Effects of Diabetes Mellitus on Lactation in the Rat (43638)

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Abstract. **Diabetes mellitus adversely affects the process of lactation. Although insulin administration restores lactation, the manner by which it does so is unknown. The goals of this study were to determine which phase of lactation, milk synthesis/supply (MS), and/or milk release (MR) was affected. Inasmuch as insulin, corticosterone, and prolactin, among other hormones, are involved in milk synthesis *in vitro*, this study investigated their probable roles in the suppression of lactation in diabetes *in vivo*. Diabetes was induced in rats on Day 3 postpartum with streptozotocin (50–60 mg/kg, ip). Milk synthesis/supply and milk release were *indirectly* monitored using body weight gain of pups during a timed-feeding period on postnatal Days 8/9 and 13/14. Plasma corticosterone, insulin, C-peptide, and prolactin were measured by radioimmunoassay in control, diabetic, and insulin-replaced diabetic animals. Insulin replacement was provided by means of an osmotic minipump implanted subcutaneously in the nape of the neck. In addition, several parameters of maternal behavior were monitored in order to determine whether diabetes affected maternal behavior and, therefore, whether such changes played a role in the alterations of lactation observed during diabetes.**

Diabetes significantly suppressed MS and MR. However, the decrease in MR, which was restored after partial insulin replacement, was a reflection of the reduced MS. There were no significant differences between the parameters of maternal behavior monitored in control and diabetic animals. Blood glucose in diabetic dams was significantly increased over that of controls. Although the levels of plasma glucose in the insulin-replaced groups were significantly lower than those of their uncontrolled diabetic counterparts, they also remained higher than that of controls. Corticosterone was not significantly altered after induction of diabetes. Insulin and C-peptide levels were significantly reduced in the uncontrolled diabetics; however, insulin levels were corrected in the insulin-replaced groups. Serum levels of prolactin decreased in all diabetic groups and insulin failed to restore these levels to those of control animals.

In conclusion, it appears that diabetes decreases lactation through a suppressive effect on MS rather than on MR, with insulin replacement correcting for such deficiency. In addition, despite the lactogenic importance of glucocorticoids, prolactin and insulin demonstrated *in vitro*, lactation *in vivo* can be corrected without returning the levels of all three hormones to normal. The importance of another factor(s), such as normoglycemia, as essential to the observed defects, needs to be investigated.

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Insulin-dependent diabetes mellitus impairs proper lactation. Studies have implicated decreased milk yield as well as altered milk composition (1–4). Proper insulin replacement, however, can correct for these deficiencies (1, 3, 5). It is well recognized that diabetes and lactation independently lead to significant hormonal changes in the organism. To what extent such alterations resulting from diabetes can affect lactation needs to be explored.

Lactation may be divided into two major processes, namely milk synthesis/supply and milk ejection. It has been suggested that normal milk supply necessitates adequate levels of prolactin, insulin, and glucocorticoids (6). Indeed, prolactin is involved in the synthesis of major milk proteins (7–10) as well as fatty acid synthesis (7, 9). The lactogenic role of insulin has been observed not only in protein expression (11), but also

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in lipogenesis (12–14) and glucose metabolism (15). Glucocorticoids are necessary for the proper maintenance of lactation, but the levels at which they should be present is unsettled (6, 16). In mammary gland cultures, glucocorticoids, like prolactin, have been shown to enhance casein gene expression (10, 17). Suckling has been shown to stimulate release of prolactin (7, 18–21), glucocorticoids (6, 22), and insulin (23–25).

Milk ejection, the second phase of lactation, is primarily a neural reflex mediated by the pulsatile release of oxytocin in response to the sucking stimulus of the pups (26). Factors such as stress come into play in hindering normal milk release (27–29). Different mechanisms of action in stress appear implicated, depending upon the nature of the stressor (29). Suppression of milk ejection during stress not only can result from central inhibition of the release of oxytocin, but also from secondary factors such as activation of the sympathetic nervous system. Indeed, the latter in inducing vasoconstriction may decrease the level of oxytocin reaching the mammary tissue (30). Maternal behavior is another factor closely tied to successful lactation. Onset and maintenance of maternal behavior is dependent upon physical contact between partners of the nursing dyad as well as adequate levels of prepartum estradiol (31, 32), prolactin (33), oxytocin (34), and progesterone (32). The importance of adequate maternal behavior lies not only in the proper maintenance of lactation, but also on its indirect effects on the development of the offspring. Rat pups require maternal contact for thermoregulation and stable behavioral, biochemical, and organ maturation (35–37). Thus, factors interfering with lactation may act via milk synthesis and/or ejection as well as maternal behavior.

