Prolactin binding in human milk at different stages of gestation and duration of lactation in relation to nutrient composition

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A prolactin binding protein was isolated and characterized in microsomes isolated from milk collected from mothers who delivered infants prematurely (28–34 wk) and at term. Scatchard analysis of (^{125}I) human prolactin binding to milk microsomal membranes revealed a single class of specific binding sites having a mean K_D and binding capacity of 4.2 ± 0.4 nM and 116.9 ± 12.4 fmol/mg membrane protein, respectively. Comparison between prolactin binding protein content and nutrient composition of milk at different stages of gestation and lactation was made. Total prolactin receptor content in milk increased with duration of lactation (0.23 ± 0.03, 0.53 ± 0.3, 0.55 ± 0.10 fmol/mL on days 4, 16, and 37 postpartum, respectively). No apparent effect of gestation on receptor content and binding affinity was observed. A negative correlation between concentration of prolactin and the lipid and lactose content of milk was observed.

Keywords: prolactin; human milk; gestation; nutrient

Introduction

The nutrient differences in milk from mothers delivering prematurely and at term[1,2] are thought to be influenced by maternal hormone status[3,4] and diet.[5-7] The primary hormone involved in initiation of lactation is prolactin. The mechanism by which prolactin exerts an effect on mammary cells is not clear. Recent evidence indicates that internalization of the prolactin receptor-complex in plasma membrane may be responsible for initiation of these actions.[8-10] Prolactin[11-13] and a prolactin binding protein[14,15] are present in human milk and milk of several mammalian species.[16,17] Prolactin may enter milk through mammary alveolar cells bound to intercellular protein.[8,18-20] These hormone-protein complexes may represent the internalization of a prolactin receptor at the cell surface or internal sites of prolactin action within the mammary cell,[9,10] indicating that an intracellular mechanism for prolactin action may exist in the mammary gland.

The physiological significance of prolactin and prolactin receptor in milk is unknown. Animals studies have shown that milk prolactin levels contribute significantly to neonatal prolactin levels,[16] suggesting a role for maternal prolactin in newborn endocrine development. For humans there is little information regarding hormonal parameters that influence partitioning of nutrients between mother and infant. The presence of prolactin and a prolactin receptor in milk allows assessment of maternal hormonal events leading to lactation. Thus, this study assessed effect of changes in maternal hormone status in lactation by measuring prolactin and prolactin receptor levels in milk at different lactation stages.

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Methods and materials

Subject selection and milk collection procedures were approved by the University of Alberta Hospital Human Ethics Committee. Milk samples were collected from 15 mothers delivering at term and from 15 mothers delivering at 28–34 wk gestation. Average age of mothers delivering prematurely and at term was 28.5 ± 4.1 and 25.9 ± 4.2 yr, respectively. The mean gestational age, weight, and length of preterm infants (31.8 ± 2.2 wk, 1806 ± 566 g, and 42.3 ± 5 cm, respectively) were significantly lower than fullterm infants (39.1 ± 1.5 wk, 3341 ± 488 g, and 49.3 ± 4.1 cm, respectively; $P < 0.05$). General information regarding the mother's background and course of pregnancy and delivery were recorded. All mothers were normal healthy individuals producing enough milk to meet their infants' energy requirements.

Two complete expressions were collected from the left breast using a mechanical Gerber Precious Care Breast Pump® (Reedsburg, Wisconsin, USA) during the first early morning expression (6–7 a.m.; T1) and approximately 2 hr later (8–9 a.m.; T2) on days 4, 16, and 37 postpartum. The first collection was preceded by a period of approximately 6 hr since the last nursing. Volume, time of expression, and the time of last nursing or expression were recorded by the subject. Milk was collected in this manner to ensure that adequate samples of milk were collected and diurnal variations in nutrient composition were accounted for. Milk samples were initially stored in glass bottles at 4° C for less than 24 hr until transported on ice to the laboratory. Samples were measured, aliquoted and stored at −70° C until assay.

Properties of prolactin binding were determined in pooled preterm ($n = 5$) and pooled fullterm ($n = 7$) milk. The remaining preterm ($n = 10$) and fullterm ($n = 10$) samples were used to determine prolactin receptor, prolactin, and nutrient composition of milk at different stages of gestation and duration of lactation. Preliminary analysis determined that 60–80 mL milk was required to provide sufficient membrane for all analyses. Thus, milk from two subjects was consistently pooled for the same day and time on days 4, 16, and 37 to ensure that total volume of milk in each group exceeded 60 mL ($n = 5$ for each group). Fullterm samples collected on day 4 were very small (less than 20 mL per expression), so milk from four subjects was pooled for each collection time ($n = 3$ for fullterm group on day 4).

Milk membrane isolation

Pooled milk samples (12–15 mL) were thawed and mixed with 20–25 mL isolation buffer containing 25 mmol/L TRIS-HCl and 10 mmol/L magnesium chloride (pH 7.5), then homogenized for 30 sec using a Polytron P-10 homogenizer (Brinkman Instruments, Westbury, New York, USA) and centrifuged at 12,700 rpm (20,000g at r_{av}) for 30 min at 4° C in a Beckman L8-70 M Ultracentrifuge and SW28 rotor (Beckman Instruments Ltd., Palo Alto, CA USA) to remove fat and particulate matter.[15] The defatted supernatant was diluted with 1–10 mL isolation buffer and centrifuged at 27,500 (100,000g at r_{av}) for 90 min at 4° C in a Beckman SW28 rotor. The 27,500 rpm (100,000g) pellet was resuspended in 2 mL of 5 M $MgCl_2$ containing 0.1 M TRIS-HCl buffer (pH 7.5) at 23° C. After 5 min the sample was diluted with 30–35 mL isolation buffer and centrifuged at 27,500 rpm (100,000g at r_{av}) at 4° C for 60 min in the same rotor to sediment membranes. The supernatant was aspirated, and the pellet resuspended in isolation buffer and recentrifuged at 27,000 rpm for 60 min at 4° C to remove excess $MgCl_2$. Membrane pellets were collected, pooled, and resuspended in 600–800 μL isolation buffer, and stored at −70° C until assay. Treatment of the 100,000g milk membrane with 5 M $MgCl_2$ removes endogenously bound prolactin from the binding site.[21] Our preliminary studies show that tr^eatment of milk membranes with 5 M $MgCl_2$ increases subsequent prolactin binding by 60–83%[14] and decreases membrane protein by 50% (unpublished data).

