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Food Restriction, Gonadotropins, and Behavior in the Lactating Rat

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McGUIRE, M. K., H. PACHÓN, W. R. BUTLER AND K. M. RASMUSSEN. *Food restriction, gonadotropins, and behavior in the lactating rat.* PHYSIOL BEHAV **58**(6) 1243–1249, 1995.—This study sought to quantify effects of undernutrition on behaviors and to relate these to gonadotropin and prolactin concentrations in the lactating dam. Dams were studied in a 2 × 3 factorial design with litter size and food intake as the two factors. Behavioral data were collected from each dam and her litter on day 9, day 14, and day 19 of lactation, and maternal blood samples collected. Plasma was analyzed for luteinizing hormone, follicle stimulating hormone and prolactin. On day 15 of lactation, percent time nursing, number of pups actively nursing, total number of pups nursing and dam location acted as mediating factors of the effect of diet group on plasma luteinizing hormone concentration. No such relationships were seen for plasma follicle stimulating hormone, and only nest condition score appeared to be a mediator for plasma prolactin concentration. In conclusion, this analysis suggests that food restriction indirectly influences plasma concentration of luteinizing hormone, but not follicle stimulating hormone, by changing maternal and pup behaviors. The relationship among diet, behavior and circulating prolactin was less clear.

Rat Lactation Nutrition Gonadotropins Prolactin Behavior

INTRODUCTION

THE EFFECTS of food restriction on maternal and pup behaviors have been well documented in the rat, such that food-restricted, lactating dams have been shown to spend more time with their pups and engage in a greater number of maternal activities than do dams allowed to consume food in ad lib amounts (2,3,6,9,16,17). Similarly, the impact of lactation on circulating gonadotropin and prolactin concentrations have also been well documented in the rat (7,8,18,19).

However, to our knowledge, the relationship among food intake, behavior and circulating reproductive hormone concentrations in the lactating animal has not been quantified in a single study. A better understanding of this interaction in any species is important for two reasons. First, a more thorough knowledge of effectors of the physiological strategies used by various species to inhibit conception is desirable, because altering fertility is of biologic, social and economic importance. Second, these data may provide information useful in predicting possible effects of food supplementation programs aimed toward the pregnant and/or lactating woman. For example, if it were shown that food restriction delays ovulation in the lactating animal via an increase in suckling frequency, public health officials might be compelled to encourage undernourished lactating women enrolled in food supplementation programs to nurse their infants more frequently to decrease the chance of an unwanted pregnancy.

Therefore, the objective of this study was to examine the relationships among dietary intake, nursing behaviors and circulating concentrations of luteinizing hormone, follicle stimulating hormone and prolactin in lactating rats. Ovariecomized animals were used so that we could examine an animal model that does not experience hypothalamic negative feedback from ovarian steroids during lactation: a model that more closely resembles the human. We hypothesized that dietary restriction would result in changes in observed maternal and pup behaviors, and consequent differences would be associated with maternal gonadotropin and prolactin concentrations.

MATERIALS AND METHODS

Because a detailed description of animal care, experimental design and methods has been published elsewhere (10,14), only a brief summary is provided here.

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Animal Care and Experimental Design

Female Sprague–Dawley rats (35 day old: $n = 125$) were purchased from Charles River Laboratories (Kingston, NY). Care of all animals was in compliance with applicable National Institutes of Health and institutional guidelines.

On day 42 of life, animals were assigned randomly to one of three dietary treatment groups: control ($n = 43$), mildly food-restricted (R-85%: $n = 39$), or more severely food restricted (R-70%: $n = 43$). Control animals were given free access to diet AIN-76A™, and the food-restricted groups (R-85% and R-70%) were fed modified forms of diet AIN-76A™ (Dyets, Bethlehem, PA) that contained 15% and 30% more, respectively, of the usual percentages of vitamins and minerals. Food-restricted rats (R-85% and R-70%) were fed 85% or 70% (by weight), respectively, of the mean ad lib intake of the control rats. All animals were weighed twice weekly before breeding, at specific days during pregnancy and daily during lactation.

Females were bred with males of the same strain obtained from the same supplier beginning after 64 day of age. The first day that newborn pups were observed with the dam was designated as day 0 of lactation. On this day, dams were assigned systematically, within dietary treatment group, to nurse either 5 or 8 pups. This experimental design was used to increase the range of suckling stimuli and overall maternal behaviors experienced by the dams. On day 1 of lactation, excess pups were removed and killed by carbon dioxide overdose. Thus, a 3×2 factorial design was implemented to study 6 groups. Litters were weighed daily and, as much as possible, were not allowed access to nonmilk food sources.