During diabetes, various hormonal changes are known to occur (38). Studies have shown increases (39–41) and decreases (42) in glucocorticoids as well as normal and decreased levels in prolactin (43–45). There also exists an interaction between glucocorticoids and prolactin (46, 47): adrenalectomy increases both basal and stress-induced levels of prolactin, whereas administration of adrenocortical steroids are inhibitory.

With the knowledge, on the one hand, that Type I diabetes induces changes in available insulin and also may alter the levels of glucocorticoids and prolactin and, on the other, that these hormones are lactogenic and interact with each other, it is conceivable that these hormonal alterations are implicated in the suppression of lactation in diabetics. Therefore, the goals of the present study were to investigate specifically (i) the nature of the lactational deficiency observed during diabetes, namely which phase(s) of lactation, milk supply, and/or milk ejection is adversely affected; and (ii) to what extent alterations in maternal behavior and/or changes in circulating insulin, glucocorticoids, and prolactin are implicated in such suppression.

Materials and Methods

General Methods. *Animals.* Timed-pregnant Sprague-Dawley rats (Crl:CD(SD)BR) were obtained from Charles River Laboratories (Wilmington, MA). Upon arrival, the animals were housed individually in opaque cages with food (Ralston Purina 5001, St. Louis, MO) and water *ad libitum*. With room temperature maintained at 21°C ± 1°C, the animals were kept on a 12:12-hr light:dark cycle with lights on at 0600 hr. On the day of parturition (Day 0 postpartum [pp]), the cages were checked every 2 hr for births. On Day 1 pp, all litters were culled to eight pups (four males and four females whenever possible). Litters with less than eight, but more than five, pups were given additional pups from other litters of the same age and appropriate gender whenever possible to achieve a total of eight pups with four males and four females.

Induction of diabetes mellitus. Diabetes was induced after treatment with streptozotocin (Sigma, St. Louis, MO). Intraperitoneal injection of streptozotocin (STZ) at a dose of 50–60 mg/kg was given on Day 3 pp, inasmuch as studies have established that the first 3 days after birth are critical for the initiation and proper expression of subsequent maternal behavior (31). On the day of treatment, the animals were fasted for 6 to 9 hr before STZ administration. Vehicle-treated controls received equivalent volumes of 100 m*M* citrate buffer (pH 4.5). Three groups emerged from the study: control (C), diabetic (STZ+), and sham diabetic (STZ−), i.e., a group that received STZ but failed to become diabetic. Diabetic animals were identified by the presence of glycosuria as tested with Clinistix (Miles, Inc., Elkhart, IN). These dams were tested every other day thereafter for sustained glycosuria. Final verification of diabetes was assessed from blood samples collected at one time point at the end of the experiments, when the animals were sacrificed by decapitation. Multiple blood sampling was not performed in order to minimize undue stress and disturbance of the mother-young dyad. Plasma/serum glucose levels were measured using an enzymatic kit (Boehringer Mannheim Diagnostics No. 189197, Indianapolis, IN). Levels greater than 200 mg/dl with attending glycosuria confirmed the diabetic state of the dams.

Study 1: Effects of Diabetes on Milk Ejection and Supply. *Milk ejection.* Milk ejection was *indirectly* assessed on Days 8/9 and 13/14 pp, as described in an earlier study (48). Body weight increments of pups were measured after a 1-hr feeding period. The same litters were used for both experimental days. Such a test-weighing method has been validated for humans as well as animals. It has been shown that milk intake of the young during a feeding period as measured by the

difference in pre- and postfeed body weight, although indirect, adequately reflects milk ejection from the mother/dam (49, 50). In the rat, this methodology was originally described and validated by Houpt and Epstein (50) and used subsequently by other laboratories as well as our own with minor modifications (48). On the day of the experiment, dams and pups were isolated for 5 hr to allow equal time for accumulation of milk supply of the dams and a concurrent 5-hr fast of the young. The latter were kept in a styrofoam bucket at nest temperature, 33–35°C (35). The 1-hr refeeding period began at 1300 hr. Milk intake was calculated as the difference between the pre- and posthour refeed. During any test-weighing procedure, it was important to ensure that voiding of urine as well as the metabolism of the young be taken into consideration because body weight changes would be affected by such factors. Dams routinely lick the anogenital area of some of their young, thereby emptying their bladder. As differences of 0.3–0.5 g/pup can result from such occurrence, it was important to standardize this discrepancy by the systematic voiding of all the pups. Thus, before each weighing, urination was induced in each pup by stroking the anogenital area with a tissue. As the litters were left undisturbed during the feeding period, it was believed that the energy consumed during that period of time was basal and similar among all pups. In this manner, a constant error was introduced in the measurement of the body weight of all the young, justifying the use of their weight change as an *indirect* measure of milk intake. On Days 13/14 pp, at the end of the 1-hr feed, maternal blood samples were collected after decapitation.

