactation became established.

Milk prolactin concentrations for women with IDDM and control women in our study compare favorably with morning values reported by Gala et al (23), $62.3 \pm 5.4 \mu\text{g/L}$, but values for our reference women are somewhat higher. As in our study, Healy et al (24) and Adamopoulos and Kapolla (8) found elevated milk prolactin concentrations at day 3. Taketani and Mizuno (22), Forsbach et al (27), and Yuen (21) defatted milk before analysis. Taketani and Mizuno's value at day 2 was $87.8 \pm 8.2 \mu\text{g/L}$, similar to the value for our reference women at day 2; Forsbach et al reported a value of $31.85 \mu\text{g/L}$ at day 105; a value similar to the day-84 value in our reference women. However, Yuen reported a milk prolactin concentrations of $11.0 \pm 1.4 \mu\text{g/L}$ at days 98–280 postpartum, a value about one-third less than in our reference women. Except for an increase from days 2–3 postpartum reported by Taketani, all researchers reported a decrease over time in milk prolactin concentrations, which is similar to our study.

The significantly lower milk prolactin concentrations for women with IDDM than for control and/or reference women during the first week postpartum could be related to elevated serum glucose concentrations. Elevated postprandial capillary glucose explained much of the variance in milk prolactin measurements for mothers with IDDM at day 2 postpartum. Evidently, good glycemic control during pregnancy and the early postpartum period was associated with higher perinatal milk prolactin values. The negative correlation of serum prolactin concentrations with HbA_{1c} during lactogenesis at day 3 for women with IDDM corresponded to the findings of Jovanovic and Peterson (5), associating poor glycemic control during pregnancy with lower concentrations of serum prolactin; however, in our study, the lower concentrations were found postnatally.

Because poor glycemic control may have affected prolactin concentrations adversely in women with IDDM, those women with IDDM who breast-fed more frequently may have increased their prolactin concentrations (8), thereby counteracting the

negative effect of hyperglycemia. Early breast-feeding episodes and the number of breast-feedings in the first 12 h were critically important for women with IDDM during initiation of lactation (10). Whiclow and Doddridge (28) found that early initiation of infant suckling was the most important factor for successful lactation for both diabetic and nondiabetic mothers. If breast-feeding began within 12 h of delivery, weaning of the infant was delayed beyond 3 mo. Breast-feeding activity in the first hours after birth stimulates prolactin secretion, which is essential to lactogenesis. Goda et al (29) reported higher basal prolactin concentrations in intense lactators at days 2 and 4 postpartum than in women with delayed lactogenesis.

Tyson et al (30) demonstrated that thyrotropin-releasing hormone stimulated prolactin secretion in lactating women and increased both milk production and the fat content of their milk through a positive effect on lipogenesis. Lipid is elevated in galactorrhea milk and correlated with the coincident hyperprolactinemia (31). Similarly, milk lipid concentrations in the present study were associated with milk prolactin at several time points.

One marker for the onset of copious milk secretion at 24–48 h postpartum is a surge of lactose in milk (32). Arthur et al (33) reported that the peak in milk lactose occurred significantly later for women with IDDM (72 ± 13 h postpartum) than for nondiabetic women (53 ± 12 h postpartum), suggesting that lactation was delayed for women with IDDM. Healy et al (24) reported a significant negative correlation between milk prolactin and milk lactose ($r = -0.59$). Similarly, in our study the negative correlation of milk lactose with milk prolactin was at day 2 postpartum for reference women, indicating normal lactogenesis for this group. Milk prolactin declined from $92 \mu\text{g/L}$ at day 2 to $77 \mu\text{g/L}$ at day 3 whereas milk lactose increased from 143 to 166 mmol/L (11). For women with IDDM the negative correlation occurred much later (at day 14), reflecting the delay in establishment of lactation experienced by the women with IDDM in our study.

To enhance prolactin concentrations during the establishment of lactation, women with IDDM should be encouraged to maintain good glycemic control and to breast-feed their infants early and frequently in the perinatal period. 

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