Prolactin binding

100,000g pellets treated with 5 M $MgCl_2$ were incubated with (^{125}I) human prolactin (New England Nuclear, Boston, MA USA).[15,22] (^{125}I) human prolactin was iodinated using carrier-free ^{125}I, by modifying the Hunter-Greenwood method[23] and purified by gel column chromatography. Membrane protein (300 μg) was incubated in 12 × 75 mm glass tubes with 100 μL (^{125}I) human prolactin (0.045 μCi/tube or 1–2 ng/tube) in the absence or presence of varying levels of unlabeled prolactin (0–40 ng, 15 μg/mL, Friesen: Batch 84-7-20) in the assay buffer (25 mmol/L TRIS-HCl, 10 mmol/L $MgCl_2$, 0.1% (wt/vol) bovine serum albumin pH 7.5) in a final volume of 0.5 mL at 23° C for 16–18 hr. Prolactin binding was stopped by adding 1 mL of cold assay buffer and centrifuging in a Beckman J2-21 centrifuge (JA-21 rotor) at 5000 rpm (3000g at r_{av}) for 30 min at 4° C. Polyethylene glycol was not used because it results in nonspecific sticking of (^{125}I) human prolactin to experimental test tubes. The supernatant was decanted and tubes were inverted and drained on absorbent paper. Pellets were counted in a Beckman LS-8000 gamma counter with a counting efficiency of 75%. Specific binding was determined by subtracting counts bound in the presence of excess (1 μg) unlabeled human prolactin (nonspecific counts) from counts bound in the absence of excess unlabeled hormone (total binding). Specific and nonspecific binding represented 35–40% and 45–65% of the total counts bound, respectively. Scatchard analysis of samples was performed with 6–11 point duplicate determinations over a range of 0–40 ng added cold prolactin, and was used to calculate binding capacity and dissociation constant by linear regression ($P < 0.05$).

Prolactin determination

Prolactin content of milk was assayed using a human prolactin radioimmunoassay kit distributed by National Institute of Arthritis Metabolism and Digestive Diseases (NIAMDD). Methods used were similar to those suggested by NIAMDD and Mulloy and Malven.[24] Pooled milk samples were diluted 1:2.5 in assay buffer (0.01 M phosphate buffered saline containing 0.01% (wt/vol) sodium azide, 1% (wt/vol) 0.15 M NaCl and bovine serum albumin, pH 7.5) and centrifuged at 5000 rpm for 30 min at 4° C in a Beckman J2-21 centrifuge. The lipid layer was removed and the defatted diluted milk samples were stored at −20° C until assay.

Defatted milk samples (50 μL) or standard human prolactin antigen (NIAMDD-hPRL-RD-1) (100 μL) of varying concentrations (1–500 ng/mL) were added to 200 μL human prolactin antiserum (NIAMDD-anti-hPRL-3; 1:400,000); 100 μL (^{125}I) human prolactin (0.0045 μCi/tube; 0.1 ng/tube) from New England Nuclear; and 300–350 μL 1% (wt/vol) bovine serum albumin-phosphate buffered saline for a final volume of 700 μL. After 48 hr at 4° C, 2000 μL goat-antirabbit gammaglobulin (Sigma Chemical Co., St. Louis, MO USA) diluted 1:25 was added to each tube. The tubes were incubated for 48 hr at 4° C, when 1 mL of cold phosphate buffered saline was pipetted into each tube and the tube centrifuged at 5000 rpm for 30 min at 4° C. The supernatant was decanted and the precipitate counted in a gamma counter. Blanks contained isotope, assay buffer, and antirabbit gamma globulin.

The validity of milk prolactin estimates was assessed by

recovery experiments in which 0.1–10 ng/mL standard human prolactin was added to a standard fullterm milk sample (collected and pooled 4, 16, and 37 days postpartum at T1 and T2; n = 7). The interassay and intra-assay coefficients of variation (mean ± S.D.) were 17.4±2.1 and 2.5±1.1, respectively. The percentage of prolactin recovered in recovery experiments was 109 ± 4.8%.

Protein and lactose determination

Protein and lactose determinations were done in diluted and defatted milk samples. Pooled milk samples were diluted 1:2.5 and 1:50 in 25 mmol/L TRIS-HCl containing 10 mmol/L $MgCl_2$ (pH 7.5) and deionized water for protein and lactose determinations, respectively. Diluted samples were centrifuged at 5000 for 30 min at 4° C. The lipid layer was discarded and the defatted supernatant stored at −20° C until assay. Protein content was determined.[25] The lactose content of milk was assayed using a modified method of Kotler et al.[26] Defatted samples (50 μL) or 50 μL standard β-lactose (Sigma Chemical Co.) of varying concentrations (0–1.27 mmol/L) were added to 100 μL β-galactosidase (80 μg/μL reaction mixture; EC 3.2.1.23; Sigma Chemical Co.) and 1 mL incubation buffer containing 0.1 M sodium phosphate (pH 7.4). Tubes were incubated for 2 hr at 30° C in a shaking water bath. Free glucose content was determined by adding 5 mL peroxidase-glucose-oxidase enzyme-color (0-dianisidine dihydrochloride) solution (Sigma Chemical Co.) to each tube. Corrections were made for blanks and presence of endogenous glucose in milk.