Milk supply. Milk supply was evaluated in another series of animals by a procedure described in an earlier study (48), on the same lactational days as those for milk release. Briefly, once the pups attached to the nipples after the 5-hr isolation period, as described above, the dams were quickly anesthetized with sodium pentobarbital (Nembutal; Abbott Laboratories, Chicago, IL) at a dose of 4 mg/100 g body wt intraperitoneally. The return of the pups to a conscious dam before the induction of anesthesia was necessary because the young, at the ages studied, do not readily succeed to attach without maternal help because the nipples are recessed after the lack of stimulation during the isolation period. Synthetic oxytocin (150 mU; Sigma) was administered intraperitoneally and every 15 min thereafter for a total of 750 mU. The anesthesia eliminated maternal participation present with conscious dams and the pharmacologic dose of oxytocin used ensured maximal milk removal by the pups. The young exhibited a sustained stretch response after each oxytocin injection. Although the characteristic stretch response should not be used as a quantitative index of actual milk release, its occurrence appears to result from mammary myoepithelial contraction (51) and, as such, is indicative of an oxytocin effect on mammary tissue. Under these conditions, the weight gain of the pups during the 1-hr refeed, determined in the same manner as the milk ejection study, was used as an *indirect* measure of maternal milk supply. At the end of each experiment, all the mammary glands were exposed after incision and skin retraction, and complete depletion of the glands was verified upon visual inspection of the mammary tissue for the absence of white milk residuals. Each dam was used on only one experimental day because this protocol is deleterious to postexperimental lactation. At the end of the experiment, blood samples were immediately collected on Day 8/9 and 13/14 pp by decapitation.

Study 2: Hormonal Profile in Diabetic Dams. This study was conducted on only Day 13/14 pp. Once diabetes was confirmed by glycosuria, the dams were either sham-operated or implanted subcutaneously on Day 5 pp with an osmotic minipump (Alzet 2002; Alza, Palo Alto, CA) with a constant delivery rate of 0.5 μl/hr for 14 days. Pumps were filled with insulin (Iletin II U-500; Eli Lilly, Indianapolis, IN) at a concentration such as to deliver 9 units/kg body wt/day. Insulin was diluted in 7 mg/ml L-glutamic acid (crystalline, Sigma) and bovine serum albumin (Sigma), 2% final volume in physiological saline. Sham-operated animals were implanted with a wax pellet resembling the minipumps in size and shape. Under ether anesthesia, pumps or pellets were placed subcutaneously in the base of the neck of the dams. In addition, each animal in the diabetic group received a bolus of 0.1 units/kg ultra lente insulin (Iletin I U-100; Eli Lilly) subcutaneously at the time of the pump implantation. Control animals received saline injections. Diabetic animals were tested daily for glycosuria and, if present, were given additional doses of ultra lente insulin twice daily at 0.1 units/kg. Five groups of animals emerged from this study: untouched control, nondiabetic sham-operated treated with STZ (STZ−), sham-operated untreated diabetic (STZ+), and two operated insulin-replaced groups. The first of these latter two groups became aglycosuric after implantation of the pumps (STZ+P), suggesting adequate insulin replacement, while the other group remained glycosuric to some extent (STZ+PI) and periodically required insulin injections for the remainder of the study. Milk release was measured in these groups of animals on Days 13/14 pp, as described in Study 1, along with the following parameters of maternal behavior: time taken by the dam to retrieve the pups to the nest upon their reunion, time required by the dam to calm down and suckle, and percent time spent in the nest in contact with the young during the 1-hr refeed (31). At the end of the study, in order to obtain basal levels of serum corticosterone, the animals were removed from the experimental room

and sacrificed within 1 min of approaching the cage. Sera were aliquoted into four tubes and frozen at −20°C for subsequent analyses of glucose, corticosterone, insulin, C-peptide, and prolactin. Corticosterone was measured by competitive protein binding assay (52). Insulin and C-peptide were assayed in duplicates using a double-antibody radioimmunoassay by Dr. R. Gingerich, St Louis, MO. Prolactin determinations were also measured in duplicates by radioimmunoassay using NIDDK-rat PRL-RP-1 as reference preparation (from Dr. A. Berkowitz, University of Texas, Health Science Center at Houston, TX). The pups' growth was monitored after routine weighing three times a week.

