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Protein Intake in the Rat Across Various Phases of the Lactation Period

Leslie Renée Cohen

A Thesis

in

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of

Psychology

Presented in Partial Fulfillment of the Requirements
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ABSTRACT

Protein Intake in the Rat
Across Various Phases of the Lactation Period

Leslie Renée Cohen, Ph.D.
Concordia University, 1993

Rats have the ability to regulate food intake and macronutrient intake in response to changes in reproductive state; during lactation both hyperphagia and a selective increase in protein intake is observed. In a series of experiments, factors affecting protein intake in the lactating rat were investigated, including placentophagia, suckling stimulation, milk delivery, and the effects of the light/dark cycle. In the first experiment placentophagia was shown not to alter protein preference which normally decreases immediately postpartum. In the second and third experiments, the relative importance of litter size and milk delivery on protein selection were compared in the first two weeks of lactation. It was found that when litter size increased, protein intake also increased. When suckling stimulation was present but milk delivery was prevented, protein intake still increased across lactation, and by week two exceeded levels of nonsuckled rats. In the fourth experiment protein intake across the light/dark cycle was investigated in lactating dams, and it was found that protein intake is greater during the dark than during the light phase of the cycle and greater in the second than in the first week of lactation. These studies serve as a foundation for the future investigation of the effects of hormones, such as prolactin, and neurotransmitters, such

as serotonin, on protein choice during lactation. The role of these neurochemical mechanisms as well as the possible role of sensory factors such as taste, odor, and texture cues are discussed.

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I want to thank Barbara Woodside, not only for her part in this research and her help and guidance in the preparation of this thesis, but more importantly, for her understanding and patience. Completing this dissertation through times of personal hardship and full-time employment have made the process a slow one, and I appreciate having been given the opportunity to take my time.

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I also extend my gratitude to my family: to my parents, Shirley and Avron Cohen, and my brother, Alan, and sister, Robin. Thanks Mom and Dad for all of your help, financial and otherwise (Marley thanks you too).

Dedications

I would like to dedicate this thesis to three very special people whom I have had the great honor of knowing. They have each taught me so much about life and what is important in it, and have shared with me their knowledge and wisdom of experience.

...to Dr. Louis Schwartz, whose fascination with all things scientific and with nature has many times inspired me to learn more.

...to Miss Nadine Haslam, whose love of teaching and love for other people have helped me to be a better teacher, and I hope, a better person.

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...and posthumously, to Professor Morris Shames, who gave me encouragement and support during the preparation of this thesis, and advice regarding career planning. I will remember that one cannot aggravate a person, but one can aggravate a condition, and that one cannot dress warmly, but can dress in warm clothing. I will miss you.

Table of Contents

List of Figures

Page

List of Tables

The number of behavioral and physiological mechanisms involved in maintaining the internal milieu of mammals is remarkable. It is not surprising, therefore, that there is still much to learn about these processes. Even in a well-studied species such as the laboratory rat the control of food intake and the regulation of energy balance are still not well understood (cf Friedman, 1990; Stellar, 1990).

Under normal circumstances, rats do not eat constantly; rather they eat in bouts or meals, and maintain a fairly consistent caloric intake from one day to the next. If the food presented ad libitum contains a higher or lower caloric density than the usual diet, rats modify their food intake to compensate for the change (see Swiergel & Cabanac, 1989), perhaps employing a long-delay learning mechanism as described by Rozin and Kalat (1971). Neither the stimuli controlling this behavior or the variable being controlled are known (for a review see Friedman, 1990). A number of possibilities have been suggested. For example, the existence of a body-weight set-point has been discussed at length (cf Wade, 1976). In the short-term, glucose levels can serve as a signal for feeding, and over longer periods of time there is evidence that fat stores may be monitored (Friedman, 1990).

This system is not infallible; if a low calorie diet is presented during a restricted feeding schedule (i.e., two hours per day) then rats do not increase their food intake so as to match the caloric intake of controls (Swiergel & Cabanac, 1989). Swiergel and Cabanac (1989) argue that this indicates that caloric intake under these conditions is affected by the palatability of the diets and not

by mechanisms involving metabolic signals. Low calorie/diluted diets appear to be less palatable than higher calorie diets. Nevertheless, under usual feeding conditions (i.e., 24 h food availability), the rat is capable of selecting the amounts of food necessary to maintain an appropriate level of daily caloric intake (e.g., Collier, Leshner, & Squibb, 1969).

Although it is interesting that the rat can regulate caloric intake, what is even more impressive is that the rat may also be able to regulate both macro- and micronutrient intake. Indeed, it has long been documented that the rat has the ability to select a diet beneficial to its well-being (for a review see Overman, 1976). Richter's (Richter & Barelare, 1938) cafeteria selection procedures demonstrated that, even when each dietary mineral, vitamin, and macronutrient was presented in a separate container, rats were able to choose amounts of each foodstuff that would be comparable to what is found in a standard laboratory chow and would result in normal growth and health. Moreover, Richter and Barelare (1938) reported that rats can alter their pattern of food choice to meet the demands of a change in organismic state, such as a change in reproductive state. For example, some dietary elements, such as protein, were selected in larger quantities during pregnancy and lactation than before impregnation.

The changes in ingestive behavior that Richter observed are possibly best reviewed in the context of the other behavioral and physiological changes that accompany reproduction in female rats. Rats have a 22 or 23 day gestation period. In the first two weeks of pregnancy, the demands placed on the dam are not great; but, in the

last week the foetuses begin to grow at a rapid rate (Stotsenburg, 1915). Energy demands are increased even further in the lactation period (Brody, Riggs, Kaufman, & Herring, 1938): the dam faces high metabolic demands as she produces milk to nourish her young. She also expends energy retrieving, grooming, and warming her pups (Wiesner & Sheard, 1933). Pregnancy is a time where the dam is relatively inactive, with the exception of nest building behaviors which are exhibited just prior to parturition. Lactation, on the other hand, is a state where the dam is very active. It is not surprising, therefore, that lactation is a period of hyperphagia. Food intake during lactation has been reported to be as high as 300 to 450% of that seen in females prior to impregnation (Cohen & Woodside, 1989; Fleming, 1976a; Munday & Williamson, 1983; Rosso, 1987).

Pregnancy and lactation also differ in terms of hormonal status (for a review see Rosenblatt & Siegel, 1981). Progesterone is the dominant hormone during pregnancy until about Day 20 when there is a dramatic decrease in progesterone levels (Pepe & Rothchild, 1974). At this time, estradiol, which has been quite low throughout pregnancy, rises sharply (Shaik, 1971). The rise in estradiol precedes a very sharp increase in prolactin on Day 22 and an even more dramatic increase on the day of parturition (Morishige, Pepe, & Rothchild, 1973) when estradiol is also high.

In contrast, pre-partum rise in estradiol is not maintained during lactation. Serum estradiol is extremely low throughout most of lactation, rising only very late in the reproductive period, around Days 15 through 20 postpartum (Smith & Neill, 1977; Taya &

Greenwald, 1982). Prolactin levels are, on the other hand, maintained and further increased until about Day 5 postpartum, after which they drop slightly, but are kept at a fairly high level until sometime between Days 15 and 20 of lactation (Amenomori, Chen, & Meites, 1970). Prolactin is necessary for milk production and is therefore vital to the lactating dam. Progesterone fluctuates throughout lactation peaking somewhere at the end of the first week postpartum, dropping sharply after that and then rising again around Day 15 (Grota & Eik-Nes, 1967; Smith & Neill, 1977; Woodside, et al., 1981).

In sum, given that pregnancy and lactation are accompanied by changes in hormonal and metabolic state of the dam, as well as the energetic costs of producing and feeding young, it is not surprising that Richter and Barelare (1938) reported that diet selection varies from pre-pregnancy, through pregnancy and lactation period. Almost 60 years later, we are still trying to understand which dietary components are essential to these episodes, and what mechanisms allow the dam to self-select a beneficial diet. Richter's findings that rats in a cafeteria selection paradigm could select a diet compatible with successful reproduction were difficult to replicate (e.g., Tribe, 1955). This problem was likely due to changes in the way some of the dietary ingredients were manufactured over the years, and possibly as well, to palatability problems associated with some of the foodstuffs. Although others, such as Chafetz, Byrne, and King (1989) have had success with the cafeteria selection procedure (i.e., rats were able to self-select an adequate diet), the self-selection method that has been more widely

used over the last twenty years is that of the two-choice test (e.g., Cohen & Woodside, 1989; Leshner, Siegel, & Collier, 1972; Wurtman & Baum, 1981).

Two-choice tests involve presenting the rat with two isocaloric diets, each of which contain equal and optimum amounts of minerals and vitamins; but, in which the percentage of particular macronutrients (i.e., protein, carbohydrate, or fat) are allowed to vary. For example, one diet may be high in protein, whereas the other diet may be high in carbohydrate. As with Richter's work, it has been shown that if changes are made to the rat's physiological state, for example, changing body temperature (Leshner, Collier, & Squibb, 1971), activity levels (Collier et al., 1969), or reproductive status (Cohen & Woodside, 1989; Leshner et al., 1972), rats adjust their intake from the two diets.

Cohen & Woodside (1989) have demonstrated that under normal circumstances the rat may be regulating caloric intake and if the rat is pregnant or lactating it also regulates the intake of specific nutrients, such as protein. Given the choice between two isocaloric diets that differ in the percentage of protein contained in them (i.e., 5% versus 45%), protein intake is increased in the last week of pregnancy compared with both pre-impregnation levels and the intake of nonimpregnated rats (Cohen & Woodside, 1989). Given these diets, on the day before parturition, and on the day of parturition, however, both food and protein intake decrease significantly. On the first day after parturition, food intake levels increase and are similar to those recorded on the third (Day 20) and second to last (Day 21) days pre-partum (see Figure 1a). There is,

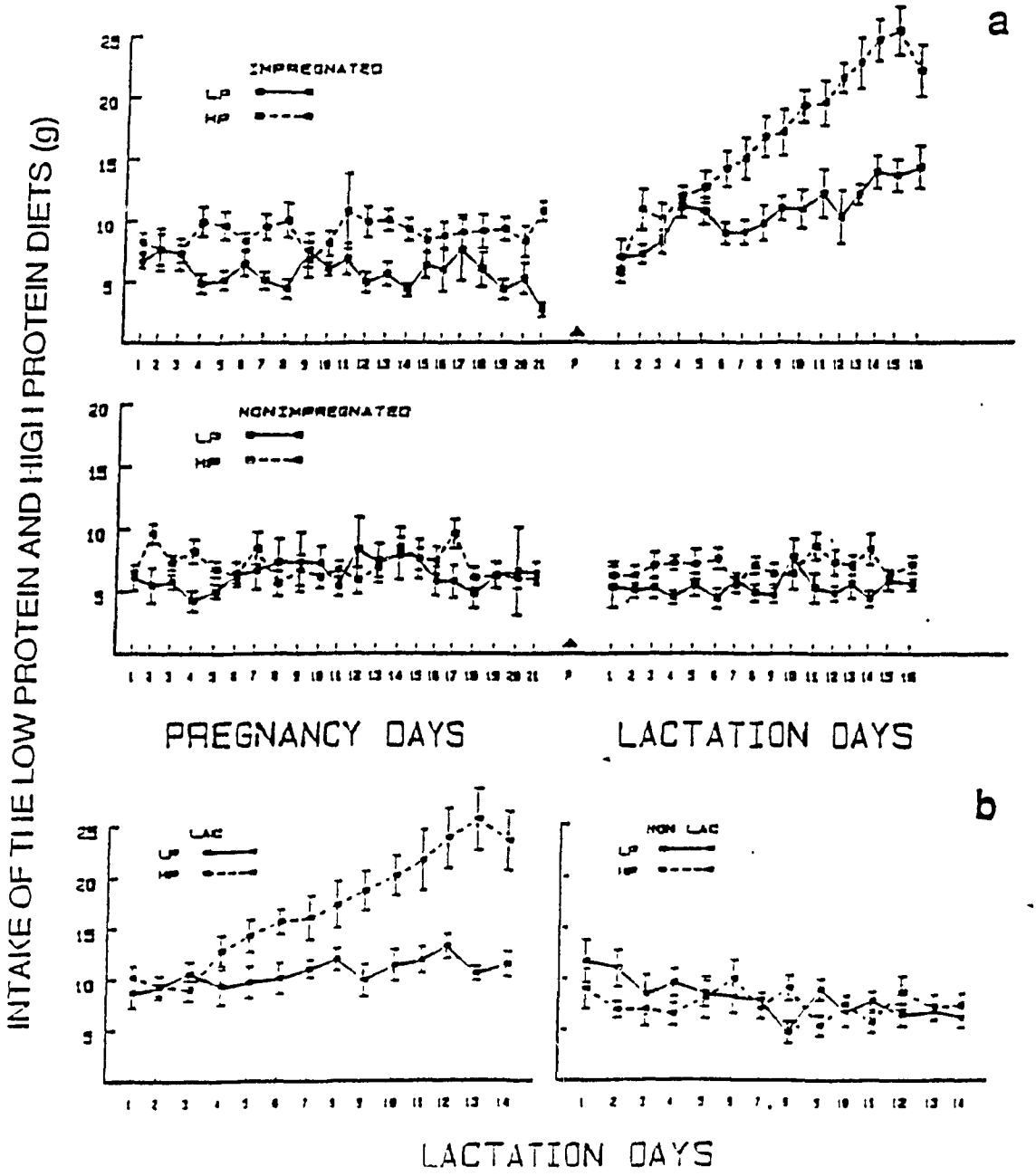

Figure 1a. Intake of low (LP) and high (HP) protein diets in impregnated and nonimpregnated rats during pregnancy and lactation. "P" = parturition. *SEMs* are shown as vertical bars (Cohen & Woodside, 1989).

Figure 1b. Intake of low (LP) and high (HP) protein diets in lactating (LAC) and nonlacting (NON LAC) rats during lactation. *SEMs* are high shown as vertical bars (Cohen & Woodside, 1989).

however, no preference for protein diet the day after parturition; if allowed to self-select between the low and high protein diets, comparable amounts are selected from both diets. From Day 2 or Day 3 of lactation to the end of the second week, there is a return to the pre-partum preference for high protein diet and intake of this diet continues to rise sharply until at least Day 16 of the lactation period. Moreover, a greater amount of protein is consumed in the second week of lactation than in the first week of lactation and than during pregnancy. It should be noted that with these particular diets, this effect has been found consistently regardless of whether the rats were presented with the diets from before impregnation (see Figure 1a) or starting on Day 1 of lactation (see Figure 1b).

The amount of protein selected during pregnancy or lactation, however, depends on the specific diets presented and whether or not a two-choice test or cafeteria selection procedure is employed (Cohen & Woodside, 1989; Leshner, et al.,1972; Richter & Barelare, 1938). In two-choice tests when the type of protein available (e.g., casein versus soybean oil meal) is varied from one diet pair to the next, differences in relative protein intake have been reported (Cohen & Woodside, 1989). It has been suggested that either palatability differences and/or differences in the way each type of protein is utilized might account for this effect. Regardless of the type of protein or dietary selection procedure employed, protein intake is higher during pregnancy and lactation than at other times of the reproductive cycle, and the level of intake observed by the end of lactation exceeds levels exhibited at the end of pregnancy.