Lipid determination

Lipid determinations in whole milk used an adapted Folch procedure described by Chappell et al.[27] Organic extracts were weighed, flushed with N_2, capped with lined caps, and stored at −70° C.

The effects of gestational age and duration of lactation on prolactin concentration, prolactin receptor content, nutrient composition of milk, and fatty acid composition of microsomal phospholipids were assessed by least-squared analysis of variance procedures.[28] Regression analysis was used to assess the relationship between maternal prolactin status (as reflected by prolactin receptor content and milk concentration) and nutrient composition of milk.[29] The relationship between fatty acid composition of microsomal phospholipids and prolactin binding was also assessed using regression analysis.

Results

Binding prolactin

Subcellular fraction. Analysis of specific binding of (^{125}I) human prolactin in subcellular fractions of fullterm milk was measured to determine the fraction in human milk containing prolactin receptor. In agreement with results by Waters et al.,[15] binding of prolactin to milk microsomes (100,000*g* pellet) treated with 5 M $MgCl_2$ was significantly greater ($P < 0.05$) than binding in the 20,000 *g* pellet and 100,000*g* supernatant. Specific binding was not detected in the 20,000*g* supernatant *(Table 1)*. Characterization of prolactin binding in milk microsomes treated with 5 M $MgCl_2$ established validity of Scatchard analysis to determine receptor numbers and dissociation constants in preterm and fullterm milk over time.

Table 1 Specific binding of (^{125}I) human prolactin in subcellular fractions of milk

Subcellular fraction	Membrane level (μg)	Binding capacity (fmole/mg)
20,000*g*	226	1.3 ± 2[a]
Pellet	452	0.97 ± 1[a]
20,000*g*	680	not distinguishable
Supernatant	1360	not distinguishable
100,000*g*	331	0.1 ± 0.09[b]
Supernatant	662	0.5 ± 0.08[b]
5 M $MgCl_2$-treated	10	5.6 ± 0.8[c]
100,000*g* pellet	250	50.9 ± 10[d]

Values without common superscripts are significantly different ($P < 0.05$).
Subcellular fractions were prepared as described in Materials and methods from a pooled milk sample collected from a mother delivering at term. The samples were collected between 6 and 7 a.m. and 8 and 9 a.m. on days 4, 16, and 37 postpartum. (^{125}I) human prolactin (25,000 cpm, 0.7 ng) was incubated with varying levels of subcellular fractions in the presence (nonspecific binding) and absence (total binding) of excess cold prolactin. Data represent mean ± SD of duplicate determinations.

Binding characteristics. Total, nonspecific, and specific prolactin binding increased with increasing membrane protein levels *(Figure 1A)*. Specific binding was not distinguishable from nonspecific binding at membrane protein concentrations less than 200 μg/mL and was maximal at concentrations above 500–600 μg/mL. Therefore, a concentration of 600 μg/mL was used for all binding assays. Specific binding increased with time of incubation at 23° C, and plateaued after 15–18 hr *(Figure 1B)*. Specific binding was markedly reduced at 4° C over the incubation period. Binding was highest at pH 6.5–7.5. Therefore, subsequent binding studies were carried out at a membrane protein concentration of 600 μg/mL at 23° C, pH 7.5 and an incubation period of 15–18 hr.

Specificity of prolactin binding. Specific binding was measured over a wide range of prolactin concentrations (0–200 ng/mL) and was also examined over a range of (^{125}I) human prolactin concentrations (0.1–10 ng/mL) to determine the level of radioligand necessary for optimal binding conditions (data not shown). A level of 2–3 ng/mL was chosen for all binding analysis, as marked variations in specific binding occurred at higher concentrations. Maximum binding was achieved at prolactin concentrations of 60–80 ng/mL. Scatchard analysis[30] of binding of (^{125}I) human prolactin to milk microsomal membrane displayed one class of low capacity (116.9 ± 12.5 fmol/mg membrane protein), high affinity (4.2 ± 0.4 nM) binding sites *(Figure 2A)*. No significant difference ($P < 0.05$) in the affinity constant (K^a) of