Statistics. For the determination of milk ejection and supply, the dams were considered the experimental unit (53). Therefore, individual weight gains of all pups sucking on a particular dam were averaged to give a single value (g/pup) for that dam. The means ± SE were then calculated for all the dams in each experimental group. For all the parameters, one- or two-way analyses of variance were first used to detect significance among all groups. Post hoc analyses for multiple comparisons among specific groups were then conducted using Fisher PLSD. Inasmuch as no differences were noted in any of the parameters measured between untouched control and STZ− animals, all statistical comparisons were made between the experimental animals and their sham counterparts (STZ−).

The growth profiles of the various groups of pups in Study 2 were examined using simple linear regression analysis. The reasons for adopting such an analytical model are based on the facts that birthing frequently occurred over 2–3 days and, in order to minimize handling of the animals, weighing was performed only 3 times a week, at the time when cages were changed. Collection of these data did not allow the measurements to be age-adjusted to equally spaced target ages (i.e., equal time intervals). As multivariate techniques, such as analysis of variance, require this criterion to be met, or that interpolation of missing observations be made, it was more efficient and accurate to compare values of the parameters derived from a regression model, as Hoel (54) has demonstrated. An additional advantage in using this technique is that the growth rate of the pups can be derived from the slopes of these equations. Accuracy of such methodology depends on the selection of a model that best fits the collected data. In the present study, body weight of the young was best fitted by a simple linear regression ($y = a + bx$) as noted by the R^2 obtained (see Results). Thus, the best fit equation was derived for each individual litter. The means of each coefficient (i.e., slope and intercept) were calculated and used to derive the regression equation presented in Figure 6 and Table III. The 95% confidence interval around the difference of the average of the slopes between experimental and STZ− animals is shown in Table III. Independent two-sample t tests with Bonferroni correction for multiple comparisons were used to compare the means of the slopes between the STZ− and the diabetic groups.

Results

Induction of Diabetes. Within 48 hr post-STZ injection, glycosuria was established in all STZ+ animals, but was absent in the STZ− group. As shown in Table I, glucose levels from both studies were not significantly different between control and STZ− animals, whereas that of STZ+ dams were significantly elevated when compared with STZ− dams ($P < 0.05$).

In Study 2 (Table I), all three experimental groups demonstrated significantly elevated glucose levels as compared with STZ− ($P < 0.05$). The levels of the two groups receiving insulin replacement (STZ+P, STZ+PI), however, were lower than those of their STZ+ counterparts ($P < 0.05$). There were no significantly different glucose levels between the STZ+P and STZ+PI animals.

Study 1: Effect of Diabetes on Lactation. As presented in Figure 1A, milk ejection was similar ($P > 0.05$) in control and STZ− dams, but significantly suppressed in STZ+ versus STZ− counterparts ($P < 0.05$) at both stages of lactation. Furthermore, milk ejection was greater on Days 13/14 pp than Days 8/9 pp ($P < 0.05$) in controls and STZ−, but not in STZ+ lactators. Figure 1B shows that milk supply was similar between control and STZ− animals. The diabetic dams (STZ+) demonstrated a significant suppression in milk supply ($P < 0.05$) at both stages of lactation (Fig. 1B) when compared with their STZ− counterparts.

Study 2: Hormonal Profiles in Diabetic Dams.

Table I. Blood (Plasma/Serum) Glucose Levels (mg/dl)

Group	Study 1
Milk ejection	
Control	149 ± 25 (6)[a]
STZ−	154 ± 14 (7)
STZ+	406 ± 21 (9)[b]
Milk supply[c]	
Control	91 ± 9 (11)
STZ−	110 ± 7 (14)
STZ+	305 ± 15 (17)[b]
Group	**Study 2**
Control	156 ± 6 (9)
STZ−	144 ± 12 (10)
STZ+	487 ± 12 (8)[b]
STZ+P	305 ± 28 (9)[b,d]
STZ+PI	382 ± 37 (6)[b,d]

[a] Number of animals.
[b] $P < 0.05$ vs. STZ− (Fisher PLSD for multiple comparisons).
[c] Values from Days 8/9 and 13/14 pp combined.
[d] $P < 0.05$ vs. STZ+ (Fisher PLSD for multiple comparisons).