These data data give rise to a number of questions. As is the

case for caloric regulation, neither the stimuli controlling this pattern of self-selection nor the physiological mechanisms underlying this ability are known.

The studies described in this thesis investigated the role of a variety of stimuli in the increase in protein selection seen during lactation. Recall that lactation itself cannot be seen as a unitary state; rather, it is characterized by change: 1) there are changes in hormonal status across the weeks of lactation as well as during the end of pregnancy and during parturition; 2) there are behavioral changes in the pattern of maternal behavior (e.g., nest building, caring for pups, etc.) across this reproductive episode; 3) energy requirements change as reflected in the amount of milk produced; and 4) there are diurnal rhythms within each day of the lactation period, and across the weeks; these rhythms coincide with changes in maternal behavior such as nursing, which may also affect diet choice.

Given the pattern of protein selection shown in Figure 1a from the Cohen and Woodside (1989) study, and the dynamic nature of the lactational state, three phases during which protein selection was shown to change or might be expected to change were the focus of this thesis: 1) pre- to post-parturition, 2) between Week 1 and Week 2 of lactation, and 3) the light half and dark half of each day within the two week lactation period. In each of these phases, there may be different mechanisms affecting protein self-selection and contributing to the pattern of protein selection observed across the lactation period.

In Chapter One, one facet of the pattern of protein selection

was examined, namely, the decrease in preference for high protein diet observed from the last week of pregnancy to the early postpartum period. It was hypothesized that placentophagia might play a role in affecting protein choice; therefore, the presence or absence of placentophagia was manipulated.

In Chapter Two, the focus was on a second feature of the pattern of intake shown in Figure 1, that is, the increasing intake of the high protein diet across the first two weeks of lactation. This increase correlates both with increases in milk production and changes in the hormonal profile of the dam. The two experiments described were designed to investigate the relative importance of suckling stimulation and milk delivery in affecting protein self-selection. In the first experiment, suckling stimulation and milk delivery were manipulated by varying litter size. In the second experiment of this chapter, milk delivery was prevented and thus the contribution of suckling stimulation and its hormonal sequelae to this pattern could be assessed.

In Chapter Three, a finer analysis of this pattern of ingestive behavior was undertaken by investigating how it changed across the phase of the light/dark cycle. In this final experiment, meal patterns were also examined on a single day of each of the first two weeks (i.e., Day 4 and Day 10) and across the light/dark cycle.

In sum, this thesis serves to explore protein self-selection in the lactating rat from the first two days postpartum across Weeks 1 and 2 of lactation, and across the light/dark cycle within the first two weeks of lactation. The possible roles of placentophagia, reproductive status, milk delivery, and diurnal rhythms are

discussed.

CHAPTER ONE

Experiment 1

As can be seen in Figure 1a, the intake of high protein diet is relatively high during the last week of pregnancy and from about Days 3-16 of lactation. In contrast, on the day following parturition, high protein intake is relatively low and is very similar to the level of low protein intake on this day (Cohen & Woodside, 1989). Total food intake on the day following parturition is very similar to that observed on the third and second to last days before parturition. Clearly, the rat is not decreasing food or caloric intake, but appears to be selectively reducing protein intake. The experiment described below was designed to investigate this shift in diet preference.

One possible explanation for the change in protein selection is that during parturition, rats have access to an alternative source of protein: the placentae. Although the placentae are not high in caloric value, they appear to be rich in protein (Klopper, 1983; Kristal, 1980). It has, therefore, been suggested (Cohen, 1984; Cohen & Woodside, 1989) that placentophagia on the day of parturition might account for the drop in high protein diet intake observed on the first postpartum day.

Let us first review what occurs at the time of parturition. Before parturition, the laboratory rat builds a nest and clears a space in the cage to be relatively free of bedding, where the dam delivers her young (Rosenblatt & Lehrman, 1963). The dam might deliver only a few pups or as many as twenty-five or more pups, and

each pup is delivered one at a time. There is a break between delivery of each pup, and this allows the dam time to clean the pup to remove the film of foetal membranes surrounding it, detach the umbilical cord, and the placenta attached to each pup. The pup is then able to breathe and the dam immediately eats the placenta. The placenta is disk shaped (Kristal, 1980) and weighs between .5 and .9 g (approximate *M* =.70 g) (personal observations).

The dam is very possessive regarding the placenta (personal observations; Kristal, 1980); the dam keeps a tight hold on the placenta and opposes any attempt to remove the placenta from the cage. It appears that eating the placentae is not just a component of maternal care; but, the consumption of a highly desired substance. Indeed, Kristal and his associates (e.g., Abbott et al., 1991; Doerr & Kristal, 1991; Kristal, 1980) have presented evidence of opioid-enhancing effects following ingestion of placentae and amniotic fluid. Presumably, this would serve to help the dam to cope with parturition, and could serve to explain why it is such a highly desired substance.

The effect of placentophagia on hormonal status of the lactating rat has been investigated (Blank & Friesen, 1980). Lactating dams who were allowed to eat the placentae were compared to dams prevented from placentophagia. On Day 1 postpartum, rats not allowed to eat the placentae had lower serum prolactin levels than dams allowed to eat the placentae. In contrast, on Days 6 and 8 postpartum, serum progesterone levels were higher for those rats prevented from eating the placentae as compared to the rats allowed to eat placentae at parturition. Blank and Friesen

(1980) have suggested that as progesterone levels inhibit the return to estrous cycling in the female rat (i.e., the ability to become impregnated) placentophagia may serve to lower progesterone levels to enable an earlier return to cyclicity.

Unfortunately these groups were different in that the former group was not disrupted during the delivery process; whereas, the latter group was interrupted so that the placentae may be removed. There is, therefore, a confounding variable which might account for observed differences between these groups in terms of serum prolactin and progesterone levels. It seems necessary to replicate these findings in a more well controlled study; however, it may be that placentophagia has a variety of functions.

The experiment described below addresses the possibility that placentophagia reduces the intake of protein in the early postpartum period. Food intake and choice between a low protein and a high protein diet were assessed in three groups of dams: 1) dams that were prevented from eating placentae at parturition, 2) dams that were allowed to eat placentae but that were disrupted during the delivery of their pups, and 3) dams that were allowed to deliver their young undisturbed. If the protein content of the placentae is influential in producing the reduction in high protein diet choice immediately postpartum, it would be expected that the placentae-removed dams would show no drop in high protein intake at this time. Alternatively, if placentophagia has an effect because of its influence on hormonal status (Blank & Friesen, 1980), which, in turn, might affect food and/or protein intake (Gerardo-Gettens, Moore, Stern, & Horowitz, 1989a; Gerardo-

Gettens, Moore, Stern, & Horowitz, 1989b; Noel & Woodside, 1991; in press), it would be expected that rats prevented from eating the placentae would eat less food and protein than dams not prevented from placentophagia.

Method

Subjects

Nineteen female Wistar rats, originally obtained from Charles River Breeding Farms, St. Constant, Quebec, and who had been through one previous reproductive episode in this laboratory served as subjects in this experiment. Multiparous rats were selected as the procedure entailed disrupting delivery and it was believed that this would be less stressful to experienced dams than it would be to primiparous dams. The lights were on at 08:00 h and off at 20:00 h. Ambient temperature was maintained at 20°C.

Diets

Low protein and high protein semi-purified diets (Cohen & Woodside, 1989) were used (see Table 1). The diets were prepared in the laboratory from ingredients obtained from ICN Nutritional Biochemicals, Cleveland, Ohio, with the exception of the fat source (CRISCO®, Procter and Gamble, Inc., Toronto, Ontario). The diets were prepared each week and stored in airtight plastic containers in a refrigerator. The low protein diet contained 5% protein (casein) and 33% fat; the high protein diet contained 45% protein and 15% fat. The diets were isocarbohydrate at 40% (dextrin) and isocaloric at 4.32kcal/g.

Apparatus

The diets were presented ad lib in glass jars (4.5 cm in diameter and 7 cm high) that were glued with epoxy to aluminum sheets (1 mm x 8 cm x 16.9 cm) bent in a S-shape. The top lip of the aluminum sheet curved over to fasten onto the side wall of the cage under the cage lid.

Table 1. Contents of the low protein and high protein diets.

	Low Protein	High Protein
Protein (Casein-Purified High Nitrogen)	5%	45%
Carbohydrate (Dextrin)	40%	40%
Fat (CRISCO®)	33%	15%
Nonutritive Bulk (Alphacel)	22%	---
Plus the following per 1 kg of diet mixture		
Vitamins (Vitamin diet fortification mixture)	22g	22g
Minerals (Rogers-Harper mineral mixture)	40g	40g
Gelling agent (Agar)	35g	35g
Water	1000ml	1000ml
Calories	4.32kcal/g	4.32kcal/g

Procedure

The rats were mated in the laboratory. On the morning when spermatazoa were seen in vaginal smears, impregnation was assumed to have occurred and this day was counted as Day 1 post impregnation. During the first two weeks of pregnancy the rats were housed in wire hanging cages with stock chow (Charles River lab chow) provided ad lib. So that the rats could become familiar with the test diets before parturition, on Day 14 post impregnation the rats were transferred to polypropylene cages (38 x 33 x 17 cm) with Beta Chip bedding and were presented with the low and high protein diets. Fresh food was added to the jars daily, and the food jars were emptied and washed with hot water every other day. The bedding was searched for any spilled diet, and if found, this was returned to the appropriate food jar. The food jars were placed on opposite sides of the cage, and in order to control for position preferences, the jars were rotated daily.

On the Day of parturition, the dams were assigned to one of three groups: 1) placentae-removed (n = 6); as the pups were delivered they were removed from the cage, the placentae detached, and just the pups returned to the cage, 2) placentae-disturbed (n = 6); as the pups were delivered they were removed from the cage, the placentae detached, and the pups and the placentae returned to the dam, and 3) undisturbed (n= 7); the dam was allowed to deliver her pups undisturbed by the experimenter.

Group Assignment. On the expected date of parturition, the experimenter watched the dam and as the rat delivered her pups, the experimenter attempted to remove the placentae as described above.

As each pup is delivered, the dam very quickly eats the placenta and is immediately protective of her young; therefore, making it very difficult to remove all placentae from the cage. Consequently, dams were allowed to eat two placentae and still be assigned to the placentae-removed group. If the dam ingested more than two placentae, the procedure of removing pups and placentae was continued; but, all retrieved placentae were later returned to the dam who was then assigned to the placentae-disturbed group. Thus, the extent of the disruption at parturition was comparable for both placentae-removed and placentae-disturbed groups. Undisturbed dams were those who delivered their pups when the experimenter was not present or who delivered once a sufficient number of subjects had already been assigned to placentae-removed and placentae-disturbed groups. Because of the above described constraints, it was not possible to match groups in terms of body weight or any other measure; nevertheless, a one-way Analysis of Variance (ANOVA) indicated that at the start of the data collection period (i.e., Day 14 post impregnation) there was no significant difference (F (2,16) = 1.549, $p > .05$) in dam weight across groups (Placentae removed: M = 354.68 g ± 5.36 g; Placentae disturbed: M = 365.67 g ± 12.44 g; Undisturbed: M = 381.39 g ± 12.16 g) (see Appendix A1).

Measures. Food intake (i.e., total amount of diet eaten), dam weight, and where applicable, litter weight were recorded from Day 14 post impregnation up to, and including, the day of parturition (Day 0 of lactation), and from Days 1 to 16 of the lactation period. As water accounted for 50% of the weight of the diets, food intake

data were divided in half prior to analysis (Cohen & Woodside, 1989).

The type of ANOVA employed for each measure is indicated below in the results section. In Chapters One, Two, and Three of this thesis, ANOVAs were calculated using microcomputer programmes: ANOVA II for Apple, and CLR ANOVA for Macintosh. In Chapter Three some of the ANOVAs were calculated using BMDP 2V. When post hoc comparisons were made, Tukey's honestly significant difference (*HSD*) was calculated (Hays, 1981).

Results

Litter size and pup weight at parturition

A one-way ANOVA (see Appendix A2) showed that there were no differences across Groups in the number of pups delivered (F (2,16) < 1.00), and the average number of pups across groups was 12.21 ± .78. A similar analysis of pup weight on the day of parturition (see Appendix A3) showed no significant differences across Groups (F (2,16) = 1.682, p >.05) with an overall average pup weight of 6.03 g ± .14 g.

Feeding Behavior

Although food intake data were collected from Day 14 post impregnation through Day 16 postpartum, the primary period of interest in this experiment is the first day postpartum; therefore, data analyses are presented for this day and for Day 2 postpartum as a comparison. In addition, where a pre-partum comparison period was necessary, the third to the last pre-partum day was employed. Food intake has previously been shown to be lower on the day of parturition than during the last week of pregnancy (see Cohen & Woodside, 1989). Usually, food intake levels start to decline the day before parturition, and are still relatively high the third day prior to parturition. The data collected in this experiment are consistent with such reports. The amounts of low protein and high protein diet selected by each group from five days prior to parturition and including the day of parturition are listed in Appendix A4. There was no difference in the amount of diet selected between the fifth and the third to last days prior to parturition (Days F (2,32) = 1.630, p >.05; see Appendix A5);

therefore, the third day prior to parturition was deemed to be an appropriate pre-partum baseline day, and was used in comparison with postpartum intake values.

The only measure where the entire 16-day postpartum period was analyzed is the intake of the low protein and high protein diets.

Percentage Protein Intake. Percentage protein intake was calculated by dividing total grams of protein selected by the total amount of food eaten and multiplying by 100 (i.e., ((low protein x .05) + (high protein x .45)/total food intake) x 100)

Pre-partum. Percentage protein intake for the three days preceding the day of parturition was calculated for each animal within each group, and these data were analyzed using a two-way Split-Plot ANOVA (Between: Groups(3); Within: Days(3); see Appendix A6). Prior to the manipulation at parturition all three groups were eating similar percentages of protein (Groups: F (2,16) = .584, p > .05). There was no significant Days effect (F (2,32) = 2.139, p >.05) or Groups x Days interaction (F (4,32) = 1.395, p > .05). The average percentage protein intake across all groups was 31.30%.

Postpartum. Percentage protein intake on Days 1 and 2 of lactation was analyzed across groups using a two-way Split-Plot ANOVA (Between: Groups(3); Within: Days(2); see Appendix A7). There was no significant difference across groups in percentage protein intake (F (2,16) = 1.221, p > .05). Only the Days effect was significant (F (1,16) = 4.595, p < .05) with a lower percentage protein intake exhibited on Day 2 (24.99%) as compared to Day 1 (28.63%). The Groups x Days interaction was not significant (F (2, 16) = 1.404, p > .05).

Intake of the Low Protein and High Protein Diets.

Pre-Partum. Intake of the two diets was assessed for the three days prior to parturition and analyzed using a three-way Split-Plot ANOVA (Between: Groups(3), Within: Diets (2), Days(3); see Appendix A8). In contrast to the results for percentage protein intake, there was a significant Groups effect (F (2,16) = 5.506, p < .05; *HSD* (3,16) = 2.45) which reflects differences in total food intake across groups (note: percentage protein intake can be consistent across groups regardless of differences in total food intake; see Appendix A9). The undisturbed group ate more food than the placentae-removed and placentae-disturbed groups.