Surgical Procedures

Between day 2 and day 5 of lactation, surgery was performed both to implant an indwelling catheter in the carotid artery and to perform a bilateral ovariectomy. Surgical anesthesia was provided via a ketamine (66–88 mg/kg body weight, Fort Dodge Laboratories, Inc., Fort Dodge, IA)—xylazine (1.8–2.6 mg/kg body weight; Rompun, Mobey Co., Shawnee, KS) mixture injected intramuscularly, with postoperative analgesia achieved using butorphanol tartrate (50 mg/kg body weight; Fort Dodge Laboratories, Inc., Fort Dodge, IA). Following surgery, animals moved freely around their cages and were able to nurse their litters successfully.

Blood Sampling

Blood samples (300 μL each) were taken every 10 minutes for 2 h (13 samples) between 0800–1200 h on days 10, 15, and 20 of lactation. This strategy was taken, because the hormones of interest are known to exhibit considerable variation, and this blood sampling scheme allowed us to obtain samples that would be representative of a longer period of time. Furthermore, preliminary data (McGuire & Rasmussen, unpublished data) indicated that clear LH pulsatility was not expected in our animal model. The pooling of samples also allowed the analyses of several hormones of interest.

Following removal of plasma, blood cells were resuspended in saline and reinfused through the indwelling catheter. Plasma samples were pooled so that a single analytical determination of each hormone of interest could be done. All samples were stored at -20°C until analyzed.

Measurement of Plasma LH, FSH and Prolactin

Plasma LH concentration was measured in duplicate (40 μL plasma/tube) using a radioimmunoassay (Amerlex™-M rLH assay system, Amersham Corp., Arlington Heights, VA). Plasma FSH concentration was measured in duplicate (50–100 μL plasma/tube) using radioimmunoassay (Amerlex™-M rLH assay system, Amersham Corp., Arlington Heights, VA). Plasma prolactin concentration was measured in duplicate (25–100 μL plasma/tube) using a radioimmunoassay (Amerlex™-M rLH assay system, Amersham Corp., Arlington Heights, VA).

Behavioral Observations

Behaviors and locations of dams and their litters were observed and recorded once a minute for 45 min three times during the lactation period: day 9, day 14 and day 19 between 1730–2130 h. Observations were made under red lights, and dams were transferred to the observation location at least 30 min before the first observation was made.

Immediately before the first and after the last observations, the condition of the "nest" was assessed as either (i) pups together at one end of the box; (ii) pups apart but all at one end of the box; or (iii) pups scattered at both ends of the box. At each observation, dam location, dam activity and litter activity were recorded, and the activity of each pup was documented and was said to be active if voluntary movement was observed.

Statistical Analyses

Except for information concerning weight at randomization, only data obtained from animals contributing blood samples are presented here. Behavioral variables were defined and constructed as follows.

Mean Nest Condition. Nest condition before and after each observation period was coded as 0 (pups together at one end), 1 (pups scattered at one end) or 2 (pups scattered on both ends). These scores were then averaged to obtain a "mean nest condition" for each animal at each observation period.

Mean Dam Location. Dam location at each of the 46 observation points was coded as 0 (near majority of pups) or 1 (away from majority of pups). These scores were then averaged to obtain a "mean dam location" for each animal at each observation period.

Maternal Activity Score. Maternal activity at each of the 46 observation points was coded as 0–4. These scores represented the number of "mothering" activities the dam was engaged in at that moment. Mothering activities included nursing pups, cleaning pups, carrying pups or sleeping with pups. These scores were then averaged to obtain a "maternal activity score" for each dam at each observation period.

Percent Time Nursing. Dam activity at each of the 46 observation points was coded as 0 or 100, depending on whether she was not nursing or was nursing, respectively. These scores were averaged to obtain the "percent time nursing" variable.

Mean Number of Pups Nursing. Litter activity at each of the 46 observation points was coded as 0–8, representing the number of pups observed as potentially nursing. These scores were averaged to obtain the variable "mean number of pups nursing."

Mean Number of Pups Actively Nursing. This variable is similar to "mean number of pups nursing," except that pups needed to display active, voluntary limb or body movement in addition to being potentially nursing to be included in the score.

Suckling Intensity. This variable was constructed to examine the combined effects of the average number of pups the dam nursed and the amount of time the dam nursed, and was calculated as (number of min during the 45-min observation period that the dam was seen nursing at least one pup) $\times$ (mean number of pups nursing in that same 45-min observation period).

Data analyses were performed using SAS (SAS/STAT Version 6, SAS Institute, Cary, NC). All variables were examined for normal distribution before further analysis, and all were acceptable except for prolactin, which was transformed to its natural logarithm. For main effects, a probability value of $P < 0.05$ was considered significant, and $P < 0.10$ was considered potentially important.

To develop working statistical models for each hormonal variable of interest, repeated measures analysis of variance was performed using the PROC MIXED procedure. Considered in each statistical model were the following main effects and their interactions: diet group, litter group and day of lactation.

To test the possible effects of behavior on endocrine status after controlling for other important variables, each behavior variable was then added individually to the models previously constructed to describe the hormone data. This was done for each sampling period separately, such that behaviors observed on day 9, day 14, and day 19 of lactation were used in the models describing hormone data collected on day 10, day 15, and day 20, respectively.