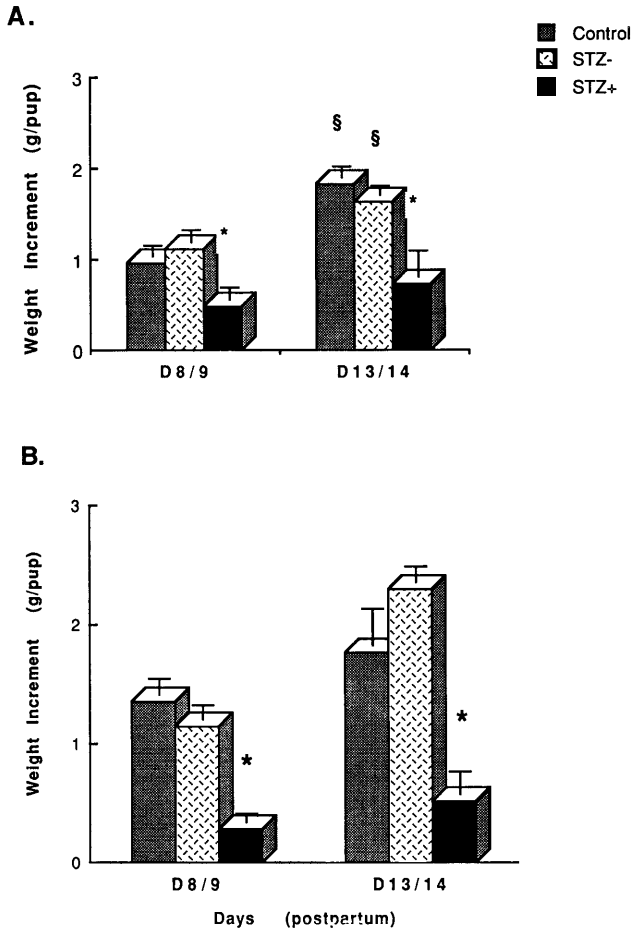

Figure 1. Means ± SE of body weight gain (g/pup) of a 1-hr feed from 5-hr-fasted pups nursing 5-hr-isolated lactators on Days 8/9 and 13/14 pp, used as indirect measure of milk release (A) and milk supply (B): Control, sham-diabetic (STZ−), diabetic (STZ+). *Fisher PLSD for multiple comparisons; $P < 0.05$ (versus STZ−). §Fisher PLSD for multiple comparisons; $P < 0.05$ (D8/9 versus Day 13/14 pp).

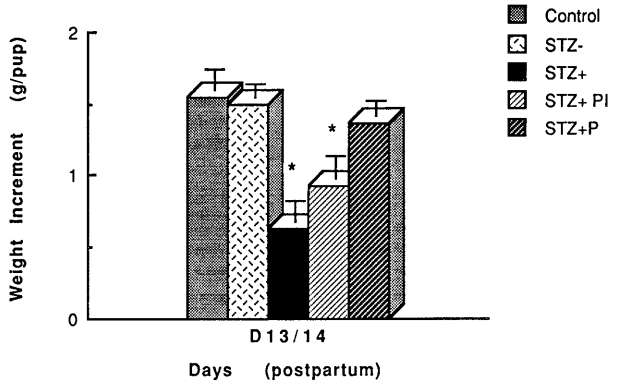

Figure 2. Means ± SE of body weight gain (g/pup) of a 1-hr feed from 5-hr-fasted pups nursing 5-hr-isolated lactators on Days 13/14 pp, used as indirect measure of milk release: Control, sham-diabetic (STZ−), diabetic (STZ+), aglycosuric insulin-replaced (STZ+P), glycosuric insulin-replaced (STZ+PI). *Fisher PLSD for multiple comparisons; $P < 0.05$ (versus STZ−).

Table II. Parameters of Maternal Behavior (Means ± SE)

Group (animals)	Pup retrieval (sec)	Calm and suckling (min)	Time in the nest (%)
Control (9)	101 ± 15	6.2 ± 0.6	83.0 ± 5
STZ− (10)	93 ± 18	5.6 ± 0.4	88.3 ± 3
STZ+(8)	106 ± 15	5.6 ± 0.6	94.8 ± 2
STZ+PI (9)	100 ± 15	4.6 ± 0.3	88.8 ± 4
STZ+P (6)	65 ± 16	4.2 ± 0.5	82.8 ± 7

The lactational performance of the animals was examined using the milk ejection protocol on Days 13/14 pp. As presented in Figure 2, there was no significant difference observed between control and STZ− dams with respect to milk ejection. Compared with their sham counterparts (STZ−), the untreated diabetic animals (STZ+) as well as diabetic rats receiving daily injections of insulin (STZ+PI) showed significant suppression in milk ejection ($P < 0.05$), whereas the aglycosuric diabetic dams (STZ+P) did not differ from the control rats in this regard.