All groups ate more high protein diet than low protein diet (Diets: F (1,16) = 21.285, p <.001; Groups x Diets: F (2,16) = 1.249, p >.05). Overall, food intake decreased from three days prior to parturition to the day before parturition (Days: F (2,32) = 17.728; p <.001; *HSD* (3,32) = 1.23). The Diets x Days interaction was significant (F (2,32) = 5.487, p <.001; *HSD* (6,32) = 2.28) with the decrease in low protein intake from the third to the second and first days prior to parturition not significant; whereas, high protein intake decreased significantly on the day prior to parturition as compared with the third and second days before parturition which did not differ significantly from each other. On each day high protein intake was always significantly greater than low protein intake. The Groups x Days (F (4,32) = 1.073, p >.05) and Groups x Diets x Days (F (2,32) = .921, p >.05) interactions were not significant.

Postpartum. Intake of the low protein - high protein

diets for the first two days postpartum was analyzed across groups using a three-way Split-Plot ANOVA (Between: Groups(3); Within: Diets (2), Days(2); see Appendix A10). There was no difference in the pattern of low protein to high protein intake seen across groups (Groups: $F(2,16) = 1.356$, $p > .05$) or intake of the diets (Diets: $F(1,16) < 1.00$). Only the Days effect was found to be significant (Days: $F(1,16) = 23.140$, $p < .001$) with all groups eating more food on Day 2 than on Day 1. No significant interactions were found (Groups x Diets: $F(2,16) = 1.372$, $p > .05$; Groups x Days: $F(2,16) = 1.594$, $p > .05$; Groups x Days x Diets: $F(2,16) = 2.618$, $p > .05$).

Difference Scores for Low Protein - High Protein Diet Intake.

Early Postpartum. In order to account for the differences in diet choice groups prior to parturition, postpartum intake of the diets was also assessed using difference scores that were calculated separately for the intake of low protein and high protein diet between Day 3 pre-partum and each of the first two days postpartum (i.e., Day 1 postpartum - Day 3 pre-partum, etc.). Day 3 pre-partum was selected as the reference point to assess change because total food intake and intake of the low protein and high protein diets, declines immediately before and on the day of parturition; Day 3 pre-partum rats show relatively high and stable levels of diet choice. (Intake of the low protein and high protein diets for the five days preceding parturition are presented in Appendix A4; similar amounts of the diets were selected, within each group, from Day 5 to Day 3 pre-partum). The difference scores from Day 3 pre-partum to the postpartum days were then analyzed using a three-way Split-Plot ANOVA (Between: Groups(3); Within:

Diets (2), Days(2); see Appendix A11).

As was the case for intake of the diets reported above, there were no significant differences among Groups in diet intake difference scores (F (2,16) = 3.031, p > .05). Similarly, more low protein and high protein diet was eaten on the second day than on the first day postpartum, which was reflected in an increase in intake of the low protein and high protein diets from Day 3 pre-partum to Day 2 postpartum with the rats eating less on the first day of lactation than they had prior to parturition (F (1,16) = 5.856, p < .05). The significant main effect for Diets (F (1,16) = 23.144, p < .05) was the result of low protein intake being increased for Days 1 and 2 postpartum compared with the pre-partum baseline; whereas, high protein intake was shown to decrease. None of the interactions was significant (Groups x Diets: F (2,16) = 1.595, p > .05; Groups x Days: F (2,16) = 2.466, p > .05; Diets x Days F (1,16) = 2.916, p > .05; Groups x Diets x Days F (2, 16) = 2.618, p > .05).

16 days of lactation. Difference scores between the third day prior to parturition and each of the 16 days postpartum for the intake of low and high protein diets were calculated (see Figure 2), and analyzed using a Three-way Split-plot ANOVA (Between: Groups (3), Within: Diets(2) x Days (16); see Appendix A12). There was a significant main effect for Groups with the placentae-disturbed group displaying a greater change in intake of the diets from pre-parturition than either the placentae-removed or undisturbed groups (Groups: F (2,16) = 5.281, p < .05). Overall, there was a greater increase in high protein diet than in low protein diet

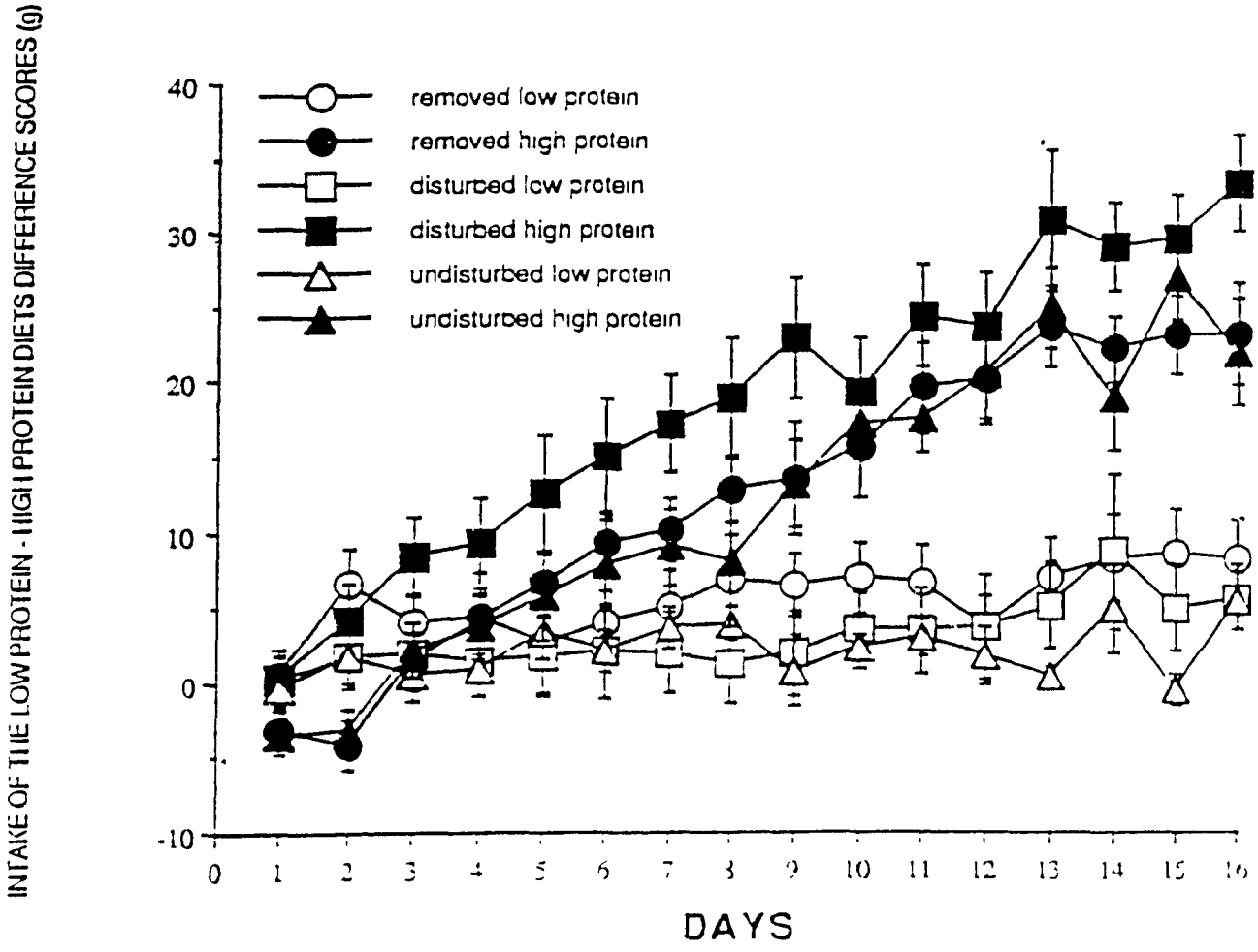

Figure 2. Difference scores of the intake of the low and high protein diets for the Placentae-removed (removed), Placentae-disturbed (disturbed), and Undisturbed groups during lactation *SEMs* are shown as vertical bars.

(Diets: F (1,16) = 279.201, p < .05 and although difference scores for the intake of both diets increased over the lactation period (Days: F (15,240) = 24.006, p < .05), there was a greater increase in high protein diet than in low protein diet (Diets x Days: F (15,240) = 21.080, p < .05). None of the other interactions was found to be significant (Groups x Days: F (30,240) = 1.134, p >.05; Groups x Diets: F (2,16) < 1.00; Groups x Diets x Days: F (30,240) = 1.179, p >.05).

Discussion

On the first two days postpartum, dams that at parturition were not allowed to eat the placentae of their pups did not display a pattern of low protein - high protein diet choice that was different from the pattern observed for dams allowed to eat placentae. In addition, the percentage of protein selected was similar across groups and was comparable to levels previously reported with these diets (Cohen & Woodside, 1989). It would appear, therefore, that protein ingested through placentophagia is not a factor affecting the decrease in high protein diet intake exhibited from the end of pregnancy to the period immediately postpartum. Similarly, if prolactin levels were different across placentae eaters and non-eaters at this time, as was reported by Blank and Friesen (1980), this did not noticeably affect food or protein intake. In addition, if as Blank and Friesen (1980) reported progesterone levels were different on Days 6 and 8 between dams allowed to eat and those not allowed to eat placentae, such differences were not reflected in differences in diet selection between placentae-removed and undisturbed groups on these days.

The fact that placentophagia does not affect protein intake is consistent with a previous report that it does not affect food intake levels in nonpregnant rats (Kristal, 1980). Ingestion of the afterbirth does however, strengthen the effect of opioid-mediated analgesia that occurs around the time of parturition (Kristal, 1980) and lasts for approximately 12 h. This is interesting, in that an opioid effect might be expected to result in an **increase** in food intake immediately postpartum. The increase in food intake

following opiate administration has been well documented. For example, Marks-Kaufman and Kanarek (1981) have reported that although there is an initial decrease in food and protein consumption following opiate (morphine) administration, by the end of six hours, intake levels have increased past that of vehicle-control animals. Similarly, Leibowitz (1987) has reported that administration of the opiate agonist morphine in the paraventricular nucleus leads to an increase in food intake and that this effect seems to be affected by the presence of corticosterone, for in the absence of this hormone, a suppression of the increase in food intake is observed.

At parturition corticosterone levels in the rat are relatively high, although not significantly different from levels exhibited during pregnancy or lactation, therefore, low glucocorticoid levels probably do not account for the suppression in food intake reported in this experiment. It is possible that other hormones interact with the opioid-mediated effect around parturition to lead to the observed suppression in food and protein choice in this early phase of the reproductive episode. Clearly, placentophagia, even though it involves some increase in protein intake, changes in hormone levels, and an enhanced analgesic effect, does not seem to be influencing food or protein intake.

Perhaps an explanation of the decrease in high protein diet immediately postpartum is best sought in the changing energetic demands on the dam or in her changing metabolism. Milk production is relatively lower early in lactation than later in the reproductive episode where the demands of the pups are greater; therefore, the dam's need for calories and protein may be lower in the first 2 days

of lactation than, for example, in the second week postpartum. Indeed, early in lactation, the dam spends 80% of her time with her pups (Woodside & Jans, 1988), which leaves little time available for feeding. The dam's metabolism is also changed from pregnancy to lactation. During pregnancy, fat stores are increased perhaps as much as 40-50%. These stores are not utilized during the pregnancy period; it appears that their purpose is to provide the dam with reserves to be used during the high cost lactation period. Even when the dam is well nourished during lactation, approximately 60% of total body fat will be lost by the end of the second week postpartum (Naismith, Richardson, & Pritchard, 1982). As the dam is capable of catabolizing her fat stores it may not be critical for her to eat a large amount of food in the early postpartum period; she would then be able to spend less time eating and more time with her young.

Protein is regulated quite differently during the reproductive episodes of pregnancy and lactation. Unlike fat, protein is not accumulated in the dam in pregnancy for catabolism during lactation; she is instead, able to use protein more efficiently. Naismith (Naismith, et al., 1982; Naismith & Robinson, 1987; Naismith & Walker, 1988) has reported that during lactation protein essential for milk synthesis is not, under normal circumstances, drawn from the dam's body stores. Instead, the rate of oxidation of amino acids is decreased, and in turn, milk synthesis is enhanced, Moreover, Naismith has suggested that prolactin is likely the hormone responsible for regulating the metabolism of protein during lactation.

From the end of pregnancy to parturition and then during

lactation, there are changes in hormonal status. This, in turn, may affect metabolism and maternal behavior. Suckling stimulation is the proximal stimulus that affects both hormonal status, and milk delivery. In the next chapter, the period of investigation is extended to cover the first two weeks of lactation, where the relative effects of suckling stimulation and milk delivery on the self-selection of protein during the postpartum period are assessed.

CHAPTER TWO

The extent of the increase in food intake during lactation is affected by the size of the litter being suckled. Ota and Yokoyama (1967) have demonstrated that dams nursing 12 or 8 pups ate more food than did dams nursing 4 or 2 pups. Similarly, nursing a larger litter also results in greater milk production (Fleming, 1976b; Knight, Maltz, & Docherty, 1986), and has been shown to affect hormonal status. Ford and Melampy (1973) demonstrated that dams that nurse litters of 12 pups exhibit higher levels of both pituitary and serum prolactin in the second week of lactation, than did dams nursing 3 or 6 pups. In sum, the size of the litter can affect both hormonal status and milk production, and either or both of these factors may play a role in affecting food intake and diet selection.

Cotes and Cross (1954) and Millelire and Woodside (1989) have reported that suckling stimulation **without** milk delivery is sufficient to increase food intake. In both of these studies a surgical procedure was used to prevent dams from delivering milk even though the litter still attached to the dam's nipples and provided suckling stimulation. These dams ate more food than did nonlactating controls, but less than amounts eaten by intact-lactating rats. These data suggest that the hormonal status of the lactating rat may contribute to the hyperphagia of lactation.

There is also evidence to suggest that the hormonal status of the lactating dam is sufficient to modulate her pattern of diet selection. Millelire & Woodside (1989) presented the results of two experiments in which they investigated the effects of size of

the litter and the effects of milk delivery, respectively, on calcium intake in lactating rats. It was found that dams nursing larger litters (i.e., 16 pups) consumed more calcium than did rats that nursed smaller litters (i.e., 4 pups). Further, suckled rats that were prevented from delivering milk, by a surgical procedure, selected more calcium than did nonlactating controls. It appears, therefore, that just as with food intake (Ota & Yokoyama, 1967), calcium intake increases with an increase in litter size, and that milk delivery is not essential to increase calcium preference in suckled dams.

The experiments presented below were designed, in parallel with the calcium studies of Millelire and Woodside (1989), to investigate the relative contributions of suckling stimulation and milk delivery to the selection of protein seen during lactation.