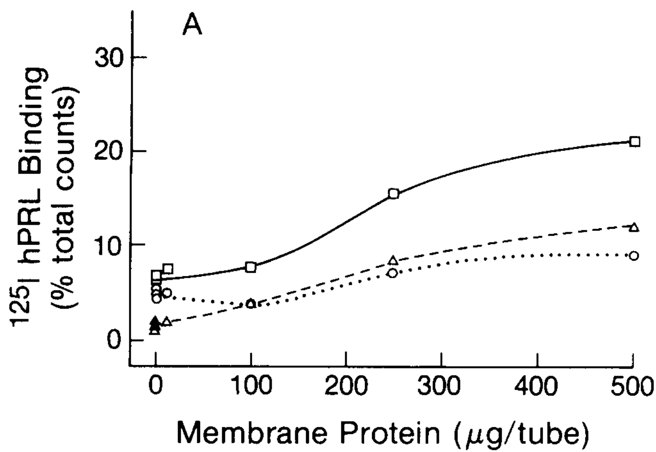

Figure 1A Changes in mean total, nonspecific, and specific binding of (^{125}I) human prolactin to 100,000g pellet treated with 5 M magnesium chloride as a function of membrane protein concentrations. (^{125}I) human prolactin (25,000 cpm, 0.6 ng) was incubated in the presence and absence of cold prolactin (2 μg/mL) with varying amounts of 100,000g pellet treated with 5 M magnesium chloride. 100,000g pellets were isolated from fullterm milk collected at 4, 16, and 37 days postpartum between 6 and 7 a.m. and 8 and 9 a.m. as described in Methods and materials. The nonspecific and specific binding represented 35–45% and 46–65% of the counts bound, respectively. Total prolactin binding was 15–25% of the total counts added. Values are means ± SD of duplicate determinations. Total binding: —□—□—; nonspecific binding: ··○··○··; specific binding: --Δ--Δ--.

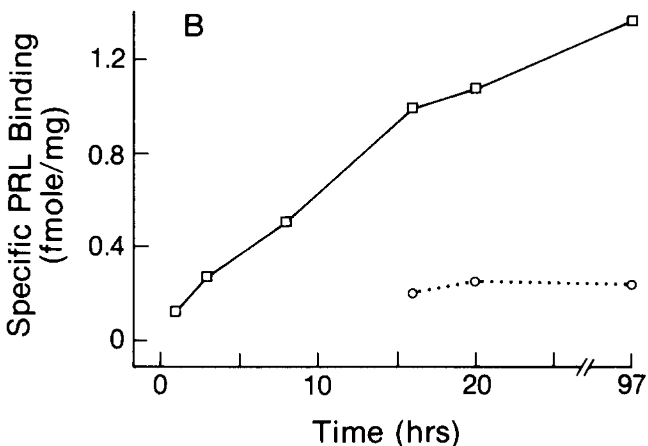

Figure 1B The effect of time and temperature on prolactin binding in 100,000g pellet. The amount of (^{125}I) human prolactin (25,000 cpm, 0.6 ng) bound over time at 23° C and 4° C. 100,000g pellets were prepared from pooled fullterm (n = 7) collected on 4, 16, and 37 days postpartum between 6 and 7 a.m. and 8 and 9 a.m. as described in Methods and materials. Values are means of duplicate determinations. T = 23° C: —□—□—; T = 4° C: ··○··○··.

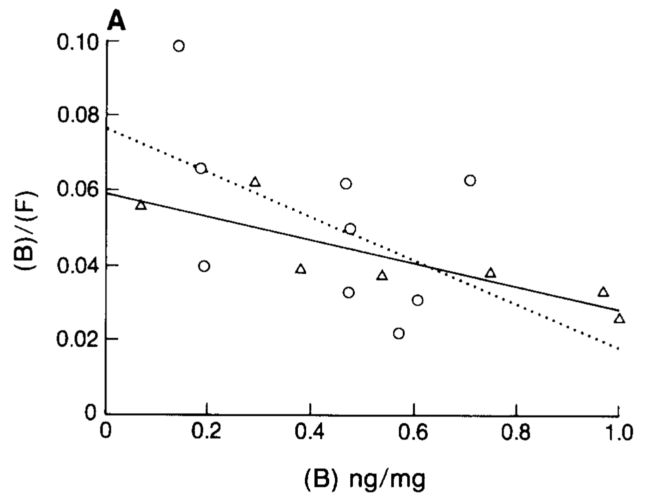

Figure 2A Scatchard analysis of prolactin binding to 100,000g pellets. 100,000g pellets treated with 5 M $MgCl_2$ were prepared from preterm (pooled) and fullterm (pooled) milk collected 16 days after delivery between 6 and 7 a.m. (T1) as described in Methods and materials. Values are means of duplicate determinations. The binding capacity and dissociation of prolactin binding was calculated from linear regression analysis. Preterm: —Δ—Δ—; fullterm: ··○··○··.

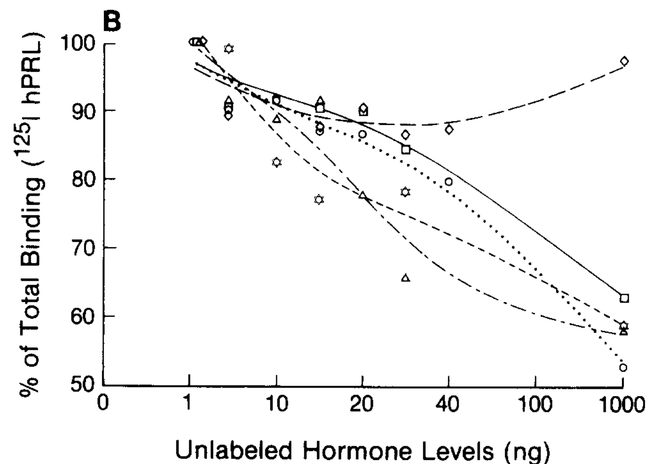

Figure 2B Competitive inhibition of bound (^{125}I) human prolactin to 100,000g pellets treated with 5 M magnesium chloride by unlabeled human prolactin (hPRL), human growth hormone (hGH), porcine prolactin (pPRL), and porcine insulin. Two concentrations of (^{125}I) were used to determine specificity of binding. Human prolactin, pPRL, and porcine insulin were incubated with (^{125}I) hPRL (1.2 ng/mL, 55,000 cpm) and 300 μg of membrane protein as described in Methods and materials. Human growth hormone and human prolactin were incubated with (^{125}I) hPRL (4.4 ng/mL, 62,000 cpm) and 300 μg of membrane protein. Membranes were prepared from pooled fullterm milk samples (n = 7) collected 4, 16, and 37 days postpartum between 6 and 7 a.m. (T1) and 8 and 9 a.m. (T2). Values represent means of duplicate determinations at each level of competing hormones. hPRL (1.2 ng/ml): —□—□—; pPRL: ··○··○··; porcine insulin: --◇--◇--; hPRL (4.4 ng/ml): --☆--☆--; hGH --Δ-Δ--.

the receptor between preterm and fullterm milk samples was detected.