Maternal behavior as measured by retrieval time of the pups to the nest, "calm and suckling," and percent time spent by the dam in the nest were not different between STZ− and any of the diabetic groups (Table II).

The levels of corticosterone measured were similar among all five groups, 17.07 ± 3.11 μg/dl (means ± SE). Insulin levels (Fig. 3A) were not significantly different between control and STZ− animals, nor between the latter and their STZ+P and STZ+PI counterparts. Only STZ+ dams demonstrated significantly reduced levels of insulin when compared with their STZ− counterparts ($P < 0.05$). Levels of C-peptide (Fig. 3B) were decreased in all three diabetic groups versus STZ− dams ($P < 0.05$). However, STZ+P animals demonstrated higher levels than STZ+ ($P < 0.05$). Prolactin, as shown in Figure 4, was similar between control and STZ− dams, but was significantly reduced ($P < 0.05$) in all three diabetic groups versus their sham counterpart (STZ−).

Figure 5 presents the growth profile of the litters. There was a strong correlation between body weight and age (R^2 of all the groups were greater than 0.96). After Bonferroni correction for multiple comparisons, there were no significant differences in the slope between control and STZ−, and between STZ+P and STZ− (Table III). On the other hand, STZ+ and STZ+PI diabetic animals showed a significantly slower rate in weight gain than STZ− counterparts ($P < 0.05$). The means ± SE of the pups' body weight on postnatal Day 1/2 for each group are presented in Table III.

Discussion

The aims of the present study were, first, to identify whether diabetes affected lactation through milk ejec-

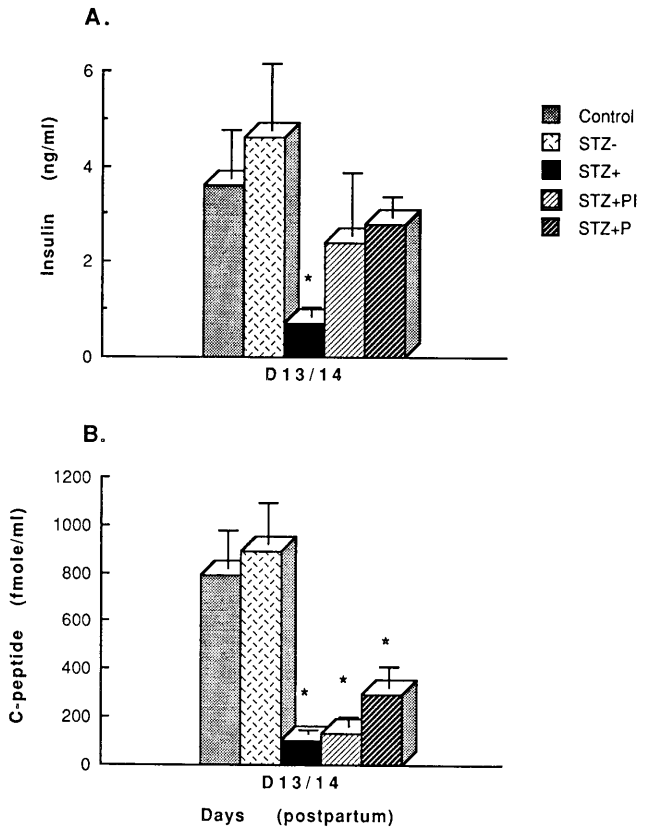

Figure 3. (A) Serum insulin levels (ng/ml); (B) serum C-peptide levels (fmol/ml) of lactators on Days 13/14 pp. *Fisher PLSD for multiple comparisons; $P < 0.05$ (versus STZ−).

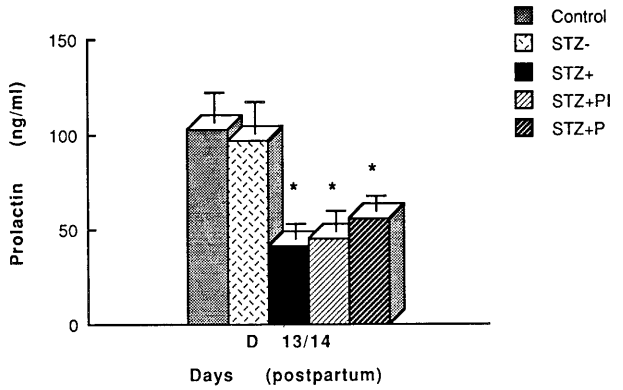

Figure 4. Serum prolactin levels (ng/ml) of lactators on Days 13/14 pp. *Fisher PLSD for multiple comparisons; $P < 0.05$ (versus STZ−).

tion and/or milk supply and, second, to what extent changes in maternal behavior and/or levels of the lactogenic hormones, insulin, corticosterone, and prolactin, which are altered during diabetes as well as lactation, play a role in the well-acknowledged suppressed lactation.