To explore more thoroughly the observed relationships among dietary treatment group, behavior variables and plasma hormone concentrations, we used further path analysis. When a behavior variable appeared to explain a significant portion of the variation of a hormone variable (as determined by ANOVA described previously), the association among diet group, behavior and plasma hormone concentrations was partitioned statistically into (i) the direct effect of the dietary treatment on the hormone; and (ii) the indirect effect of the dietary treatment on the hormone via changes that could be attributed to changes that dietary treatment had upon the behavior variable (Fig. 1).

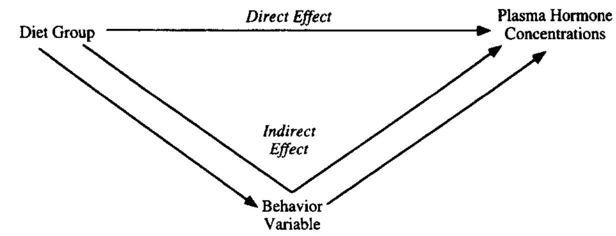

FIG. 1. Diagram of three-component theoretical model used in path analyses. The total effect of dietary treatment group on plasma hormone concentration was partitioned statistically between "direct" and "indirect."

TABLE 1

MEAN DAM AND LITTER WEIGHTS (G ± SEM) ON DAY 10, 15, AND 20 OF LACTATION

Variable and Group	Day of Lactation 10	15	20
Dam Weight			
R-70%			
5 pups	280 ± 4* (10)	280 ± 8* (8)	260 ± 11* (7)
8 pups	276 ± 5* (10)	272 ± 5* (4)	263 ± 4* (5)
R-85%			
5 pups	309 ± 9 (7)	332 ± 11 (3)	317 ± 10 (5)
8 pups	335 ± 7 (8)	332 ± 7 (8)	312 ± 20 (5)
Control			
5 pups	362 ± 13 (5)	327 ± 14 (4)	360 ± 13 (2)
8 pups	331 ± 7 (9)	325 ± 14 (7)	347 ± 15 (7)
Litter Weight			
R-70%			
5 pups	80 ± 6 (10)	124 ± 5 (8)	164 ± 5† (7)
8 pups	127 ± 6 (10)	177 ± 8 (4)	227 ± 9† (5)
R-85%			
5 pups	78 ± 9 (7)	106 ± 15 (3)	154 ± 14† (5)
8 pups	138 ± 9 (8)	203 ± 11 (8)	257 ± 27 (5)
Control			
5 pups	94 ± 6 (5)	135 ± 13 (14)	213 ± 3 (2)
8 pups	122 ± 9 (9)	177 ± 19 (7)	266 ± 24 (7)

Numbers in parentheses are cell sizes. Dams were offered food in ad lib amounts or were fed 70% or 85% of their expected ad lib intake (R-70% and R-85%, respectively). Litters were culled to 5 or 8 pups.

* Value differs ($P < 0.05$) from that of control animals nursing litters of the same size on the same day of lactation.

† Value differs ($P < 0.05$) from that of similar sized litters nursed by control dams on the same day of lactation.

RESULTS

Weights at Randomization

To determine if biases were introduced due to unbalanced assignment to treatment group and/or self-selection in the form of biased drop-out characteristics, analysis of variance was performed on weights at randomization (day 42 of age). Based on these analyses, our randomization scheme was adequate.

Dam and Litter Characteristics for Animals Contributing Blood Samples

Of the 33 animals assigned to the experimental control group, 13 were excluded from the study, leaving 20 animals from whom samples were obtained at one or more sampling periods. Of the 39 animals assigned to the R-85% group, 21 were excluded from the study, leaving 18 animals from whom samples were obtained. Of the 43 animals assigned to the R-70% group, 19 were excluded from the study, leaving 24 animals from whom samples were obtained. Dams were generally excluded from the study due to loss of catheter patency.

Data indicate that R-70% dams, regardless of litter size, weighed significantly less throughout lactation than did the control animals (Table 1). Dams nursing 5 pups in the R-85% group tended to weigh less than control dams nursing 5 pups, although this difference was not significant. Dams nursing 8 pups in the R-85% group were similar in size to control dams nursing 8 pups, except in late lactation when controls tended to be heavier.

There was no consistent effect of diet group on litter weight except in the 8-pup litters of the 85% group (Table 1).