The specificity of prolactin binding was examined by measuring displacement of (^{125}I) human prolactin by increasing concentrations of a variety of cold competitors *(Figure 2B)*. The most effective inhibitor was human prolactin, followed by porcine prolactin and human growth hormone. Porcine prolactin and human growth hormone displaced bound (^{125}I) human prolactin with similar potencies. Displacement of (^{125}I) human prolactin by human prolactin was significantly greater ($P < 0.05$) than observed for human growth hormone levels (1–15 ng) examined, demonstrating greater affin-

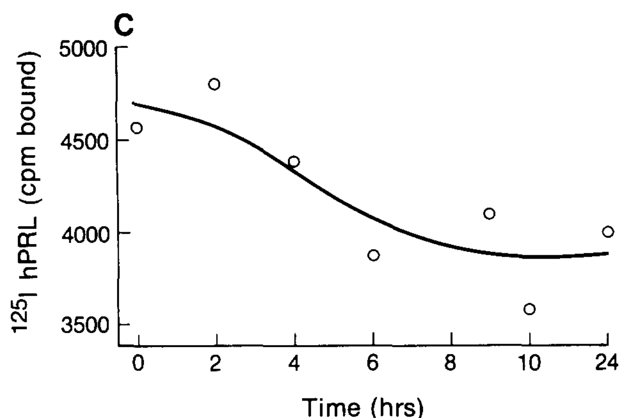

Figure 2C Reversibility of prolactin binding to 100,000*g* pellets treated with 5 M $MgCl_2$. The amount of (^{125}I) human prolactin (50,000 cpm, 1.6 ng) by 1 μg cold prolactin over time was measured in microsomes prepared from a pool of six fullterm milk samples (collected 4, 16, and 37 days postpartum between 6 and 7 a.m. (T1) and 8 and 9 a.m. (T2)). To insure equilibrium conditions, membranes were incubated with (^{125}I) human prolactin for 16–18 hrs before the addition of excess prolactin. Values represent means of duplicate determinations.

ity of the receptor for human prolactin. No significant displacement by insulin was noted at higher concentrations (2 μg/mL), although minimal displacement was observed at lower concentrations (20–80 ng/mL). This displacement profile shows the specificity of this receptor preparation for lactogenic hormones.

Reversibility of prolactin binding to the microsomal receptor was assessed by measuring displacement of (^{125}I) human prolactin with excess cold prolactin (1 μg) over time. After 8 hr, 20% of bound prolactin was displaced by cold prolactin, showing partial reversibility of prolactin binding *(Figure 2C).*

Prolactin receptor content and binding affinity during lactation. Total receptor content of milk increased with duration of lactation in both groups (*Figure 3; $P <$ 0.05*). Mean receptor content (mean ± S.D.) on days 4, 16, and 37 postpartum for preterm and fullterm milk samples was 66.2 ± 28.2, 138.5 ± 28.2, 126.7 ± 28.2 (fmol/mg protein); and 72.8 ± 21.3, 103.1 ± 28.2, 161.1 ± 28.2 (fmol/mg protein), respectively. No effect of gestational age on receptor binding capacity was observed over time or between collection times. However, there was a significant difference ($P < 0.05$) in receptor content between collection times on the same collection day. When receptor content was expressed per mL of milk or corrected for variations in milk volume, the same profile of change in receptor content was observed. For example, the mean receptor content of preterm milk collected at T2 was significantly higher than milk expressed at T1 on day 16. Comparison of prolactin binding at each prolactin concentration used with the stage of gestation, duration of lactation, and time of collection was done to assess gestational effects (data not shown). However, differences in prolactin binding at each prolactin level studied could only be attributed to changes occurring with maturation of lactation. These results suggest that differences in the rate of appearance of prolactin receptor in milk exist between groups. No significant difference in the affinity constant (K_a) of milk receptor was detected between groups and collection times over the course of the study.

The concentration of protein in preterm milk was significantly higher than fullterm milk ($P < 0.05$) *(Table 2)*. The mean (±S.D.) concentration of protein in preterm and fullterm milk 4, 16, and 37 days postpartum was 22.6±10.5, 20.2±4.3, 17.9±5.9 mg/mL; and 15.4±12.5, 20.2±7.9, 17.9±12.9 mg/mL, respectively. Pooling of milk may have produced large within-group variability in the concentrations of protein, lactose, and prolactin observed. No apparent effect of collection time or stage of lactation on protein concentration in milk was observed. However, a significant effect of time of expression on total protein levels (g) in milk was observed (data not illustrated). Protein content at T1 was significantly higher ($P < 0.05$) than in milk collected 2 hours later (T2). These differences reflect difference in milk volume produced at each expression.

Lactose concentration in preterm milk was significantly higher ($P < 0.05$) than fullterm milk, regardless of lactation stage *(Table 2)*. Mean (±S.D.) lactose concentration of preterm and fullterm milk 4, 16, and 37 days postpartum was 101.2±31.0, 123.9±57.3, 111.9±40.5 mmol/L; and 83.6±57.6, 85.3±50.7, 91.3±24.3 mmol/L, respectively. No apparent effect of duration of lactation or collection time on milk lactose concentration was observed. However, when values were corrected for variations in volume of each expression, a significant difference in lactose content was observed between collection times (data not illustrated). Lactose content of milk at T1 was significantly ($P <$ 0.05) higher than at T2.