Experiment 2

The experiment described below was designed to examine the changes in protein intake from Week 1 to Week 2 of lactation, in dams nursing either relatively large (16 pups) or small (4 pups) litters. These weeks were selected for comparison because suckling stimulation, milk output, and hormone levels change from Week 1 to Week 2 of lactation (Millelire & Woodside, 1989). It was expected that dams nursing 16 pups would eat more food and grams of protein than did dams nursing 4 pups. How the intake of the low and high protein diets would be affected by litter size was difficult to predict. It was possible that the preference in both groups for high protein diet would be similar to that seen previously in dams nursing a litter of 8 pups (Cohen & Woodside, 1989). Alternatively, the number of pups suckled might modulate the preference so that dams nursing 16 pups would show a greater increase in intake of the high protein diet from Week 1 to Week 2 than would dams nursing 4 pups.

Method

Subjects

Seventeen female virgin Wistar rats, obtained from Charles River Breeding Farms, St. Constant, Quebec, served as subjects. Mating and housing procedures were as described in Experiment 1. Within 24 h of the day of parturition, rats were assigned to one of two groups: 1) litter sizes culled to 4 pups (n=9) or 2) litter sizes culled to 16 pups (n=8). The two groups were matched for weight of the dam on the first day postpartum (4-pup dams: 313.62 g ± 7.32 g; 16-pup dams 318.26 g ± 10.76 g; $t(15) = -.3564$, $p > .05$). There was no difference in pup weight between groups on Day 1 (see Results).

Diets and Apparatus

Diets and apparatus were as described in Experiment 1.

Procedure

Food intake was recorded daily for a period of 16 days. Food jars were filled, cleaned and rotated as described in Experiment 1. In order to account for the water content of the diets, food intake data were halved before being analyzed. Gram protein intake and percentage protein intake were analyzed as daily means expressed in weekly blocks (Week 1 = Days 1-8 postpartum; Week 2 = Days 9-16 postpartum) using a Split-Plot ANOVAs (Between: Litter size (2); Within: Weeks (2)). The intake of the low protein and high protein diets were calculated as daily means expressed in weekly blocks, and analyzed with a three-way Split-Plot ANOVA (Between: Litter Size (2); Within: Diets (2), Weeks (2)).

Dam weight was recorded daily for 16 days. Change in body weight was assessed by comparing the weight of the dam at the end

of the experiment to the weight recorded at the start of lactation and expressing this change in body weight as a percentage of the dam's Day 1 weight (i.e., ((Day 16 body weight - Day 1 body weight)/ Day 1 body weight) x 100). These data were compared using a t-test for independent groups.

Pup weight was recorded daily for 16 days, and at weaning (i.e., Day 25 postpartum) and post weaning (i.e., Day 40 postpartum) so that pup growth could be assessed for each group. Five days were selected for comparison: Days 1, 8, 16, 25, and 40 postpartum. Pup weight was calculated as an average weight for an individual pup within each group (i.e., mean individual pup weight) for these days and then analyzed using a two-way Split-Plot ANOVA (Between: Litter size (2); Within: Days (5)), and Tukey's (*HSD*) post hoc tests (Hays, 1981).

Results

Intake of the Low Protein and High Protein Diets

Dams nursing 16 pups ate a greater amount of food than did dams nursing 4 pups (Litter Size: $F(1,15) = 70.148$, $p < .001$). There was also a main effect of Diet: more high protein diet than low protein was eaten (Diets: $F(1,15) = 32.83$, $p < .001$; see Figure 3) Both groups significantly increased intake of the diets from the first to the second week of lactation, and in each week, a greater amount of food was eaten by the dams nursing 16 pups (Weeks: $F(1,15) = 140.667$, $p < .001$; Litter Size x Weeks : $F(1,15) = 16.146$, $p < .01$; *HSD* (4,15) = 2.06). When the entire lactation period is considered, dams with large litters ate more high protein diet than low protein diet, and there was no difference in low protein intake across the groups (Litter Size x Diets: $F(1,15) = 15.351$, $p < .01$; *HSD* (4,15) = 13.26). Both the 4-pup and 16-pup groups selectively increased intake of the high protein diet from the first to the second week of lactation; whereas, neither group displayed a significant increase in low protein intake at this time (Litter Size x Diets x Weeks: $F(1,15) = 8.073$, $p < .05$; *HSD* (8,15) = 5.95). (see Appendix B1).

Protein Intake (in grams)

As the intake of the low and high protein diets data would seem to indicate (see Figure 4), the 16-pup dams ate more protein (Litter Size: $F(1,15) = 80.984$, $p < .001$), with both groups displaying an increase in grams of protein eaten from the first to the second week postpartum (Weeks: $F(1,15) = 146.357$, $p < .001$). Dams nursing 16-pup litters exhibited a slightly larger increase in protein intake

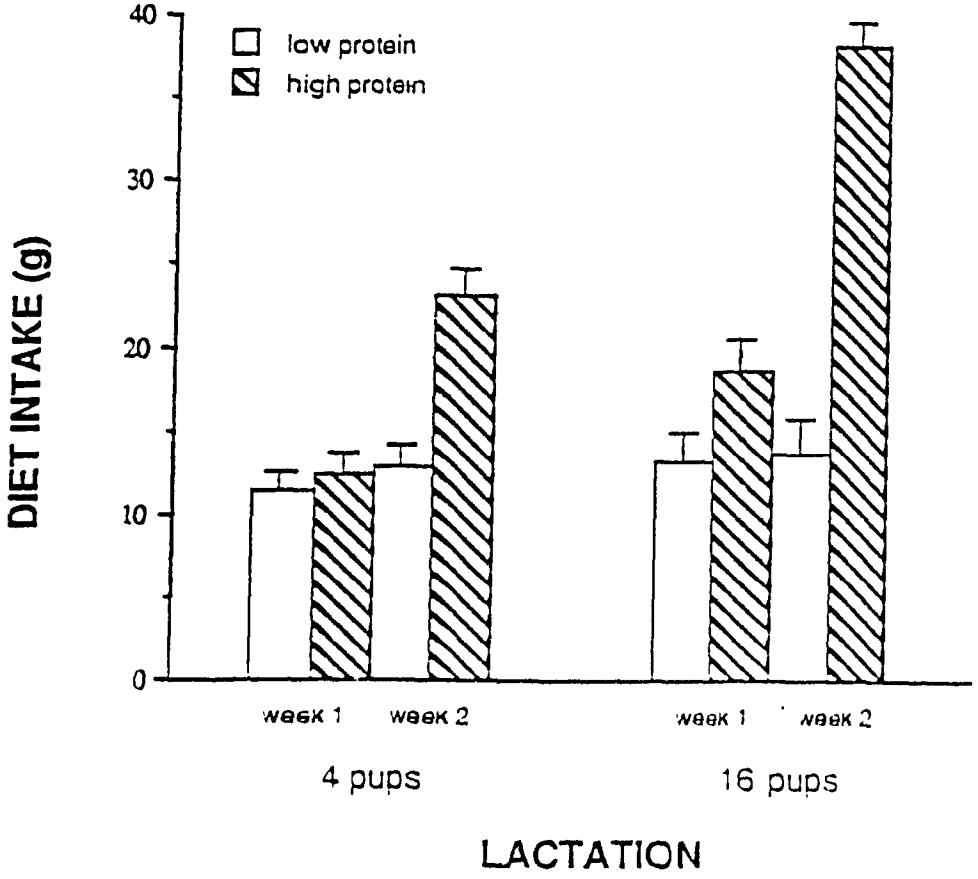

Figure 3. Mean daily amounts of low and high protein diet expressed in weekly blocks (*SEMs* are shown as vertical bars) for 4-pup and 16-pup dams during lactation.

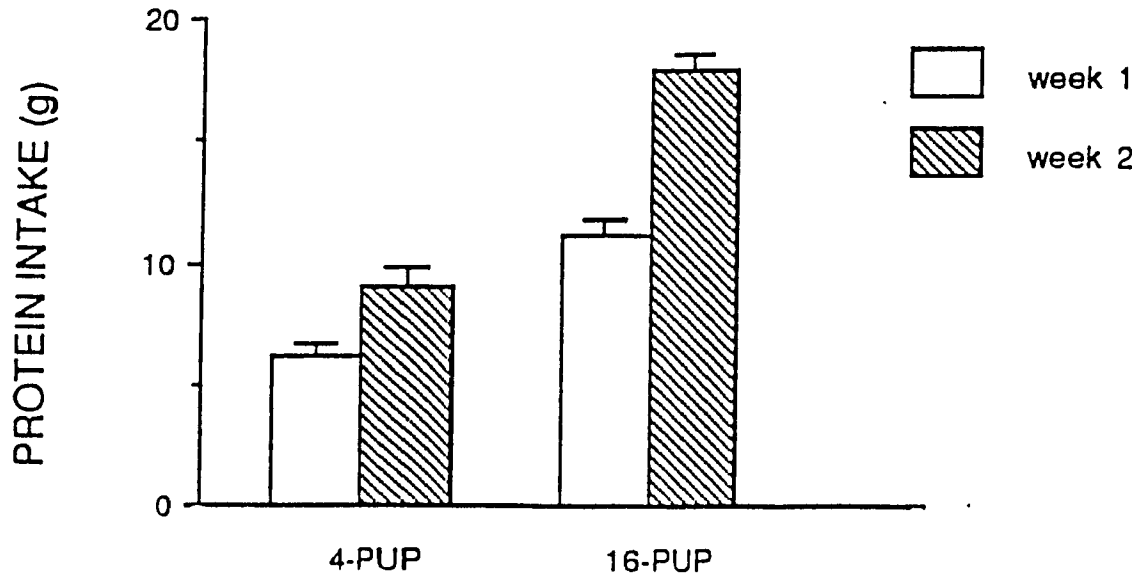

Figure 4. Mean daily amounts of protein (g) expressed in weekly blocks for 4-pup and 16-pup dams during lactation. *SEMs* are shown as vertical bars.

across weeks than did the 4-pup dams (Litter Size x Weeks F (1,15) = 24.04, p < .001; *HSD* (4,15) = 1.643; see Appendix B2)

Percentage Protein

The 16-pup rats showed a higher percentage protein (33 84%) intake than did the 4-pup dams (26.50%) (F (1,15) = 9 424, p <.05). There was no difference in percentage protein intake across the Weeks (F (1,15) <1.00) nor was there a significant Groups x Weeks interaction (F (1,15) < 1.00). (see Appendix B3)

Dam Weight

There was no significant difference in change in body weight over the lactation period between the 4-pup (M = 6 45% ±1 92%) and 16-pup dams (M = 9.00% ± 1.03%) (t (15) = -1 17, p > 05).

Pup Weight

Pups from litters of 4 weighed more than pups from litters of 16 (Litter Size: F (1,15) = 30.32, p <.001), and pup weight increased over days (Days: F (4,60) = 789.985, p <.001; *HSD* (5,60) = 6 86, see Table 2). On Days 1 and 8 postpartum, there was no difference between groups in the average weight per pup within each group (*HSD* (10,60) = 11.363); however, by Day 16, pups from 4-pup litters weighed significantly more than pups from 16-pup litters. This trend continued throughout, and even on Day 40, pups from the 16-pup litters had not caught up to the weights of the 4-pup litters (Litter Size x Days: F (4,60) = 14.987, p <.001; see Appendix B4).

Table 2. Mean weight per pup in 4-pup and 16-pup litters for Days 1, 8, 16, 25, and 40 postpartum.

		Days				
		1	8	16	25	40
Litter Size						
4	M	6.58	23.49	49.05	70.98	147.68
	SEM	.27	1.22	1.81	2.81	3.36
16	M	6.52	17.72	30.70	52.60	113.04
	SEM	.16	.75	1.72	2.55	7.60

Discussion

Dams who nursed 16 pups ate more protein and food than did dams who nursed only 4 pups, but both groups exhibited the characteristic increase in food and protein intake from the first to the second week of lactation. Moreover, the amounts of low protein diet relative to high protein diet selected by 16-pup dams was comparable to the pattern previously reported for dams who nursed 8 pups (Cohen & Woodside, 1989). Although both 4-pup and 16-pup groups increased high protein diet intake from the first to the second week of lactation, and in both groups the increase in food intake from the first to second week was largely accounted for by an increase in high protein diet (i.e., relative change in high protein diet intake), the 16-pup dams selected more high protein diet overall. In sum, the size of the litter did have an effect on the total amount of protein, as well as, the total food that were consumed. The results of this experiment, therefore, support and extend the findings of Ota and Yokoyama (1967) who reported that food intake increases with an increase in litter size.

Furthermore, the results obtained in the present experiment are consistent with those reported by Millelire and Woodside (1989) in litter size effects on calcium self-selection, where the increase in calcium intake from Week 1 to Week 2 of lactation was greater for 16-pup dams than for 4-pup dams.

Increasing litter size can have several effects, anyone of which, or any combination of which, might influence diet selection. For example, litter size has been reported to directly affect milk production (Knight, et al., 1986), and protein consumption is

expected to lead to an increase in milk production (Roberts & Coward, 1985). Therefore, dams nursing 16 pups probably produced more milk than did dams nursing 4 pups, as the former group nursed a larger litter **and** ate more protein. It is not clear, whether the protein might be selected in response to the stimulation of the increased litter size, or because the dam is in a protein deficit state with protein being lost through the milk delivered to the young. It is, therefore, important to separate the two factors of stimulation and milk delivery.

One way to determine whether suckling stimulation in the absence of milk delivery is sufficient to affect protein intake is to block milk delivery by cutting the galactophores while maintaining suckling from the pups. This then was the purpose of Experiment 3.

Experiment 3

In the previous experiment, it was demonstrated that increasing litter size does have an effect on protein intake in lactating females. It will be recalled that increasing litter size increases suckling stimulation and thereby, milk production and delivery, as well as affecting the hormonal status of the dam. The experiment described below was designed, therefore, to assess the effects of suckling stimulation and its expected hormonal consequences on protein intake during the lactation period.

In order to compare these factors it must be possible to maintain hormonal status through suckling stimulation, yet, prevent milk from being delivered. This can be accomplished by severing the galactophore, a tubule that aliows milk to pass through the nipple to the offspring. Cotes and Cross (1954) have demonstrated that dams whose galactophores have been cut 36 h after parturition, will still nurse pups, and because the nipple has not been affected by the surgical procedure, pups do not have difficulty attaching and providing the dam with suckling stimulation. The sensory input and neural signals are, therefore, the same for galactophore-cut and intact dams and as a result, their hormonal status should be similar. They would only differ in that the latter dams are able to deliver milk to their young. Cotes and Cross (1954) showed that galactophore-cut dams increase food intake during the "lactation" period, although to a lesser extent than do intact controls.

In the experiment described below, the protein selection of three groups of rats was compared. The galactophores of one group of rats had been severed prior to impregnation, but these rats gave

birth normally and nursed their pups postpartum. A second group consisted of sham-operated dams, and the third group were nonlactating rats. If suckling stimulation alone is sufficient to increase protein appetite, it would be expected that galactophore-cut dams, like lactating rats, would show a higher protein intake than nonlactating dams; but, that as has been previously demonstrated with total food intake (Cotes & Cross, 1954; Millelire & Woodside, 1989), the increase in protein intake in galactophore-cut dams would not be as great as that seen in dams actually delivering milk to their young.

Method

Subjects

Twenty-seven female virgin Wistar rats, obtained from Charles River Breeding Farms, St. Constant, Quebec, served as subjects in this study. The rats were assigned, in equal numbers, to one of three groups: galactophore-cut, sham-operated, and nonlactating control.