Plasma Hormone Concentrations

Because this report will focus mainly upon the interactions among dietary intake, behavior and hormone concentrations, only a summary of the effects of diet and litter size on plasma hormone concentrations is reported here (Table 2). A more thorough description and discussion of the effects of dietary intake and litter size on plasma hormone concentrations has been published elsewhere (10). In summary, although dietary restriction at the more moderate level (85% of ad lib) did not significantly influence plasma LH and FSH concentrations, R-70% animals had lower circulating concentrations of both go-

TABLE 2
MEAN PLASMA CONCENTRATIONS (NG / ML ± SEM) OF LH, FSH, AND PROLACTIN (PRL) ON DAY 10, 15, AND 20 OF LACTATION

Variable and Group	Day of Lactation 10	15	20
LH* R-70†			
5 pups	2.4 ± 0.3 (10)	1.8 ± 0.2 (8)	3.4 ± 0.9 (6)
8 pups	2.3 ± 0.4 (10)	2.3 ± 0.8 (4)	2.3 ± 0.2 (5)
R-85%			
5 pups	2.5 ± 0.3 (7)	3.8 ± 0.4 (3)	6.1 ± 0.7 (5)
8 pups	2.2 ± 0.3 (8)	3.4 ± 0.5 (7)	4.1 ± 1.3 (5)
Control			
5 pups	2.8 ± 0.2 (5)	3.0 ± 0.5 (4)	5.8 ± 0.7 (2)
8 pups	1.9 ± 0.3 (9)	2.6 ± 0.4 (7)	5.5 ± 0.5 (7)
FSH‡ R-70%§			
5 pups	76.8 ± 13.6 (7)	92.5 ± 16.6 (8)	139.5 ± 45.2 (6)
8 pups	39.8 ± 6.8 (4)	42.9 ± 10.5 (3)	38.9 ± 7.4 (5)
R-85%			
5 pups	106.3 ± 16.7 (7)	151.9 ± 10.6 (3)	203.4 ± 61.8 (5)
8 pups	103.9 ± 9.0 (8)	137.5 ± 19.1 (7)	163.6 ± 46.9 (5)
Control			
5 pups	139.0 ± 5.5 (4)	104.7 ± 22.7 (3)	213.9 ± 71.1 (2)
8 pups	96.3 ± 17.2 (9)	123.0 ± 9.5 (6)	159.8 ± 15.9 (5)
Prl			
R-70%	157.7 ± 65.4 (16)	108.8 ± 48.0 (11)	106.2 ± 55.1 (12)
R-85%	59.5 ± 21.8 (15)	194.3 ± 102.3 (10)	50.7 ± 37.1 (7)
Control	99.5 ± 28.4 (14)	31.2 ± 8.1 (9)	35.6 ± 17.9 (9)

Dams were offered feed in ad lib amounts or were fed 70% or 85% of their expected ad lib intakes. Litters were culled to 5 or 8 pups. Numbers in parenthese are cell sizes.

* Significant overall effect of litter size on day 20 (5 pups > 8 pups; $P < 0.05$).

† When litter groups are combined, value on day 20 is significantly less than that of control group on day 20 ($P < 0.05$).

‡ Significant overall effect of litter size (5 pups > 8 pups; $P < 0.01$); overall mean on day 10 significantly less than that at day 20 ($P < 0.0001$); overall mean on day 15 significantly less than that at day 20 ($P < 0.05$).

§ Overall mean significantly less than that of control group ($P < 0.0001$).

nadotropins than did control animals, especially in later lactation. Furthermore, data indicated that dams nursing 5 pups had significantly higher plasma concentrations of both LH and FSH as compared to those nursing 8 pups. Although not statistically significant, food restriction also appeared to increase plasma concentration of prolactin, especially in later lactation. Because no effect of litter size was apparent, data were pooled for purposes of presentation.

Maternal and Litter Behaviors

In general, dams with 8-pup litters nursed significantly more than did those with 5-pup litters (Table 3), and regardless of litter size food-restricted dams nursed significantly more on day 19 than did control dams. More pups in 8-pup litters were observed nursing at each observation than were those in 5-pup litters, and at day 19 more pups of food-restricted dams were observed nursing than those of control dams (Table 4). Similarly, more pups were observed actively nursing in 8-pup litters as compared to 5-pup litters (Table 5), and regardless of litter size, number of pups actively nursing declined significantly from day 9 to day 19. There was no effect of diet on number of pups actively nursing. When food-restricted groups were compared to control groups nursing similar litters, only differences between R-85% and control dams nursing 8 pups were noted for suckling intensity score (R-85% < control at day 9; R-85% > control at day 19; $P < 0.05$, data not shown).

TABLE 3
MEAN PERCENT OF TIME DAMS WERE OBSERVED NURSING AT LEAST 1 PUP ON DAYS 9, 14 AND 19 OF LACTATION

Group	Day of Lactation 9	14	19
R-70%†			
5 pups	78.4 ± 9.2 (10)	71.4 ± 14.2 (7)	70.5 ± 16.2 (6)
8 pups	90.3 ± 4.7 (10)	95.0 ± 3.2 (4)	59.5 ± 10.3 (4)
R-85%			
5 pups	60.9 ± 16.9 (7)	42.3 ± 3.7 (3)	58.4 ± 13.1 (5)
8 pups	60.3 ± 13.0 (8)	76.8 ± 11.9 (6)	92.0 ± 2.9 (3)
Control			
5 pups	77.8 ± 10.2 5)	49.8 ± 16.6 (4)	21.5 ± 19.5 (2)
8 pups	82.9 ± 8.4 (9)	64.3 ± 16.7 (7)	44.0 ± 18.1 (5)

Values are ± SEM; figures in parentheses are cell numbers. Dams were fed either 70% or 85% of what control animals consumed or were allowed ad lib access to food. Litters were culled to 5 or 8 pups. Significant effect of litter size (5 pups < 8 pups; $P < 0.05$).