No apparent effect of gestational age, duration of lactation, or collection time on fat concentration was observed *(Table 2)*. Mean (± S.D.) fat concentration in preterm and fullterm milk 4, 16, and 37 days postpartum was 2.3±1.1, 2.3±.8, 2.9±1.7 g/dL; and 1.6±1.3, 3.2±3.2, 2.1±.9 g/dL, respectively. Pooling of milk may have produced the large within-group variability. Correction for volume difference at each expression did not alter the pattern of results (data not shown).

Prolactin concentration decreased significantly ($P <$ 0.05) from day 4 to day 37 in both groups, perhaps reflecting the role of prolactin in the early stages of lactation. Mean ± S.D. concentration of prolactin in milk on 4, 16, and 37 days postpartum was 25.3 ± 11.7, 16.4 ± 12.1, and 13.7 ± 4.4 ng/mL, respectively. No effect of gestational age or collection time (T1 and T2) on prolactin concentration in milk was observed.

Prolactin concentration was compared with prolactin receptor content of preterm and fullterm milk to assess whether a relationship between these two milk constituents exists. Prolactin concentration in preterm and fullterm milk was negatively correlated with prolactin receptor content in milk ($P < 0.05$) in both groups over the course of the study *(Figure 4)*.

There was a strong negative correlation ($P <$ 0.05) between prolactin concentration and total fat

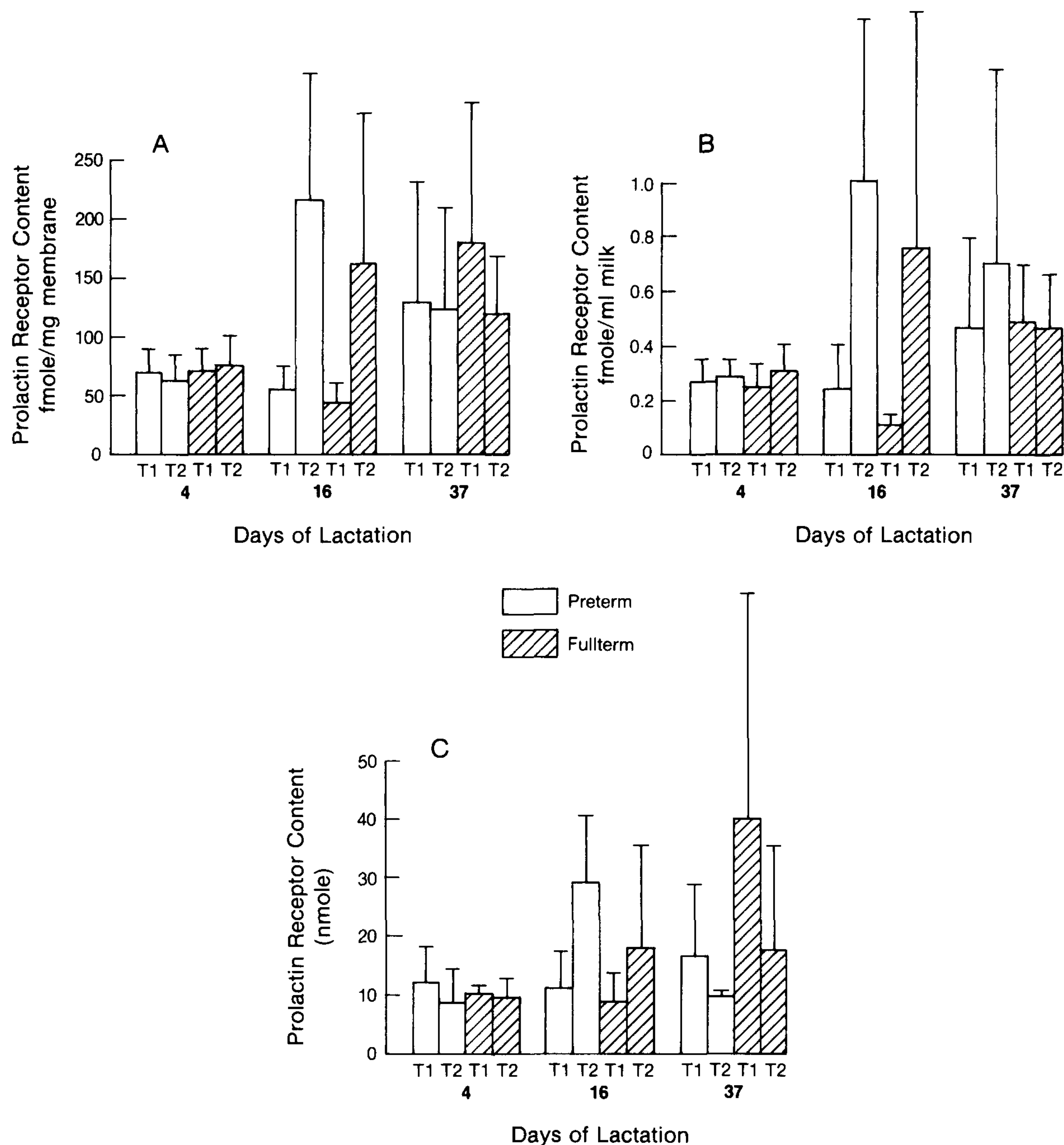

Figure 3 The effect of stage of gestation and duration of lactation on prolactin receptor content in human milk. 100,000*g* pellets treated with 5 M magnesium chloride were prepared from milk collected from mothers delivering prematurely (28–34 wk) and at term. Milk samples were collected 4, 16, and 37 days postpartum between 6 and 7 a.m. (T1) and 8 and 9 a.m. (T2) as described in Methods and materials. Milk from two subjects was pooled for the same day and time on days 4, 16, and 37 for both groups (n = 5) except on day 4 for the fullterm milk. Values are means ± SD (n = 5) determinations. A. Prolactin receptor content in milk (fmol/mg membrane protein). B. Prolactin receptor content in milk (fmol/ml milk). C. Total prolactin receptor in milk (nmoles).