Surgical Procedures

Both galactophore-cut and sham-operated rats were administered methoxyflurane (Metofane, Janssen Pharmaceuticals, Toronto, Canada) anaesthetic and for each rat two mid-line incisions were made (Millelire & Woodside, 1989). For the former group, the galactophores were then severed and tied, and for the latter group the galactophores were inspected, but left intact; the mid-line incisions were then closed. The surgery was performed prior to impregnation as per Millelire and Woodside (1989), and not 36 h after parturition as Cotes and Cross (1954) had done, so as to avoid any interruption of the nursing process.

Two weeks after the surgical procedure, these rats were mated; their litters were culled to eight pups within 24 h of parturition. The remaining eight rats were left unmated and served as the nonlactating control group. All housing conditions were as previously described in Experiment 2.

Diets and Apparatus

The diets and apparatus were as described in Experiment 1.

Procedure

Food intake and dam weight were recorded daily for a period of

16 days postpartum. In addition, pup weight was recorded every 12 h at which time litters were switched among galactophore-cut, sham-operated and foster dam triads in order to maintain healthy young. This allowed the pups 24 h of milk, followed by 12 h with a dam who could not deliver milk. Litter weights recorded from the galactophore-cut females consistently yielded a decrease in litter weight over the 12 h period; at no time was a weight gain found. This was taken as an indication that no milk was being delivered from galactophore-cut dams. Furthermore, post-mortem inspection of the galactophore-cut dams showed that the mammary tissue of these rats was flat and yellowish in color in comparision with the swollen, pink mammary tissue of the nursing/milk delivering dams. It is likely that little or no milk was produced by the galactophore-cut dams.

Dam weight was assessed as described in Experiment 2, and analyzed using a one-way ANOVA. Intake of the low protein and high protein diets and gram protein intake were analyzed as daily means expressed in weekly blocks (Week 1 = Days 1-8; Week 2 = Days 9-16). Intake of the low protein and high protein diets was then assessed using a three-way Split-plot ANOVA (Between: Groups (3) ; Within: Diets (2), Weeks (2)). Gram protein intake and percentage protein intake were analyzed using a two-way Split Plot ANOVA (Between : Groups (3); Within: Weeks (2)). Where applicable, Tukey's (*HSD*) post hoc tests were employed.

Results

Dam weight

There was an increase in body weight change over time (see Appendix C1 for average dam weights per group on Day 1 and Day16). There was a significant Groups effect of percentage of body weight change over the lactation period (F (2,24) = 8.496, p <.001; *HSD* (3,24) = 6.94) which was due to a significant difference between the galactophore-cut (M = 19.14% ± 1.79%) and nonlactating (M = 7.73% ± 2.33%) groups (see Appendix C2). The difference in body weight change between the two groups of suckled dams was not significant (sham-operated: M = 12.57% ± 1.71%)

Intake of the low and high protein diets

There was a significant difference across groups in the amounts of low and high protein diets that were consumed, because the sham-operated group ate more total food (i.e., low protein plus high protein intake) than the galactophore-cut and nonlactating groups (F (2,24)= 45.312, p <.001; *HSD* (3,24) = 6.94; see Figure 5). Overall, more high protein diet than low protein diet was selected (F (1,24) = 23.879, p <.001) and there was a significant effect for Weeks (F (1,24) = 160.501, p <.001) with more food being consumed in the second than the first week of lactation. Both the galactophore-cut and sham-operated groups increased total food intake from the first to the second week of lactation; whereas, food intake across the weeks did not change in the nonlactating group (Groups x Weeks (F (2,24) =148.685, p <.001; *HSD* (6,24) = 1.08). The significant Groups x Diets x Weeks interaction (F (2,24) = 26.412, p <.001; *HSD* (12,24) = 3.95) reflects the difference between the two

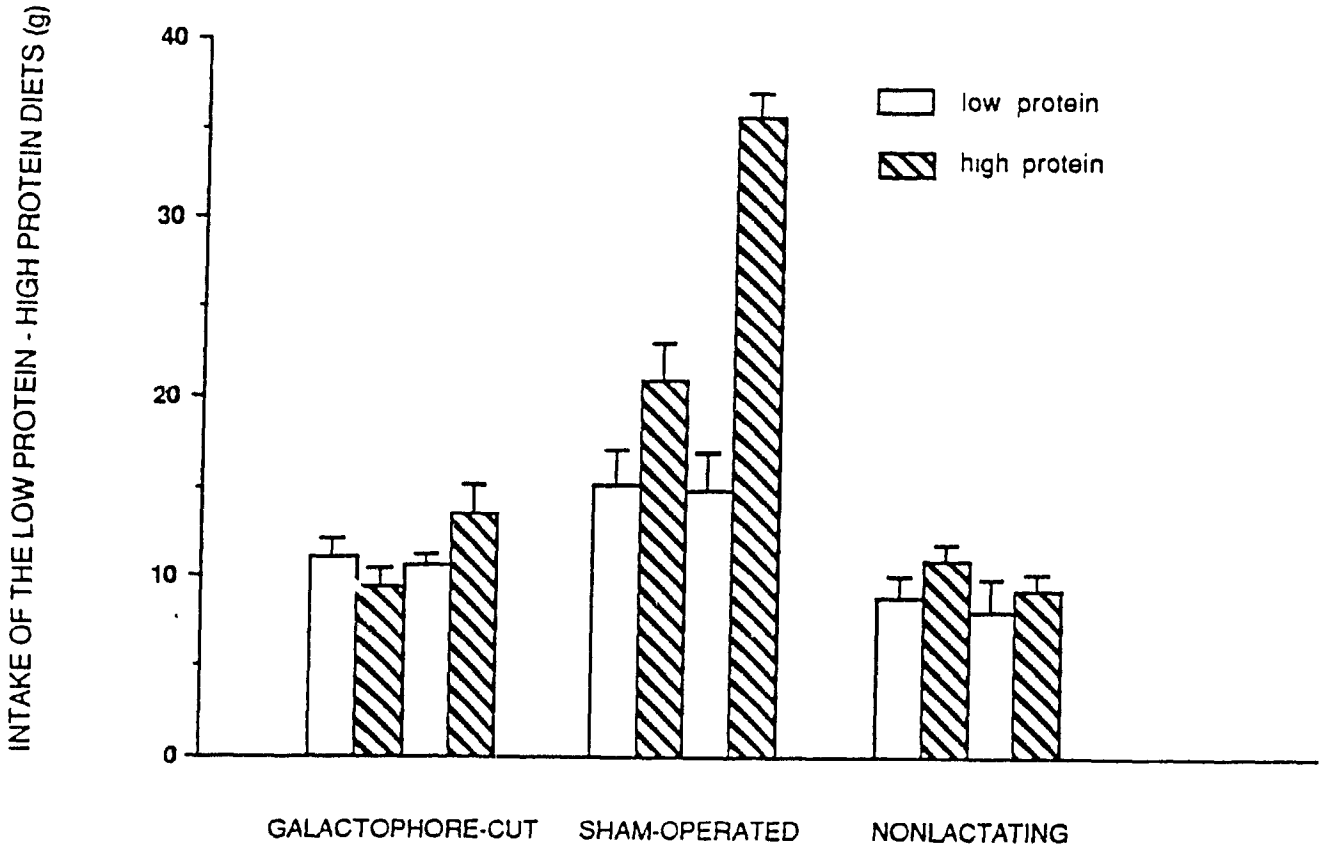

Figure 5. Intake of the low and high protein diets in galactophore-cut, sham-operated and nonlactating groups across Weeks 1 and 2 of lactation. *SEMs* are shown as vertical bars.

groups of suckled dams (i.e., galactophore-cut and sham-operated) and the nonlactating group; the galactophore-cut and sham-operated groups exhibited a significant increase in high protein diet from Week 1 to Week 2, without changing low protein intake. In contrast, the nonlactating group did not significantly change intake of either of the diets across the weeks. (see Appendix C3)

Protein Intake (in grams)

The results for protein intake are presented in Figure 6. In the first week of lactation, the galactophore-cut and nonlactating rats selected similar amounts of protein, and significantly less protein than the sham-operated dams. The galactophore-cut dams significantly increased their protein intake from Week 1 to Week 2 of lactation, as did the sham-operated dams; whereas, the nonlactating dams showed no significant change from the first to the second week of lactation, and ate significantly less protein in the second week than did the galactophore-cut rats (Groups x Weeks: $F(2,24) = 96.974$, $p < .001$; $HSD(6,24) = 1.17$; Weeks: $F(1,24) = 134.172$, $p < .001$). Overall, the sham-operated dams ate significantly more protein than did the galactophore-cut and nonlactating groups (Groups: $F(2,24) = 59.835$, $p < .001$; $HSD(3,24) = 3.04$; see Appendix C4)

Percentage Protein

There was a significant effect for Groups ($F(2,24) = 3.80$, $p < .05$; $HSD(3,24) = 5.57$) as the sham-operated ($M = 30.91\%$) and nonlactating ($M = 26.91\%$) groups consumed a greater percentage of protein than did the galactophore-cut ($M = 24.87\%$) group.

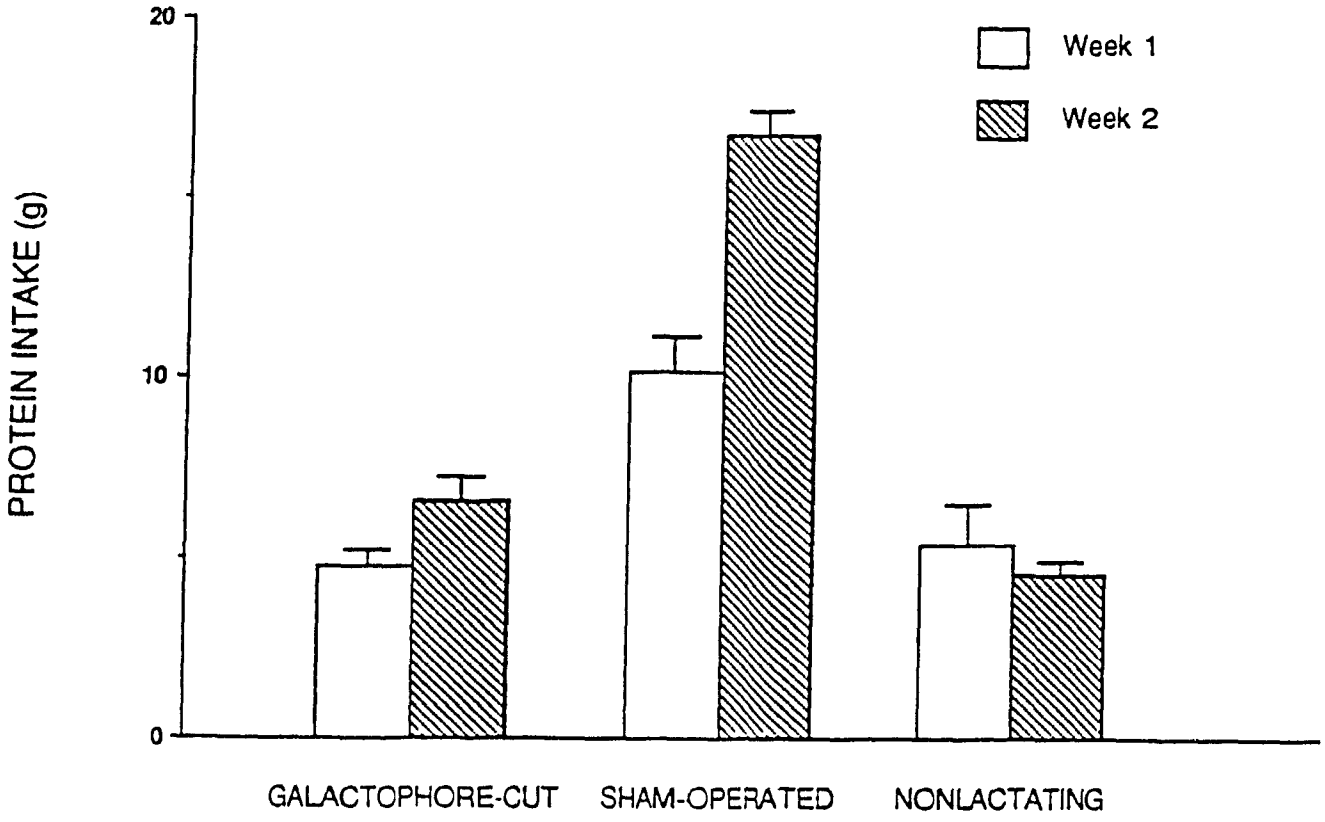

Figure 6. Protein intake (in grams) in the galactophore-cut, sham-operated, and nonlactating groups across Weeks 1 and 2 of lactation. *SEMs* are shown as vertical bars.

Percentage protein was greater in Week 2 than in Week 1 (F (1,24) = 19.367, p <.001). There was a significant Groups x Weeks interaction (F (2,24) = 8.284, p <.001; HSD (6,24) = 3.606) as both of the suckled groups significantly increased percentage protein from Week 1 to Week 2; whereas, the nonlactating group showed no change in percentage protein intake over time (galactophore-cut: Week 1 M = 22.96%, Week 2 M = 26.77%; sham-operated: Week 1 M = 28.25%, Week 2 M = 33.58%; nonlactating: Week 1 M = 27.28%, Week 2 M = 26.53%) (see Appendix C5).

Discussion

Although the greatest amount of food and protein was selected by sham-operated rats, the galactophore-cut females displayed a similar pattern of choice, and clearly exhibited the increase in food and protein intake from the first to the second week of lactation that is characteristic of lactating dams (Cohen & Woodside, 1989). The data are consistent with the idea that milk delivery is not necessary to induce the increase in protein selection seen in lactating dams although the total volume of protein consumed is greater when milk is delivered.

The galactophore-cut dams and nonlactating controls did not differ in terms of food intake or grams of protein eaten in Week 1 of lactation, but in Week 2, the galactophore-cut rats ate a greater amount of food and protein than did nonlactating rats. Percentage protein intake in galactophore-cut dams was lower than levels observed in nonlactating controls in Week 1 and then both groups matched percentage protein intake in Week 2. One interpretation of these data is that for some reason percentage protein intake is suppressed after parturition and then returns to nonlactating levels. A way to explore this possibility would be to use a litter-removed control group; that is, rats would be impregnated and their litters would be taken away immediately after delivery. It would be interesting to conduct a partial replication of Experiment 3 with both of these control groups to assess whether any differences in protein selection would be observed between litter-removed and galactophore-cut females.

Choosing the appropriate controls for this type of experiment

is somewhat problematic. Since the food intake of cycling females decreases on the night of proestrus (see Wade, 1976; Wurtman & Baum, 1980), including these data might contribute to the difference in food intake seen between nonlactating and galactophore-cut rats. One way to avoid this would be to include only the food intake for cycling females on the diestrus days. This, however, would create another potential confound because although the night of proestrus is a time of decreased food intake this is accomplished with a saving in protein intake, so that percent protein intake increases at this time (Wurtman & Baum, 1980). Therefore, one would be eliminating from the analysis the time of peak protein intake in nonlactating females, thereby favoring the hypothesis made about protein intake in this experiment. As the period of the experiment was 16 days and, therefore, included only 4 proestrus feeding episodes it was decided to include all the data.