† When litter groups are combined, value at day 19 is significantly greater than that of control group ($P < 0.05$).

TABLE 4
MEAN NUMBER OF PUPS OBSERVED NURSING (± SEM; FIGURES IN PARENTHESES ARE CELL NUMBERS) AT EACH DISCRETE OBSERVATION ON DAYS 9, 14 AND 19 OF LACTATION

Group	Day of Lactation 9	14	19
R-70%†			
5 pups	3.4 ± 0.5 (10)	2.8 ± 0.8 (7)	3.0 ± 0.7 (6)
8 pups	6.0 ± 0.7 (10)	6.5 ± 0.2 (4)	3.5 ± 0.7 (4)
R-85%†			
5 pups	2.4 ± 0.7 (7)	1.3 ± 0.1 (3)	2.2 ± 0.4 (5)
8 pups	2.6 ± 0.7 (8)	4.6 ± 1.1 (6)	5.5 ± 0.2 (3)
Control			
5 pups	2.8 ± 0.6 (5)	1.8 ± 0.7 (4)	1.0 ± 1.0 (2)
8 pups	4.8 ± 0.9 (9)	3.8 ± 1.2 (7)	2.2 ± 1.0 (5)

Dams were fed either 70% or 85% of what control animals consumed or were allowed ad lib access to food. Litters were culled to 5 or 8 pups. Significant effect of litter size (5 pups < 8 pups; $P < 0.0001$).

† When litter groups are combined, value at day 19 is significantly greater than that of control group at day 19 ($P < 0.05$).

TABLE 5

MEAN NUMBER OF PUPS OBSERVED ACTIVELY NURSING (± SEM; FIGURES IN PARENTHESES ARE CELL NUMBERS) AT EACH DISCRETE OBSERVATION DAYS 9, 14 AND 19 OF LACTATION

Group	Day of Lactation		
	9	14	19
5 pups	0.51 ± 0.09 (22)	0.23 ± 0.04 (14)	0.29 ± 0.06 (13)
8 pups	0.60 ± 0.08 (27)	0.64 ± 0.11 (17)	0.40 ± 0.09 (12)

Dams were fed either 70% or 85% of what control animals consumed or were allowed ad lib access to food. Litters were culled to 5 or 8 pups. Significant effect of litter size (5 pups < 8 pups; $P < 0.005$).

† When litter groups are combined, value at day 9 is significantly greater than that at day 19 ($P < 0.05$).

TABLE 7

MEAN DAM LOCATION SCORE (± SEM; FIGURES IN PARENTHESES ARE CELL NUMBERS) ON DAYS 9, 14 AND 19 OF LACTATION

Group	Day of Lactation		
	9	14	19
R-70%	0.12 ± 0.04 (20)	0.11 ± 0.05* (11)	0.13 ± 0.05† (10)
R-85%	0.33 ± 0.10 (15)	0.33 ± 0.09 (9)	0.17 ± 0.06 (8)
Control	0.14 ± 0.06 (14)	0.37 ± 0.11 (11)	0.36 ± 0.13 (7)

Dams were fed either 70% or 85% of what uncatheterized, lead control animals consumed or were allowed ad lib access to food. This score represents the average proximity of the dam to her pups. This score has the limits of 0–1; the lower the number, the more time she was observed situated close to her pups.

* Value is significantly different than that of control animals on same day of lactation ($P < 0.05$).

† Value is marginally different than that of control animals on same day of lactation ($P < 0.10$).

For maternal activity score, dams nursing larger litters tended to exhibit more maternal activities than those nursing smaller litters (Table 6), and R-70% dams were engaged in significantly more maternal activities compared to control dams. Dam location scores indicated that, in early lactation, R-85% dams were observed spending more time away from their pups than were control dams (Table 7); in mid and late lactation, R-70% dams remained closer to their litter than did control dams. There was no effect of litter size on dam location score. Nest condition scores suggested that litters containing 5 pups were tidier than those containing 8 pups (Table 8), and for both litter groups nest tidiness decreased between day 9 and day 19. There was no effect of diet on nest condition score.

Relationships Between Behaviors and Endocrine Status

For plasma LH concentration, mean number of pups nursing was important in the model at day 10 and day 15 (Table 9). Mean number of pups actively nursing and suckling intensity score were significant in the model at day 15. For plasma FSH concentration, none of the variables considered was significant. At day 10 and day 15, mean nest condition was significant in the model describing plasma prolactin, and important in this model at day 20.