Diabetes was induced during lactation on Day 3 pp for two reasons. Induction of diabetes postpartum removed any possibility that STZ might have an *in utero* effect. It also allowed for the full development of

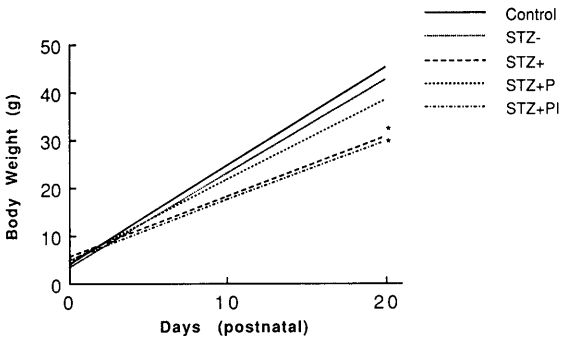

Figure 5. Simple linear regression representing the growth profile of pups. *Independent t test with Bonferroni correction for multiple comparisons; $P < 0.05$ (versus STZ−).

maternal behavior inasmuch as the first 3 postpartum days are crucial for the initiation and expression of maternal behavior (31). The sham animals that received STZ but did not become diabetic (STZ−) provided evidence that the drug per se was not a direct cause of the alterations seen in lactation and hormonal levels, as these animals and the vehicle-treated controls exhibited similar responses. Although insulin administration (STZ+P, STZ+PI) reduced circulating levels of glucose when compared with untreated diabetics (STZ+), these levels remained significantly higher than those of STZ− counterparts, even in the case of STZ+P animals which were aglycosuric. It is likely that the dose of insulin used was not sufficient to correct fully for the diabetes. The observation that STZ+P animals were aglycosuric despite relatively elevated glucose levels is puzzling. This discrepancy may lie in the fact that in these animals, glucose levels may have fluctuated near the glycosuric threshold and blood sampling was only taken at one time point at the end of the experiment, when the dam had been suckling for 1 hr.

The induction of diabetes led to a decrease in milk release, as measured by the intake of the pups on postnatal Days 8/9 and 13/14, when compared with nondiabetic counterparts. This confirms observations made in earlier reports (2–4). In addition, milk release did not increase between Days 8/9 and 13/14 pp as observed in the control and STZ− animals as well as earlier studies (48). Milk supply in the STZ+ rats also was markedly suppressed when compared with the STZ− counterparts. Inasmuch as pups ingested the same amount of milk during the 1-hr refeeding period under both milk ejection and milk supply protocols, the present study suggests that suppression of lactation in the diabetic animals is a direct consequence of decreased milk synthesis/supply rather than an alteration in the process of milk ejection. However, the results herein do not rule out the possibility that the decrease in milk supply may have resulted from an overall decrease in milk ejection since the induction of diabetes (i.e., during the previous 5–10 days). It was discussed

Table III. Regression Equations Representing the Neonatal Growth Profile

Groups	n^a	Pups' body wt postnatal day 1/2 (g)	Regression equations	95% confidence levels[b]	P^c
Control	9	6.73 ± 0.21	y = 3.80 + 2.06x	[−0.24, 0.05]	NS
STZ−	10	6.75±0.12	y = 3.23 + 1.97x	—	—
STZ+	7	7.19 ± 0.27	y = 5.53 + 1.26x	[0.17, 1.25]	0.05[d]
STZ+P	6	7.53 ± 0.23	y = 4.67 + 1.69x	[0.02, 0.52]	NS
STZ+PI	9	7.01 ± 0.25	y = 4.91 + 1.24x	[0.56, 0.89]	<0.01[d]

[a] Number of animals.
[b] 95% Confidence interval around the difference of the average of the slopes between STZ− and each of the other groups.
[c] Independent *t* test (versus STZ−).
[d] $P < 0.05$ after Bonferroni correction for multiple comparison.

above that stressful stimuli can interfere with the latter process. As no such alteration is apparent in the present study, based on the levels of corticosterone, the degree of diabetes induced did not appear to be sufficiently severe and/or diabetes does not affect this mechanism. From this observation, in the second part of this investigation, milk ejection was used as a direct reflection of changes in milk synthesis. This was purposefully done so as to allow the monitoring of maternal behavior in conscious diabetic dams.