In this experiment, both groups of suckled dams displayed a different pattern of food and protein intake than did the nonlactating controls. The effects of suckling stimulation are complex. Suckling stimulation leads to an increase in ACTH, corticosterone, prolactin, and progesterone, and a decrease in estrogen. Many of these hormones have been implicated in the control of metabolism and ingestive behavior. There is evidence relating prolactin levels both to food intake and protein intake. It is possible, therefore, that prolactin plays a role in affecting the pattern of food and protein intake observed in the sham-operated and galactophore-cut dams. Interestingly, in nonlactating rats, prolactin enhances food intake; although, if administered peripherally it can also make female rats

acyclic (Gerardo-Gettens et al., 1989a; 1989b; Noel & Woodside, 1991; Noel & Woodside, in press) which, in turn, may be affecting food intake. The effect of prolactin on food intake has been reported to be dose dependent with a larger effect found for higher (3 µg/g body wt) than with medium (1µg/g body wt) or low (0.3 µg/g body wt) doses (Gerardo-Gettens, et al., 1989a). In lactation, prolactin levels are at their highest; prolactin peaks at 133 ± ng/ml on Day 5 of lactation compared to levels of 21 ± 1.4 ng/ml on Day 10 and 33 ng/ml on Days 15 and 18 of pregnancy (Simpson, Simpson, Sinha, & Schmidt, 1973). Perhaps prolactin does not just affect total food intake, but given the opportunity to adjust macronutrient or specifically protein intake, it may be responsible for stimulating protein feeding. Some suggestion of an interaction between protein ingestion and prolactin was presented by Huang, Hawrylewicz, Kissane, and Drab (1982) who reported that young (7 weeks) female rats presented with protein deficient diets were found to have lower serum prolactin levels than did rats fed standard and high protein diets.

Perhaps of more interest in terms of the general literature on the control of macronutrient intake is that prolactin interacts with two neurotransmitters, dopamine (DA) and serotonin (5-HT), each of which has been shown to affect food intake in male rats. Dopamine, when administered into the lateral prefornical hypothalamus, leads to a suppression in protein intake (Leibowitz, 1987; Leibowitz, Shor-Posner, Maclow, & Grinker, 1986) and hypothalamic DA is a prolactin inhibiting factor (Ben-Jonathan, Neill, Arbogast, Peters, & Hoefer, 1980; Frohman, 1980; Kordon, Hery, Szafarczyk, Ixart, &

Assenmacher, 1981; Tolis, 1980).

Whereas DA can inhibit prolactin levels, 5-HT has been shown to stimulate the release of prolactin (Frohman, 1980; Tolis, 1980). Indeed, it has been demonstrated that blocking 5-HT in the lactating dam (e.g., using p-chlorophenylalanine (PCPA)), will block release of prolactin in response to suckling stimulation. Furthermore, it appears that both 5-HT and suckling are necessary for prolactin release in the lactating dam, as treatment with 5-hydroxytryptophan after the PCPA administration is not sufficient to increase the secretion of prolactin (see Kordon, et al., 1981). Kordon et al. (1981) have also reported that levels of tryptophan hydroxylase, an enzyme responsible for 5-HT biosynthesis, and 5- hydroxyindole acetic acid (5-HIAA) a serotonin metabolite, are both increased following suckling stimulation. Interestingly, however, concentrations of 5-HT outside of the area of the hypothalamus are not influenced by the suckling stimulus.

The possible effects of 5-HT on both caloric intake and macronutrient self-selection have been investigated in several laboratories over the last two decades. Anderson and his associates (e.g., Li & Anderson, 1982; 1984), as well as Richard (e.g., Fernstrom & Wurtman, 1972; Yokogoshi & Wurtman, 1986) and Judith Wurtman (e.g., Wurtman, 1985) have presented evidence to suggest that given the chance to choose between protein and carbohydrate, there is a meal-to-meal shift in macronutrient preference. That is, a carbohydrate meal will be followed by a protein meal. It appears that when the rat eats a carbohydrate meal 5-HT levels in the brain are elevated, and this, in turn, leads the rat to select a protein meal

The protein-rich meal results in a suppression of brain 5-HT, and the next meal consists of carbohydrate (see for example Li & Anderson, 1984). Therefore, it has been suggested that 5-HT affects the protein-to-carbohydrate meal-to-meal shift.

Fernstrom and Wurtman's (1972), and Anderson's (1979) explanations for meal changes in macronutrient preference was that the ratio of concentrations of some plasma amino acids was critical in influencing levels of 5-HT in the brain.

The proposed effects of 5-HT on macronutrient selection are not, however, unequivocal. It has more recently been reported (Yokogoshi & Wurtman, 1986) that these effects are true only when the carbohydrate-rich meal is totally devoid of protein. If the meal is mixed with as little as 5% protein, the plasma TRP:LNAA ratio will not be increased. Furthermore, Harper and his associates (Tackman, Tews, & Harper, 1990) have pointed out that this effect is for previously fasted rats. In addition, not all researchers believe that the ratio of TRP:LNAA is the critical factor in affecting the meal-to-meal shift in macronutrient intake.

In sum, there appears to be considerable support from most research teams to suggest that the availability of 5-HT in the brain affects macronutrient self-selection in the male rat. Leibowitz and her associates have repeatedly stressed (e.g., Leibowitz, Weiss, Walsh, & Viswanath, 1989; Stanley et al., 1989; Tempel & Leibowitz, 1990), however, that it is important to consider circadian rhythms when attempting to understand feeding and macronutrient self-selection. This is true because it has been observed that meal patterns change over the day/night cycle, and because levels in

neurotransmitters, such as 5-HT, may also show a diurnal rhythm. For example, 5-HT peaks early in the dark cycle, when carbohydrate is preferred, over protein and fat. Later in the dark cycle, protein intake is increased (Larue-Achiogotis, et al., 1991; Stanley, et al., 1988; Tempel et al.,1989).

The last chapter of this thesis continues the investigation of protein intake by examining meal patterns over the light/dark cycle.

CHAPTER THREE

Experiment 4

The pattern of diet intake shown in Figure 1 and found in Experiments 2 and 3 show that as lactation progresses, food intake increases, and that given the paradigm used, this increase in food intake is accomplished largely by an increase in high protein diet intake. In the study described below, the investigation of this pattern of intake was taken to a finer level by examining changes over the light/dark cycle of both food intake and protein intake across the first two weeks of lactation.

Laboratory rats maintained on a 12h light: 12 h dark cycle, exhibit greater locomotor activity and feeding behavior in the dark. Many aspects of maternal care also fluctuate across the light/dark cycle. For example in the dark the dam produces more milk (Grigor & Thompson, 1987), eats more food, and shows a greater increase in body weight (Munday & Williamson, 1983; Strubbe & Gorissen, 1980) than in the light portion of the cycle. By contrast, nursing bouts are longer during the light period (Woodside & Jans, 1988) and pups show a greater increase in body weight in the first half of the light hours than in any other 6 h period of the 24 h day (Grigor & Thompson, 1987). Although the lactating rat still concentrates its feeding during the night time, feeding in the light portion of the cycle is greater than when the female is nonlactating. For example, it has been shown that lactating rats presented with standard chow will select 35% of their total food intake during the light hours as compared with nonlactating rats who select only 15% (Munday & Williamson, 1983).

Strubbe and Gorissen (1980) also showed that as lactation progressed there were changes in patterns of food intake so that meal frequency increased, but meal duration did not. One issue that arises from these data is how these changes in ingestive behavior might be modified by the available diet. It has already been demonstrated that the amount of food eaten by lactating dams is affected by the diets presented (e.g., Cohen & Woodside, 1989). The current study was designed to assess whether the type of diet presented to lactating rats might affect the amount of eating exhibited in the light and dark parts of the cycle, and/or the number of meals eaten or meal duration. A secondary question was how diet quality might affect the time demands on the lactating female as reflected in time spent with her young.

The study described below also served to extend the experiments in this thesis, as well as those in the paper by Cohen and Woodside (1989), in that a new diet group was introduced. Cohen and Woodside (1989) demonstrated that rats who were allowed to increase protein intake selectively because they were presented with a choice between low and high protein semi-purified diets, ate less food and fewer calories than did rats that were presented with a standard laboratory powdered chow. These groups differed in terms of the ability or lack of ability to display a preference for protein; however, there was another difference between these groups in that the semi-purified diets have a higher caloric density than does the powdered control chow. The study described below used a lactating group presented with the low protein - high protein diets, and two control diet groups. One was

presented with the powdered lab chow, and the other was presented with a semi-purifed diet, isocaloric with the low protein and high protein diets. In this way, the effects of protein self-selection, and the effects of caloric density and other properties of the diet (e.g., texture) could be explored within an investigation of feeding and nursing behavior across the light/dark cycle and over the first two weeks of lactation.

Method

Subjects

Thirty-one primiparous Wistar rats, originally obtained from Charles River Breeding Farms, St. Constant, Quebec served as subjects. Within 24 h after parturition, litters were culled to eight pups, and dams were assigned to one of three diet groups: 1) low protein - high protein (n = 8), 2) semi-purified control diet (n = 9), or 3) powdered chow control diet (hereafter referred to as powdered-chow; n=14). Lights were on at 09:00 h and off at 21:00 h. Housing and ambient temperature conditions were as described above in Experiment 2.

Diets

The low protein - high protein diets were as described in Experiment 1. The semi-purified control diet was prepared in our laboratory from ingredients obtained from ICN Nutritional Biochemicals, Cleveland, Ohio, with the exception of fat (CRISCO®, Procter and Gamble, Toronto, Ontario). The composition of this diet was created by calculating the average percentage for each macronutrient across the low and high protein diets (e.g., 5% protein + 45% protein yields an average of 25% protein) with micronutrient percentages held constant for all semi-purified diets (see Table 3). The semi-purified control diet was stored in the same way as the low protein - high protein diets.

The powdered chow diet was obtained from Charles River, Canada and was stored at room temperature in sealed plastic bins. This diet contained 22.5% protein, 5% fat, and 51.1% total utilizable carbohydrates. The caloric value of the diet is 3.39 kcal/g.

Table 3. Contents of the semi-purified control diet.

Protein (Casein-purified high nitrogen)	25%
Carbohydrate (Dextrin)	40%
Fat (CRISCO®)	24%
Non-nutritive bulk (Alphacel)	11%
Plus the following per 1 kg of diet mixture	
Vitamins (Vitamin diet fortication mixture)	22g
Minerals (Rogers-Harper mineral mixture)	40g
Gelling agent (Agar)	35g
Water	1000ml
Calories	4.32kcal/g

Apparatus

Modified cages were used on Days 4 and 10 when meal patterns were assessed. Each food jar was placed in a holder made from aluminum, that had sides which ended just above the level of the rim of the food jar. Photocells on these aluminum sides allowed the recording of each time the rat placed its head in the food jar. The data were then relayed to an Apple IIe computer. A Panasonic videocassette recorder and camcorder was used to videotape rats during the computerized data collection procedure. The camcorder allowed for a running counter to be displayed on screen, so that meal durations could be determined. The data collected from the videotaped procedure were used to verify the accuracy of the computer collection procedure. In all, approximately 600 hours of videotape were analyzed.

Procedure

Each rat was presented with two food jars per cage. In the case of the low protein - high protein group each jar contained a different diet. For both the semi-purified control and powdered-chow groups, the same diet was placed in both jars.

Food intake was recorded every 12 h (09:00 h & 21.00 h), at the start of the light part of the cycle and and just prior to the start of the dark period. The amount of food eaten during the light hours was assessed by subtracting the amount the jar weighed just before lights out from the amount that the jar weighed when it was placed in the cage at the start of the light period. The amount of food eaten during the dark hours was calculated by subtracting the amount the jar weighed at the start of the light period from the amount the jar

weighed at the start of the previous dark part of the cycle. Data were collected for a period of 16 days postpartum. The first day following parturition was designated as Day 1 of lactation. Dam weight and litter weight were recorded daily for the same period.

Food intake values for the semi-purified diets were divided in half in order to account for the water content of the diets. As the semi-purified diets were not isocaloric with the powdered chow, food intake values were converted to caloric intake. Caloric intake, gram protein intake, and intake of the low protein and high protein diets were all analyzed with aid of the BMDP 2V statistical package and when the assumption of sphericity was violated the Greenhouse-Geisser corrected probability values were used. Caloric intake, and gram protein intake were analyzed using three-way Split Plot ANOVAs (Between: Groups (3), Within: Light Cycle (2), Days (16)). Intake of the low protein and high protein diets was analzyed for the appropriate group using a Repeated Measures ANOVA (Diets (2) x Light Cycle (2) x Days (16)).

On Days 4 and 10 of lactation, meal patterns were assessed via computer data collection and videotaped procedures (see Apparatus above). This necessitated placing the dam in a different but similar cage to her home cage. In order to allow for adjustment to the new surroundings, dams and their litters were placed in these cages 12 h prior to testing (i.e., 21:00 h Days 3 & 9). Data were collected by the computer for a 24 h period and when possible, some of the rats were videotaped for the first 8 h of both the light and dark parts of the cycle (i.e., 09:00 h - 17:00 h & 21:00 h - 06:00 h).

The videotapes were scored for the amount of time spent

eating and the jar from which the rat was eating. The time that the dam spent with her pups was also recorded. Computer printouts indicated when there was activity at a food jar, which jar was involved and for how long the activity occurred. Inter-meal intervals were defined as 15 min without any eating being observed (Strubbe & Gorissen, 1980).

Dam weight was assessed as described in Experiments 1 and 2, and analyzed using a one-way ANOVA. Litter weights on Day 1 and Day 16 were analyzed using a Split-Plot ANOVA (Between: Groups (3); Within: Days (2)) so that pup growth could be compared across groups.

Results

Dam Weight

The average weight of dams for each group on Day 1 and Day 16 are shown in Appendix D1. There was no significant difference in the change in body weight of the dams in the low protein- high protein (M = 11.32% ± 1.47%), control (M = 15.53% ± 2.49%), and powdered-chow groups (M = 9.21% ±1.80%) across the lactation period (F (2,27) = 2.457, p >.05) (See Appendix D2).

Litter Weight

The days effect was significant reflecting growth of the pups across lactation (Days: F (1,28) = 1090.862, p <.001). There was a significant difference across groups in litter weight (Groups: F (2,28) = 5.168, p <.05; HSD = 24.66) and a significant Groups x Days interaction (F (2,28) = 6.081, p <.01; HSD = 40.80) because the litters of the powdered-chow dams (M = 277.38 g ± 10.20 g) did not weigh as much as the litters nursed by the low protein-high protein (M = 342.26 g ± 11.94 g) group on Day 16. On this day, the litters in the two semi-purified diet groups (Control: M = 317.54 g ± 20.33 g) did not differ significantly, nor did the litters across the powdered -chow and control groups. Litter weight on Day 1 did not differ significantly across groups. (see Appendix D3)

Caloric Intake

The powdered-chow group (M = 83.32 kcal ± 9.02 kcal) ate significantly more calories than did the low protein-high protein (M = 73.34 kcal ± 7.90 kcal) and control groups (M = 75.44 kcal ± 4.86 kcal); these latter groups did not differ significantly from each other (Groups: F (2,28) = 5.84, p < .01; HSD (3,28) = 7.96;

see Figure 7; see Appendix D4). Overall, more calories were consumed in the dark phase (M = 97.28 kcal ± 2.57 kcal) of the cycle than in the light period (M = 57.46 kcal ± 1.87 kcal) (Cycle: F (1,28) = 212.97, p <.0001; HSD (3,28) = 37.88), and caloric intake increased over the days of lactation (Days: F (15,420) = 115.42, p <.0001).