Because most differences and significant associations among hormones and behaviors occurred between the R-70% animals and controls at day 15 of lactation, further analyses were performed comparing these groups at this time point. Only the behavior variables previously found important in the models explaining variation in plasma hormone concentrations were investigated further. Exploration using the path analysis technique suggested that indirect effects of dietary treatment on percent time nursing, dam location score and number of pups nursing each explained approximately 25% of the total effect of dietary treatment group on plasma LH concentration (Fig. 2). All additive combinations of these variables did not explain a greater proportion of this indirect effect. Number of pups actively nursing and suckling intensity did not appear to be important mediators of the effect of dietary treatment group on plasma LH concentration (< 5% of effect could be attributed to each of these variables).

Approximately 30% of the differences in plasma prolactin concentration due to dietary treatment group could be attributed

TABLE 6

MEAN MATERNAL ACTIVITY SCORE* (± SEM; FIGURES IN PARENTHESES ARE CELL NUMBERS)

Group	Litter Size	
	5 pups	8 pups
R-70%†	1.27 ± 0.14 (23)	1.47 ± 0.11 (18)
R-85%	0.95 ± 0.16 (15)	1.22 ± 0.15 (17)
Control	1.02 ± 0.14 (11)	1.19 ± 0.15 (21)

Dams were fed either 70% or 85% of what control animals consumed or were allowed ad lib access to food. Litters were culled to 5 or 8 pups. Marginal effect of litter size (5 pups < 8 pups; $P < 0.10$).

* This score represents the mean number of mothering activities the dam was engaged in at at each individual observation. Mothering activities included nursing pups, cleaning pups, carrying pups or sleeping with pups. This score has the limits of 0–4; the lower the number, the fewer maternal activities the dam was observed doing.

† When litter groups are combined, value is significantly greater than that of control group ($P < 0.05$).

TABLE 8

MEAN NEST CONDITION SCORE* (± SEM; FIGURES IN PARENTHESES ARE CELL NUMBERS) ON DAYS 9, 14 AND 19 OF LACTATION

Group	Day of Lactation		
	9†	14†	19
5 pups	0.05 ± 0.03 (21)	0.07 ± 0.05 (14)	0.27 ± 0.15 (13)
8 pups	0.28 ± 0.12 (27)	0.24 ± 0.11 (17)	0.63 ± 0.12 (12)

Litters were culled to 5 or 8 pups. Significant effect of litter size (5 pups < 8 pups; $P < 0.01$).

* This score reflects whether the pups were observed together or scattered immediately before and after the 45 min observation period. The limits for this score are 0–2, where the lower the score, the tidier the nest (i.e., pups all together).

† When litter groups are combined, value is significantly less than that at day 19 ($P < 0.05$).

TABLE 9
PROBABILITY VALUES FOR THE SIGNIFICANT EFFECTS OF NURSING BEHAVIORS ON PLASMA CONCENTRATIONS OF LH, FSH AND PROLACTIN

Behavior (independent variable)	Plasma Hormone (dependent variable) LH	FSH	Prolactin
Mean nest condition	–	–	0.0300 (day 10) 0.0426 (day 15) 0.1089 (day 20)
Mean dam location	0.0984 (day 10)	–	–
Mean dam activity	–	–	–
% time nursing	0.0688 (day 15)	–	–
Mean # pups nursing	0.0630 (day 10) 0.0544 (day 15)	–	–
Mean # pups actively nursing	0.0121 (day 15)	–	–
Suckling intensity	0.0119 (day 15)	–	–

Probability values are followed by the time at which they were found to be important. Models were run separately for each independent variable at each time point.

to differences in nest condition score caused by dietary treatment group (Fig. 2).

DISCUSSION

Our data clearly indicate that dietary treatment group influences both the amount of time the dam spends with her litter and the suckling "intensity" she experiences. Although suckling intensity remains a vague term referring to a combination of suckling duration, number of pups nursing and the many physical and neural attributes that describe the nature of the nursing bout, we use this term to describe generally the overall amount of nursing stimulus experienced by the dam. Our data show that, at least in late lactation, food-restricted dams are suckled by more pups than are dams fed ad lib. Food-restricted dams also spend more time with their pups and engage in a greater number of maternal activities than do control dams. This finding is in agreement with other reports of the effects of maternal malnutrition on behaviors during lactation (2,3,6,9,16,17).

Similarly, these data confirm the hypothesis that dams allowed to nurse more pups indeed experience greater suckling intensity as compared to those nursing fewer pups. Dams with 8-pup litters nurse a greater number of pups than do those with 5-pup litters, and more pups in the larger litters are observed actively suckling than are those in the smaller litters. Dams with larger litters nurse more frequently, and engage in a greater number of caregiving, or mothering, activities than do dams with smaller litters.