($r = -0.55$) and lactose ($r = -0.55$; $n = 12$) content in preterm and fullterm milk over the course of the study *(Figure 5)*. These relationships may show a declining role for prolactin in stimulation of nutrient synthesis in milk as lactation matures. There was also a weak positive correlation between milk prolactin receptor content and protein content of milk (data not shown). However, these relationships were not significant over the course of the study. Large within-group variability in milk receptor content and nutrient composition of milk on days 16 and 37 indicate that a range of individual responses may occur.

Discussion

Differences exist between the macronutrient composition of milk produced by mothers delivering prema-

Table 2 Effect of state of gestation and duration of lactation on the concentration of protein, fat, and lactose in milk

	DAY 4		DAY 12		DAY 37	
	T1	T2	T1	T2	T1	T2
A. Protein (mg/mL)						
Preterm[a]	18.6 ± 9.8	26.5 ± 3.8	23.0 ± 4.9	17.4 ± 4.2	18.4 ± 2.0	16.9 ± 5.6
Fullterm[b]	20.3 ± 10.1	10.4 ± 7.3	7.4 ± 2.0	11.6 ± 7.7	16.0 ± 12.4	9.4 ± 3.7
B. Lactose (mmol/L)						
Preterm[a]	95.6 ± 23.0	106 ± 20.8	116.9 ± 34.8	130.9 ± 45.5	96.2 ± 20.1	127.6 ± 35.2
Fullterm[b]	97.9 ± 35.6	69.2 ± 45.3	81.2 ± 27.2	89.3 ± 42.8	85.8 ± 16.9	96.7 ± 17.5
C. Fat (g/dl)						
Preterm	2.1 ± 0.6	2.4 ± 0.9	2.1 ± 0.4	2.4 ± 0.7	2.4 ± 0.8	3.4 ± 1.3
Fullterm	1.9 ± 1.1	1.3 ± 0.6	3.6 ± 2.9	2.7 ± 1.2	1.5 ± 0.4	2.7 ± 0.9

a versus b: preterm significance different than fullterm within time and day ($P < 0.05$).
Protein and lactose determinations (A and B) were done in diluted and defatted milk samples as described in Methods and materials. Fat determinations (C) were done in whole milk samples as described previously. Milk samples were collected from mothers delivering prematurely (28–34 weeks of gestation) and at term between 6 and 7 a.m. (T1) and 8 and 9 a.m. (T2) on days 4, 16, and 37 postpartum. Milk samples were pooled as described in Methods and materials. Data represent mean ± SD of duplicate determinations.

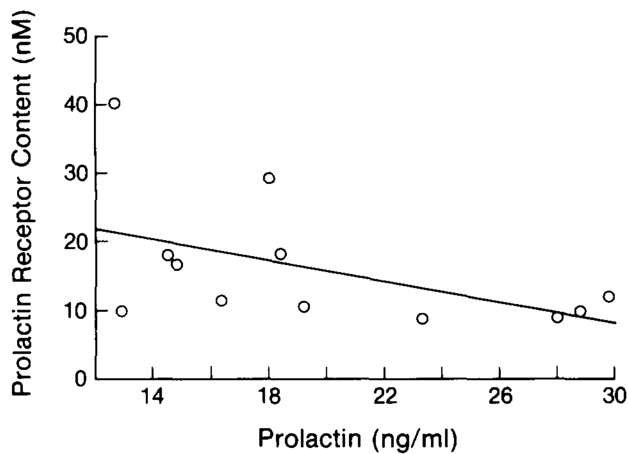

Figure 4 The effect of stage of gestation and duration of lactation on the concentration of prolactin in human milk. Prolactin was measured in defatted milk samples diluted 1:2.5 in phosphate buffered saline/1% (wt/vol) bovine serum albumin (pH 7.45) as described in Methods and materials. Milk was collected 4, 16, and 37 days postpartum between 6 and 7 (T1) a.m. and 8 and 9 a.m. (T2). Values illustrated are means ± SD (n = 5) except on day 4 for fullterm milk samples (n = 3). The decrease in the concentration of prolactin milk over the duration of lactation was significant ($P < 0.05$).

turely and at term.[1,2] Maternal hormonal status[3,4] and dietary intake[5–7] may influence milk composition. Prolactin stimulates the initiation of lactation in the mammary gland, but the mechanism by which prolactin exerts these effects is unclear. Evidence supports the hypothesis that internalization of the prolactin receptor-complex at the plasma membrane may be responsible for initiation of these actions.[8–10] The presence of biologically potent prolactin and a prolactin binding protein in milk[14,15] provide indirect evidence that an intracellular mechanism for prolactin action exists in the mammary gland. Postpartum increases in levels of mammary receptors have been associated with suckling-induced rises in serum prolactin,[31] suggesting that prolactin plays a direct role in auto regulation of receptor numbers. Assay of milk prolactin and milk prolactin binding pro-