There was a significant Groups x Cycle interaction (F (2,28) = 18.46, p <.0001; HSD = 14.13) as each group ate significantly more calories in the dark part of the cycle than they did in the light hours (low protein-high protein, light period: M = 53.84 kcal ± 3.07 kcal; dark period: M = 92.85 kcal ± 3.45 kcal; control, light period: M = 64.81 kcal ± 3.32 kcal; dark period: M = 86.07 kcal ± 3 29 kcal; powdered-chow, light period: M = 53.74 kcal ± 2.51 kcal; dark period: M = 112.91 kcal ± 8.85 kcal). In the light part of the cycle, all the groups ate similar amounts of calories; but, in the dark period the powdered-chow group ate significantly more calories than did the control diet group. Neither of the control diet groups differed significantly from the low protein-high protein diet group The significant Groups x Days interaction (F (30,420) = 3.03, p < .001) is likely accounted for by the powdered-chow group which exhibited a much greater increase in calories over lactation than did the two semi-purified diet groups, which on most days selected similar amounts of calories. Moreover, there was a sharper increase in caloric intake over days during the dark part of the cycle than in the light portion (Cycle x Days: F (15,420) = 6.56, p < .0001), which was largely due to the feeding behavior of the powdered-chow group during the dark hours; the Groups x Cycle x Days interaction fell short of significance (F (30,420) = 1.55, p > .05).

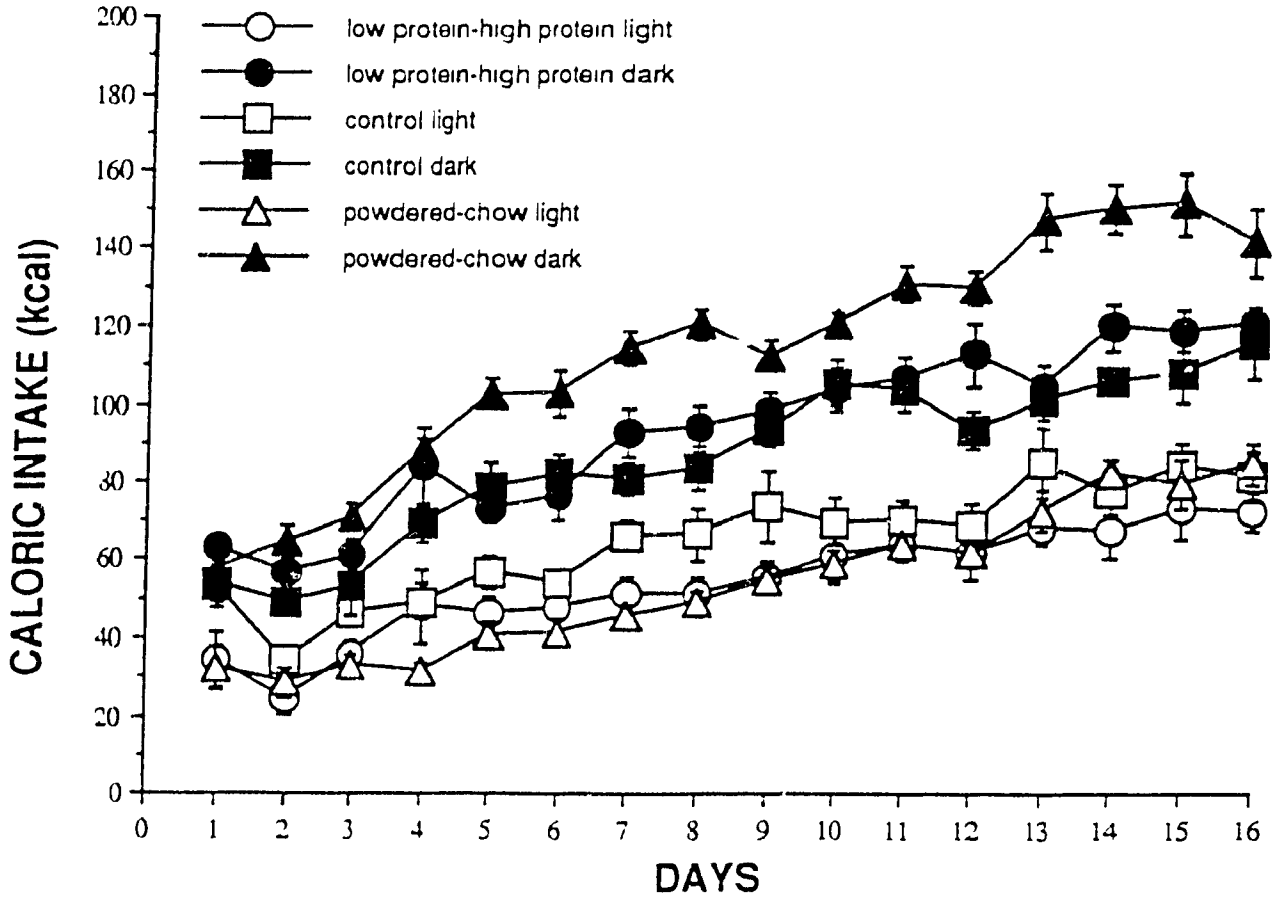

Figure 7 Caloric intake (kcal) of the low protein-high protein, control diet, and powdered-chow groups in the light/dark cycle over the days of lactation. *SEMs* are shown as vertical bars.

Protein Intake (in grams)

For protein intake as for caloric intake, all main effects and two-way interactions were significant; only the three-way interaction was not significant (Groups x Cycle x Days: F (15,420) = 1.04, $p > .05$). Overall, the low protein - high protein diet group (M = 5.96 g ± .40 g) and the powdered-chow group (M = 5.48 g ± .44 g) ate similar amounts of protein (see Figure 8), and ate significantly more protein than did the semi-purified control diet (M = 4.37 g ± .21 g) group (Groups: F (2,28) = 17.91, $p <.0001$; HSD (3,28) = .63). There was a significant increase in gram protein intake over lactation (Days: F (15,420) = 98.61, $p <.0001$); but the control diet group appeared to exhibit a smaller increase in gram protein intake over time than did the other two groups (Groups x Days: F (30,420) = 3.26, $p < .001$).

More protein was eaten in the dark (M = 6.59 g ± 30 g) part of the cycle than in the light (M = 3.91 g ± .18 g) period (Cycle: F (1,28) = 155.81, $p <.0001$; HSD (2, 28) = 1.38) and the significant Groups x Cycle interaction (F (2,28) = 13.60, p =.0001; HSD (6,28) = 1.12) reflects the fact that each group ate more grams of protein in the dark period than in the light part of the cycle (low protein high protein, light period: M = 4.40 g ± .38 g; dark period : M = 7.47 g ± .47 g; control, light period: M = 3.75 g ± .21 g; dark period: M = 4.98 g ± .30 g; powdered-chow, light period: M = 3.57 g ± 32 g, dark period: M = 7.39 g ± .48 g). All of the groups ate similar amounts of protein in the light period; in the dark part of the cycle the low protein-high protein and powdered-chow groups did not differ significantly from one another and both ate more protein than did

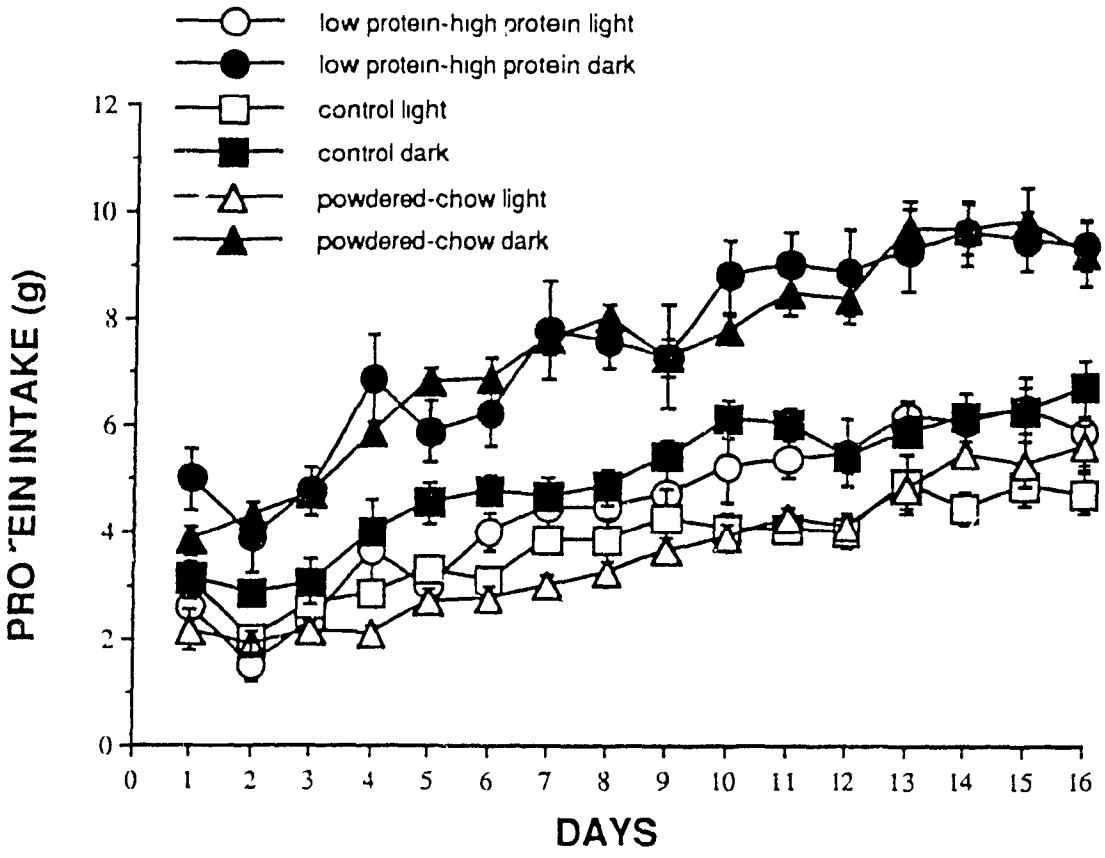

Figure 8. Protein intake (g) of the low-protein-high protein, control diet, and powdered-chow groups in the light/dark cycle over the days of lactation. *SEMs* are shown as vertical bars.

the control group. Across lactation, there was a greater increase in protein intake during the dark hours than during the light (Cycle x Days: $F(15,420) = 3.88$, $p < .001$; see Appendix D5).

Intake of the Low Protein and High Protein Diets

Overall, more food (i.e., low protein and high protein diet intake combined) was eaten in the dark (M = 13.56 g ± 1.36 g) part of the cycle than in the light (M = 3.74 g ± .90 g) period (Cycle: $F(1,7) = 48.98$, $p < .001$), and more high protein diet (M = 17.31 g ± 1.35 g) was eaten than low protein diet (M = 5.18 g ± .53 g) (Diets: $F(1,7) = 94.50$, $p < .0001$; see Figure 9) There was also a significant main effect for Days with food intake increasing over the lactation period (Days: $F(15,105) = 28.82$, $p < .0001$), and a significant Diets x Days interaction, where high protein intake increased to a greater extent over lactation than did the intake of low protein diet ($F(15,105) = 8.44$, $p < .001$). Interestingly, there was a significant Cycle x Diets interaction (Cycle x Diets: $F(1,7) = 10.70$, $p < .05$; HSD (4,7) = 2.74) where the difference between low protein diet intake across the light and dark parts of the cyle was not significant; but, there was a significant difference between high protein diet intake across the phases of the light/dark cycle (low protein, light: M = 2.91 g; low protein, dark: M = 5.51 g; high protein, light: M = 9.55 g; high protein, dark: M = 15.98 g). The difference between low and high protein diet intake within each phase of the cycle was significant. The Cycle x Days ($F(15,105) = 1.77$, $p > .05$) and Cycle x Diets x Days interactions were not significant ($F(15,105) < 1.00$) (see Appendix D6).

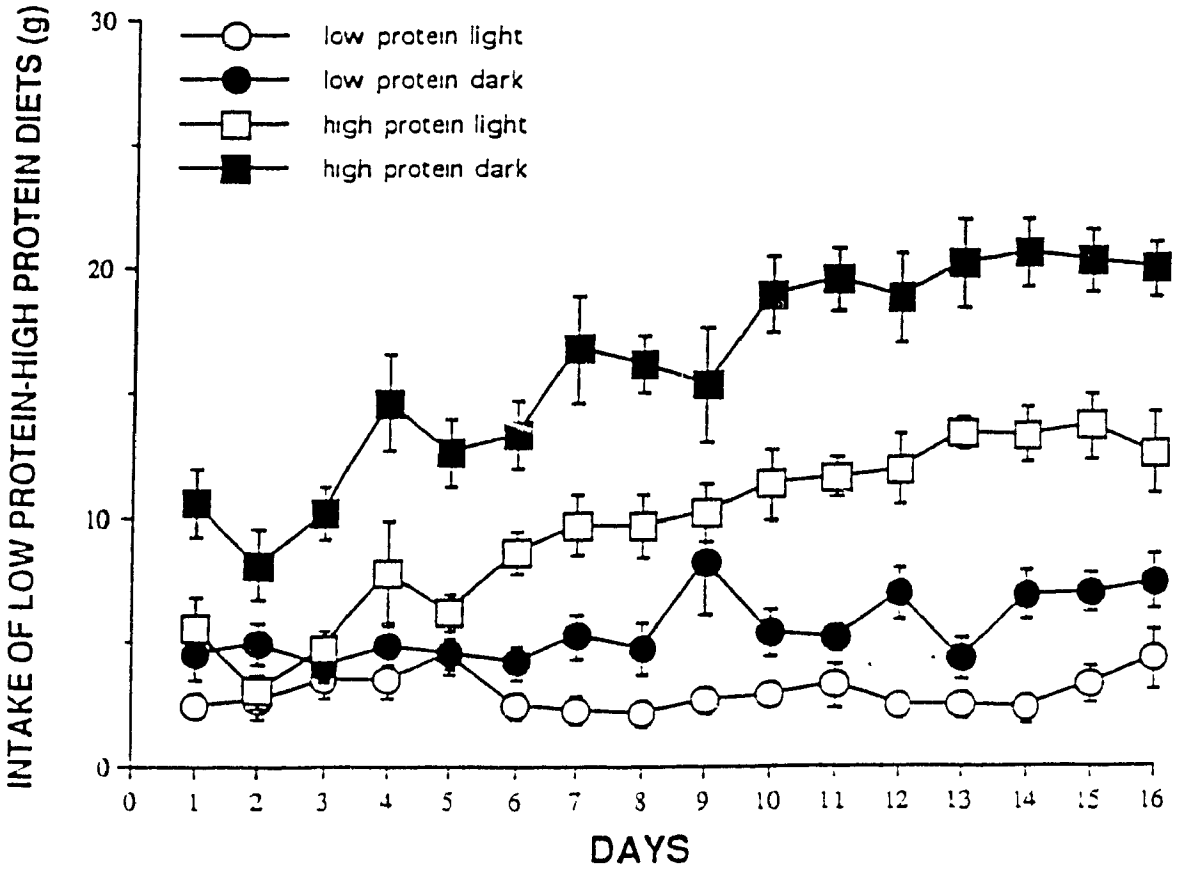

Figure 9. Intake of the low protein and high protein diets in the light/dark cycle across lactation. *SEMs* are shown as vertical bars.