Interestingly, we found several significant relationships between the behaviors observed and hormone concentrations measured within an individual animal, especially in mid-lactation (d 15), when both behaviors and hormones begin to be differentially expressed between dietary treatment groups. The more exploratory, post hoc path analyses of the observed relationships present among behavior and endocrine variables yielded interesting results. These analyses indicated that diet-induced changes in nursing and "mothering" behaviors explain approximately 25% of the total effects of dietary treatment on plasma LH concentration in the more food-restricted group. In contrast, no behavior variable proved important in predicting FSH, suggesting differential responses of these two gonadotropins to the suckling stimulus. Although this was not predicted, it does help to explain the differential response of the two gonadotropins to lactation, such that LH has been shown to be much more depressed during this period than has been FSH (as reviewed in reference 11).

Finally, although we did not find the expected relationships among nursing behaviors and plasma prolactin concentrations, we did find consistent evidence of an interaction among dietary treatment group, nest condition score and plasma prolactin concentrations. However, one can rearrange the path of action such that nest condition score becomes dependent upon the direct effect of dietary treatment plus the indirect effect of dietary treatment on plasma prolactin concentration (which then directly influences nest condition). If one then does path analysis using this theoretical model, it can be demonstrated that approximately 60% of the variation in nest condition score can be attributed to indirect effects of dietary treatment group upon plasma prolactin concentration, suggesting that this theoretical path may be more physiologically relevant. Although the directionality of the causal relationship between prolactin and maternal behavior is not completely understood, evidence suggests that higher circulating prolactin does not directly result in more intensive mothering behaviors (as reviewed in reference 15). Conversely, it is well docu-

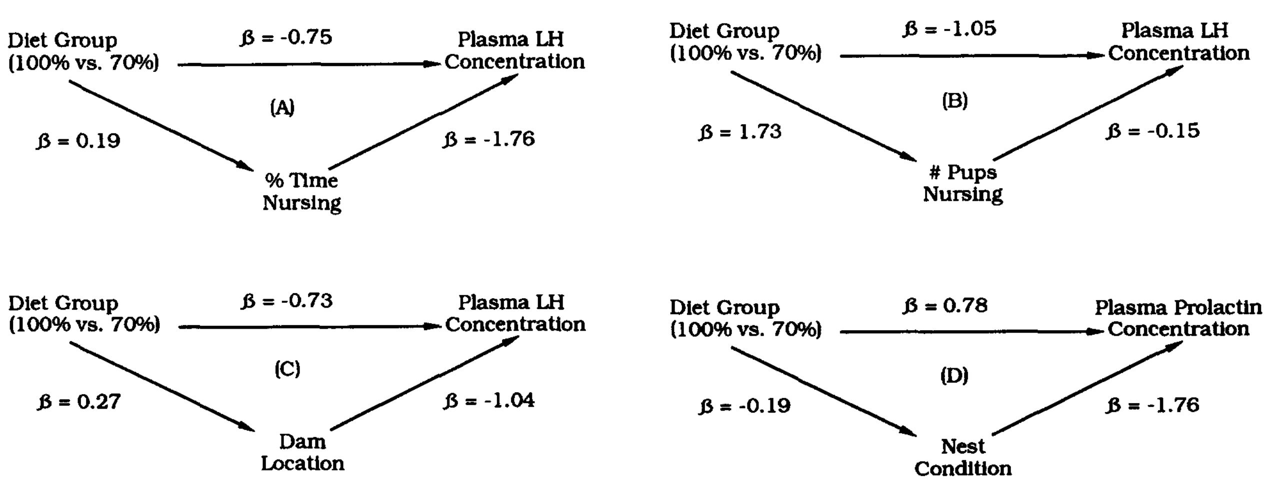

FIG. 1. Diagram of beta coefficients obtained via path analysis to explore direct and indirect effects of dietary treatment (control vs. R- 70%) and behavior variables (A: percent time nursing; B: # pups nursing; C: dam location score; D: nest condition score) in determining plasma LH (A-C) and prolactin (D) concentrations on day 15 of lactation.

mented that suckling and nonsuckling-related, exteroceptive stimuli can result in prolactin release from the pituitary gland of the lactating rat, especially in mid-lactation (4,5,12). Further work is required to determine the nature of these relationships in this animal model.

Although we were able to demonstrate several strong relationships among diet, behavior and endocrine status, there are several limitations to these findings. Observations were made during the night before the morning that blood samples were obtained. It was decided to observe animals during the night, because we expected that this nocturnal species would be more active at this time. Blood samples were taken during the morning, because this is the conventional period of the day to examine plasma gonadotropin and prolactin concentrations. Inaccuracies due to this approach may explain why we found very little relationship between suckling behaviors and plasma prolactin concentration. In future research, it would be preferable to take blood samples during the dark phase immediately following the period of behavioral observations.