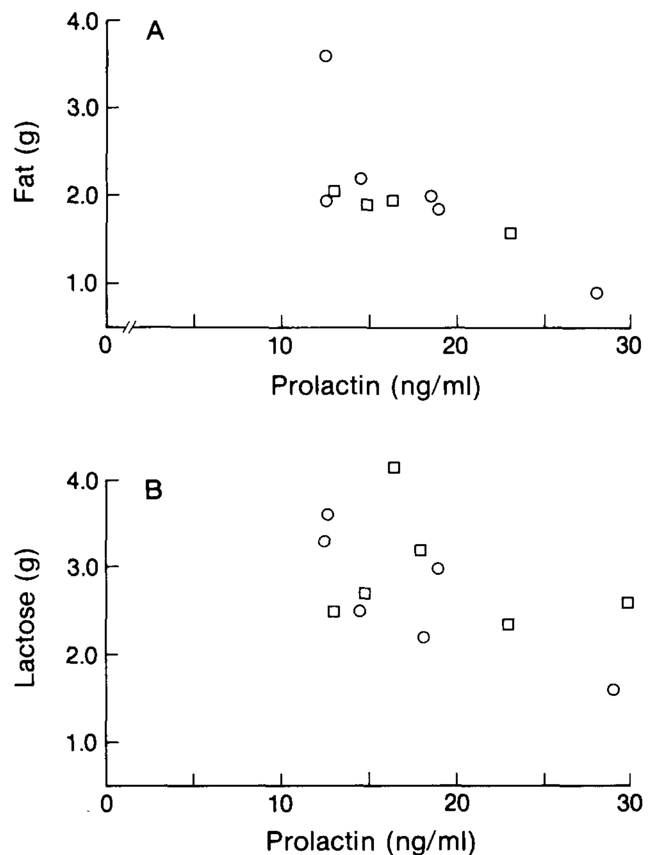

Figure 5 Relationship between the concentration of prolactin (ng/mL) and the fat and lactose content of milk (g) collected from mothers delivering prematurely (28–34 wk) and at term. Milk was collected between 6 and 7 a.m. (T1) and 8 and 9 a.m. (T2) on days 4, 16, and 37 as described in Methods and materials. Values are means (n = 5) for all days and times in both groups except on day 4 for fullterm milk (n = 3). Preterm and fullterm milks are represented by □ and ○ respectively. *Figure A* represents fat values, while *Figure B* represents lactose values.

tein levels at different stages of gestation and lactation may help characterize the relationship between prolactin action and nutrient composition of milk. Maternal diet may also interact with these hormonal effects. In this regard, dietary fat has been shown to affect receptor-mediated functions by altering the fatty acid composition of membrane phospholipids.[32]

This study demonstrates that a prolactin binding protein capable of selectively binding prolactin exists in human milk, in agreement with sites previously reported in rabbit milk[15] and rabbit mammary gland.[22,31,33-35] Prolactin binding increased with treatment of microsomal membrane with $MgCl_2$. This procedure results in removal of endogenously bound prolactin from the binding site.[21] Conditions for maximum specific binding were similar to those reported earlier.[15,22,33] Binding increased with membrane protein concentration and length of incubation. Binding of prolactin to its receptor was saturable, partially reversible, and specific for human prolactin, indicating that Scatchard analysis was appropriate for determination of binding capacity and affinity. This analysis revealed the existence of a low capacity, high affinity prolactin binding site in milk microsomal membrane in agreement with those reported in rabbit milk[15] and rabbit mammary gland.[33,35,36] Binding specificity obtained with the milk prolactin receptor preparation was similar to that observed in other tissues that respond to prolactin.[15,22,36] Results indicate that this receptor is only responsive to lactogenic hormones. The fact that porcine prolactin also bound to the receptor preparation suggests a common structural property in prolactin binding sites between species.[37]

The prolactin receptor content of milk increased with maturation of lactation over the first 4 wk of lactation in a pattern similar to rabbit milk.[15] Prolactin receptor levels in preterm milk achieved maximum levels by day 16 of lactation and declined by day 37. The receptor content of fullterm milk achieved maximum levels by day 37. These changes in receptor content occurred with a marked decrease in prolactin concentration in milk after day 4, suggesting a declining role for prolactin as lactation progresses.[11,12]

Waters et al.[15] demonstrated that milk prolactin receptor content correlates with mammary gland prolactin receptor content over the duration of lactation. It is possible that the change in milk prolactin receptor content over the duration of lactation reported in this study reflects change in mammary gland receptor content as shown by Djiane et al.[33] The drop in concentration of prolactin in milk observed may also reflect a coinciding decrease in serum levels of prolactin.[24] This decrease in prolactin concentration may trigger a signal to modulate receptor function in the mammary gland by increasing the number of available binding sites. Changes in binding affinity are unlikely as no apparent effect of gestational age, duration of lactation, and time of collection on binding affinity was observed.

The present study demonstrates a negative correlation between prolactin concentration and lipid or lactose content of milk, in agreement with earlier reports.[12] Prolactin receptor level tended to follow change in protein and lactose content of milk. These results suggest that a relationship exists over the duration of lactation between maternal hormonal status, as reflected in prolactin receptor content and prolactin concentration in milk, and nutrient composition. However, as gestational effects were not observed, it is likely that other factors in addition to prolactin play a role in determining milk nutrient composition. Progesterone and estradiol have a direct effect on prolactin receptor function in the mammary gland.[31,38,39] Further research is required to establish the role of other hormonal influences on prolactin receptor function at different stages of gestation and duration of lactation.

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