The maintenance of lactation necessitates proper maternal behavior (31). As the latter, in turn, can be altered by various types of stressful situations (55), it was of interest to determine whether maternal behavior was altered in the diabetic dam, thereby leading to an alteration in lactation. From the results obtained, this does not appear to be the case because the maternal behavioral parameters monitored were not affected by the degree of diabetes induced.

Although studies have demonstrated that the lactational deficiency observed during diabetes can be reversed after insulin replacement, none has concurrently measured the levels of the lactogenic hormones, insulin, glucocorticoids, and prolactin to determine whether alterations in the latter could be correlated to the observed phenomenon. As discussed earlier, *in vitro* studies have implicated these hormones as requirements for optimal milk supply (6, 8–15, 17); however, information is lacking in regard to their corrective roles *in vivo*. In the present study, the levels of corticosterone, unlike previous works, were not altered by the degree of diabetes induced here nor by the insulin treatments used (39–42, 56). However, comparison is made difficult as male and female nonlactators were used in the aforementioned studies versus lactators in the present one. This is particularly pertinent knowing that adrenocortical activation in response to various stimuli differs between lactators and nonlactators (30, 57).

Insulin levels were returned to that of STZ− animals in both groups of diabetic dams receiving insulin, i.e., STZ+P and STZ+PI, despite the presence of glycosuria in the latter. Malfunctioning of the osmotic minipumps (decreased insulin delivery) likely led to the sustained glycosuria in the STZ+PI animals. Indeed, the daily dose of additional insulin given subcutaneously twice a day may have been sufficient to correct for the circulating levels of insulin at the time of blood sampling, but not for the glycosuria. The low levels of C-peptide in all three diabetic groups confirmed the expected destruction of the β cells of the pancreas by STZ. However, the observation that the STZ+P animals had higher levels of C-peptide than their uncorrected diabetic counterparts would suggest that correction of their insulin level was a partial result of endogenous production. In this case, it is tempting to speculate that the ability of these animals to retain a certain degree of endogenous insulin secretion allowed them to restore lactation, thus implying that insulin exerts a primary effect on lactation. It is also conceivable that the thresholds for the insulin effect on glucose homeostasis and lactation are dissimilar.

The induction of diabetes (STZ+) suppressed the levels of circulating prolactin, in agreement with observations made in humans and animals (43, 45, 58). Although sampling was only done at one time point (i.e., at the end of the 1-hr feed), it is representative of the level normally measured in nonisolated dams that have remained with their young (59). Indeed, Arey *et al.* (59) showed that after a 6-hr separation of dam and young, the prolactin levels 1 hr after a refeeding session were similar to that before the isolation period. The two insulin-replaced groups (STZ+P, STZ+PI) did not correct for the decreased prolactin, an observation also made by Botta *et al.* (43) on diabetic women receiving insulin therapy and Ikawa *et al.* (58) on rats. As milk release was fully restored in the STZ+P group while the levels of prolactin remained suppressed, it would appear that normal levels of prolactin are not essential for the restoration of lactation. It is conceivable that only maintenance of a certain threshold level of prolactin is important. The suppressed prolactin levels cannot be attributed to changes in glucocorticoids as the latter were not altered, nor to insulin as its replacement did not correct for the low prolactin levels. It is conceivable

that prolactin was inhibited by the sustained hyperglycemia, which remained elevated despite insulin replacement, or by the suppression of a prolactin-releasing factor, or increase of a prolactin-inhibiting factor. Collectively, therefore, this study confirms that lactation can be reinstated with insulin replacement (1, 2, 5). However, it suggests that normal levels of insulin, prolactin, and/or glucocorticoids are not necessary for a corrective effect. These hormones may have permissive effects on each other via threshold levels. Although the focus of this study has been on changes occurring in insulin, prolactin, and glucocorticoids, it is not disregarding the possibility that other hormones, such as thyrotropin-releasing hormone or estrogen/progesterone (60, 61), which are also affected during diabetes, can also be responsible for the lactational deficiency noted in diabetes. Whether normoglycemia, in conjunction with as yet undetermined elements, plays a role also merits further investigation.

In summary, the well-acknowledged decrease in lactation during diabetes appears to reflect a suppression of milk synthesis rather than milk ejection. In addition, consideration should be given to the possibility that in *in vivo*, unlike in *in vitro*, conditions, glucocorticoids, prolactin, and insulin may not exert their lactogenic properties independently via adequate circulating levels, but rather through an interactive mechanism that requires only subnormal levels of each hormone.

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