Taken together, this experimental evidence strongly supports the widely held theory that nutritional status may partially influence the duration of postpartum infecundity by influencing suckling behaviors. Anecdotal evidence suggests that poorly nourished women living in nonindustrialized societies practice more frequent and prolonged breastfeeding and experience longer periods of lactational amenorrhea than do better-nourished women living in more developed societies, who have often adopted more regimented schedules for nursing their young. However, prolonged lactational amenorrhea has been documented in a group of well-nourished women living in the United States who breastfed on demand for extended periods of time (1). Indeed, nursing characteristics have long been known to be important in determining the duration of lactational infecundity in well-nourished women (7). Moreover, in more controlled animal studies researchers have provided compelling evidence that the simple physical proximity of the cow to her calf, without any suckling, can prolong the period of postpartum anestrus (13,20).

Together these data and observations suggest that, although maternal nutritional status probably does have an important direct effect upon the return of ovulation during lactation, one must never disregard the possible mediating and sometimes over-riding effects of suckling behaviors or even the mere proximity of the mother to her young.

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REFERENCES

1. Cable, T. A.; Rothenberger, L. A. Breast-feeding behavior patterns among La Leche League Mothers: A discriptive survey. Pediatrics 73:830–835;1984.
2. Crnic, L. S. Models of infantile malnutrition in rats: Effects on maternal behavior. Dev. Psychobiol. 13:615–628;1980.
3. Fleischer, S. F.; Turkewitz, G. Alterations of maternal behaviors of female rats caring for malnourished pups. Dev. Psychobiol. 14:383–388;1981.
4. Grosvenor, C. E. Evidence that exteroceptive stimuli can release prolactin from the pituitary gland of the lactating rat. Endocrinology 76:340–342;1965.
5. Grosvenor, C. E.; Maiweg, H.; Mena, F. A study of factors involved in the development of the exteroceptive release of prolactin in the lactating rat. Horm. Behav. 1:111–120;1970.
6. Hennessy, M. B.; Laughlin, N. K.; Weiner, S. G.; Levine, S. In: Maternal influences and early behavior. Spectrum Publications, Inc.; 1980:211–234.
7. Kliewer, R. L.; Rasmussen, K. M. Malnutrition during the reproductive cycle: Effects on galactopoietic hormones and lactational performance in the rat. Am. J. Clin. Nutr. 46:926–935;1987.
8. Maeda, K.-I.; Uchida, E.; Tsukamura, H.; Ohkura, N.; Ohkura, S.; Yokoyama, A. Prolactin does not mediate the suppressive effect of suckling stimulus on luteinizing hormone secretion in ovariectomized lactating rats. Endocrinol. Japon 37:405–411;1990.
9. Massaro, T. F.; Levitsky, D. A.; Barnes, R. H. Protein malnutrition in the rat: Its effects on maternal behavior and pup development. Dev. Psychobiol. 7:551–561;1974.
10. McGuire, M. K. Control of postpartum fecundity by natural food intake and suckling stimulus in the laboratory rat. Ph.D. Dissertation. Ithica, NY, Cornell University; 1994.
11. McNeilly, A. S.; Glasier, A.; Howie, P. W. Endocrine control of lactational infertility. I. In: Dobbing, J., ed. Maternal nutrition and lactational infertility. New York; Vevey/Raven Press; 1985:1–24.
12. Mena, F.; Grosvenor, C. E. Release of prolactin in rats by exteroceptive stimulation: Sensory stimuli involved. Horm. Behav. 2:107–116;1971.
13. Mukasa–Mugerwa, E.; Tegegne, A.; Franceschini, R. Influence of suckling and continuous cow-calf association on the resumption of postpartum ovarian function in Bos indicus cow monitored by plasma progesterone profiles. Reprod. Nutr. Dev. 31:241–247;1991.
14. Pachón, H.; McGuire, M. K.; Rasmussen, K. M. Nutritional status and behavior during lactation. Physiol. Behav. 58:393–400; 1995.
15. Rosenblatt, J. S.; Siegel, H. I. Maternal behavior in the laboratory rat. In: Maternal influences and early behavior. Spectrum Publications, Inc.; 1980:155–199.
16. Smart, J. L. Maternal behaviour of undernourished mother rats toward well fed and underfed young. Physiol. Behav. 16:146–149;1976.
17. Smart, J. L.; Preece, J. Maternal behaviour of undernourished mother rats. Physiol. Behav. 21:613–619;1973.
18. Smith, M. S.; Neill, J. D. Inhibition of gonadotropin secretion during lactation in the rat: Relative contribution of suckling and ovarian steroids. Biol. Reprod. 17:255–261;1977.
19. Taya, K.; Greenwald, G. S. Mechanisms of suppression of ovarian follicular development during lactation in the rat. Biol. Reprod. 27:1090–1101;1982.
20. Viker, S. D.; McGuire, W. J.; Wright, J. M.; Beeman, K. B.; Kiracofe, L. M. Cow-calf association delays postpartum ovulation in mastectomized cows. Theriogenology 32:467–474;1989.