Meal Patterns for Days 4 and 10 of Lactation

Because of limitations in the amount of data that could be collected by the computer in any 24 h period, and problems with the computer during data collection, meal patterns were assessed for only some of the rats (low protein - high protein, n=4; control, n=5; powdered-chow, n=4).

Meal Frequency. Across the diet groups, there were no differences in the number of meals eaten (Groups: $F(2,10) = .954$, $p > .05$; see Figure 10). A greater number of meals were eaten in the dark cycle than in the light cycle (Cycle x Days: $F(1,10) = 36.899$, $p < .001$; $M_{Light} = 7.26$; $M_{Dark} = 10.77$). None of the other main effects and interactions were significant (Days: $F(1,10) = 1.260$, $p > .05$; Groups x Days: $F(2,10) = 1.312$, $p > .05$; Groups x Cycle: $F(2,10) = 2.448$, $p > .05$; Cycle x Days: $F(1,10) = .682$, $p > 05$; Groups x Cycle x Days $F(2,10) = .717$, $p > .05$; see Appendix D7).

Meal Duration. The powdered-chow group ($M = 1038.38$ s $\pm$ 129.86 s) ate longer meals than did the semi-purified controls ($M = 801.53$ s $\pm$ 87.85 s) and the low protein - high protein group ($M = 635.56$ s $\pm$ 66.62 s); the semi-purified groups did not differ significantly from each other (Groups: $F(2,10) = 5.456$, $p < .05$; $HSD(3,10) = 347.84$; see Figure 11).

Meal duration was significantly longer in the dark period than in the light period (Cycle: $F(1,10) = 33.793$, $p < .001$) and there was a significant Groups x Cycle interaction ($F(2,10) = 5.447$; $p < 05$, $HSD(6,10) = 473.83$) largely due to the powdered-chow group, which more than doubled meal duration from the light ($M = 630$ 21 s) to the dark ($M = 1446.56$ s) part of the cycle. There were no significant

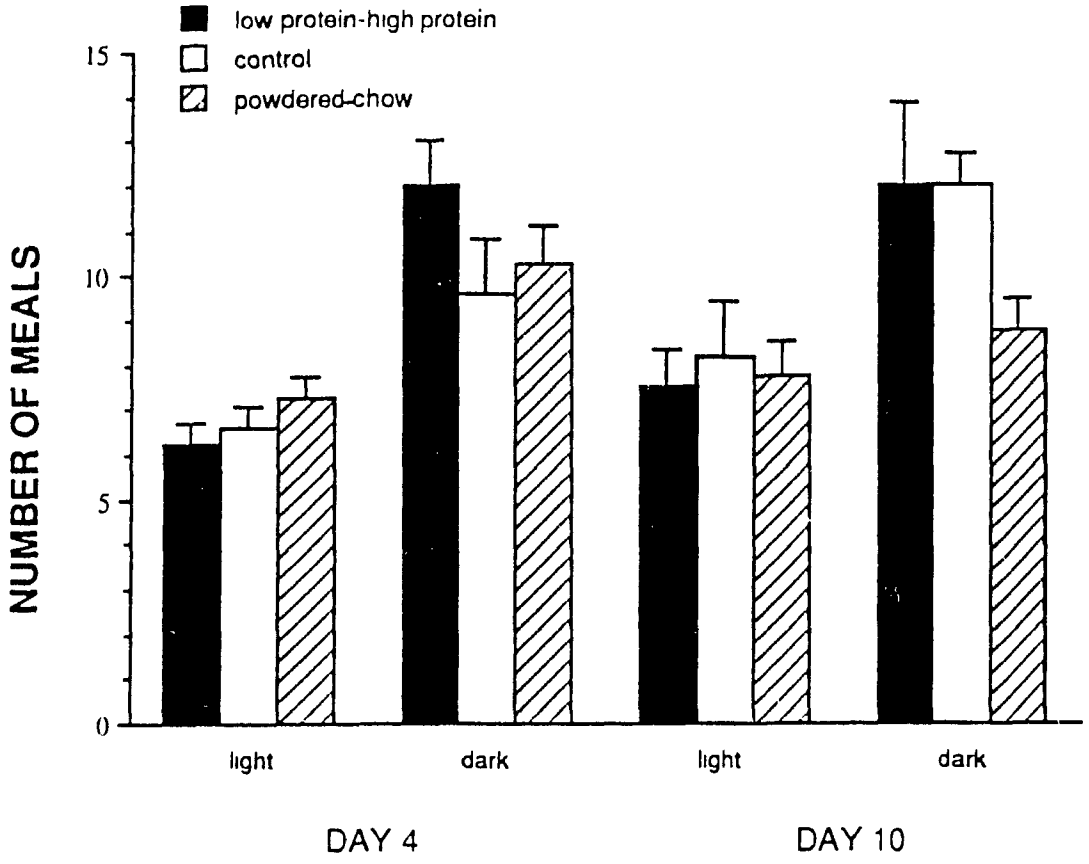

Figure 10. The number of meals eaten by low protein-high protein, control diet, and powdered-chow groups in the light/dark cycle on Day 4 and Day 10 of lactation. *SEMs* are shown as vertical bars.

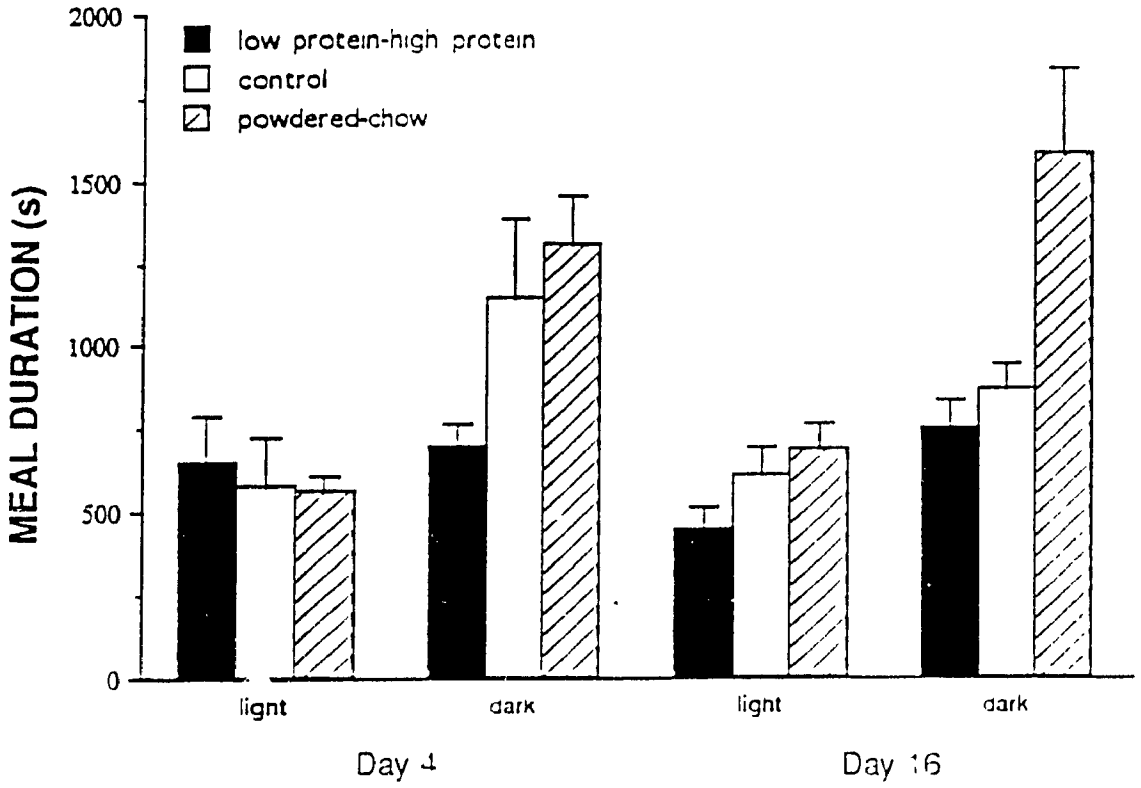

Figure 11. The duration of meals eaten by low protein-high protein, control diet, and powdered-chow groups in the light/dark cycle on Day 4 and Day 10 of lactation. *SEMs* are shown as vertical bars.

differences in meal duration across the light and dark portions of the cycle in the low protein-high protein (light: *M* = 548.03 s; dark: *M* =723.69 s) and control (light: *M* = 597.81 s; dark: *M* =1005.25 s) groups. As was the case for meal frequency, there was no significant Days effect ($F(1,10)=.001$, $p > .05$) nor were the remaining interactions found to be significant (Groups x Days: $F(2,10) = 1.54$, $p > .05$; Cycle x Days: $F(1,10) = .074$, $p > .05$; Groups x Cycle x Days: $F(2,10) = 1.785$, $p > .05$; see Appendix D8)

Total Time Eating. The results for total time eating paralleled those reported for meal duration. The powdered-chow group (powdered-chow: *M* =8988.37 s ±1311.47 s) spent more time eating than did the low protein-high protein group (*M* =5972.94 s ± 1003.94 s); the semi-purified control group (*M* = 7306.60 s ± 1197.71s) was not significantly different from either the low protein-high protein or powdered-chow groups (Groups: $F(2,10) = 5.892$, $p < .05$; *HSD* (3,10) = 2500.09; see Figure 12). More time was spent eating during the dark part of the cycle than during the light hours (Cycle: $F(1,10) = 156.252$, $p < .001$) and the Cycle x Groups interaction was significant ($F(2,10) = 4.127$, $p < .05$; *HSD* (6,10) = 3229.11). In each of the groups more time was spent eating in the dark period than in the light, and the powdered-chow dams spent more time eating in the dark part of the cycle than did either of the semi-purifed diet groups (low protein-high protein, light: *M* = 3551.12 s; dark: *M* = 8394.75 s; control, light: *M* = 4226.90 s; dark: *M* =10386.30 s; powdered-chow, light: *M* = 4762.87 s; dark: *M* =13213.87 s). There was no difference in total time eating across Day 4 and Day 10 of lactation (Days: $F(1,10) = .973$, $p > .05$) and none of the remaining

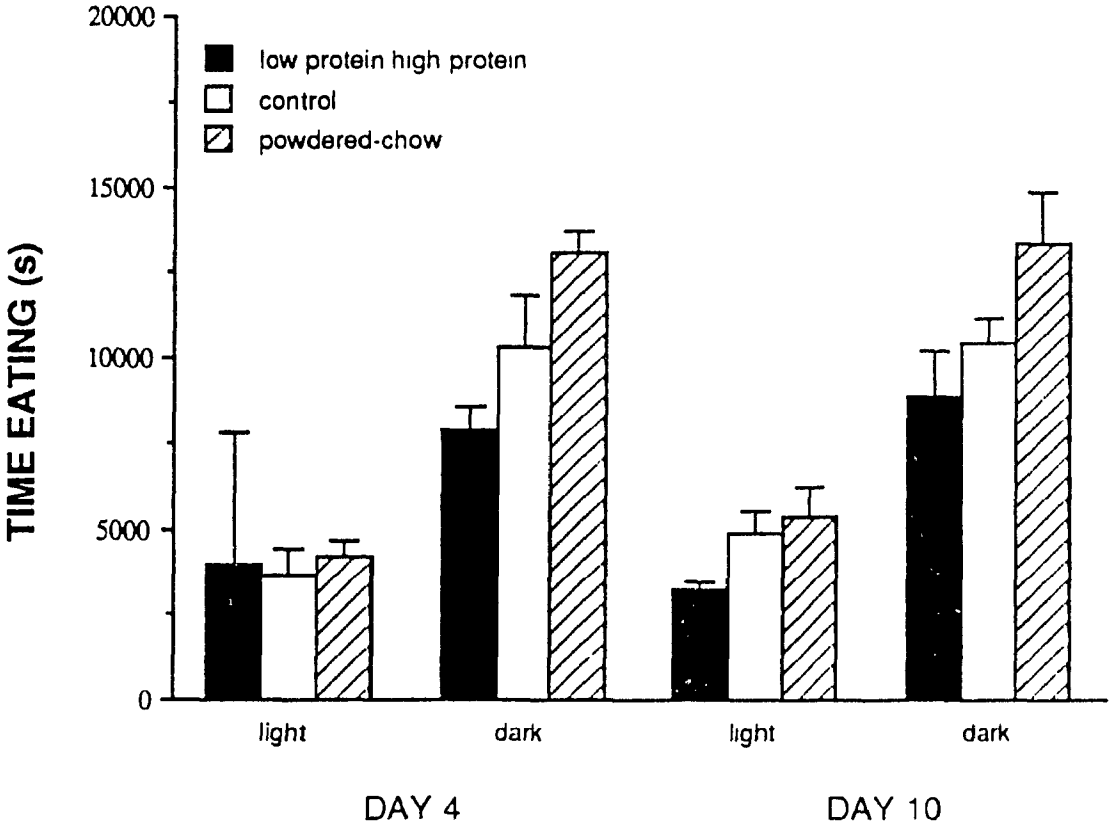

Figure 12. Total time spent eating (in s) by low protein-high protein, control diet, and powdered-chow groups in the light/dark cycle on Day 4 and Day 10 of lactation. *SEMs* are shown as vertical bars.

interactions was found to be significant (Groups x Days: $F(2,10) = .147$, $p > .05$; Cycle x Days: $F(1,10) = .01$, $p > .05$; Groups x Cycle x Days: $F(2,10) = 1.323$, $p > .05$; see Appendix D9).

Analysis of Videotapes

As there are individual differences among rats in diet selection, and it was assumed this would be the case for meal patterns, and amount of time spent with pups, these measures should have been analyzed using a within-subjects comparison for each individual rat across the four possible time periods; namely, Day 4 light and dark portions of the cycle, and Day 10 light and dark portions of the cycle, respectively. Unfortunately, due to technical limitations in taping the subjects during the meal patterns data collection procedure, vidoetapes do not exist for all four time periods for all rats. In all, analyses have been performed for four animals in the low protein-high protein diet groups and for four animals in the semi-purified control diet group. As for the powdered-chow group, there were too few rats videotaped for all four times. Although not ideal, results for these animals have been calculated as means (and *SEMs*) for each time period, that is, regardless of which subjects were recorded in that time period. Note that the number of subjects contributing to the data differed in each time period. Although the low protein-high protein and control groups were analyzed separately from the powdered-chow data, Figure 13, displays the time spent with pups for all of these groups so as to allow for some comparison across these diets conditions.

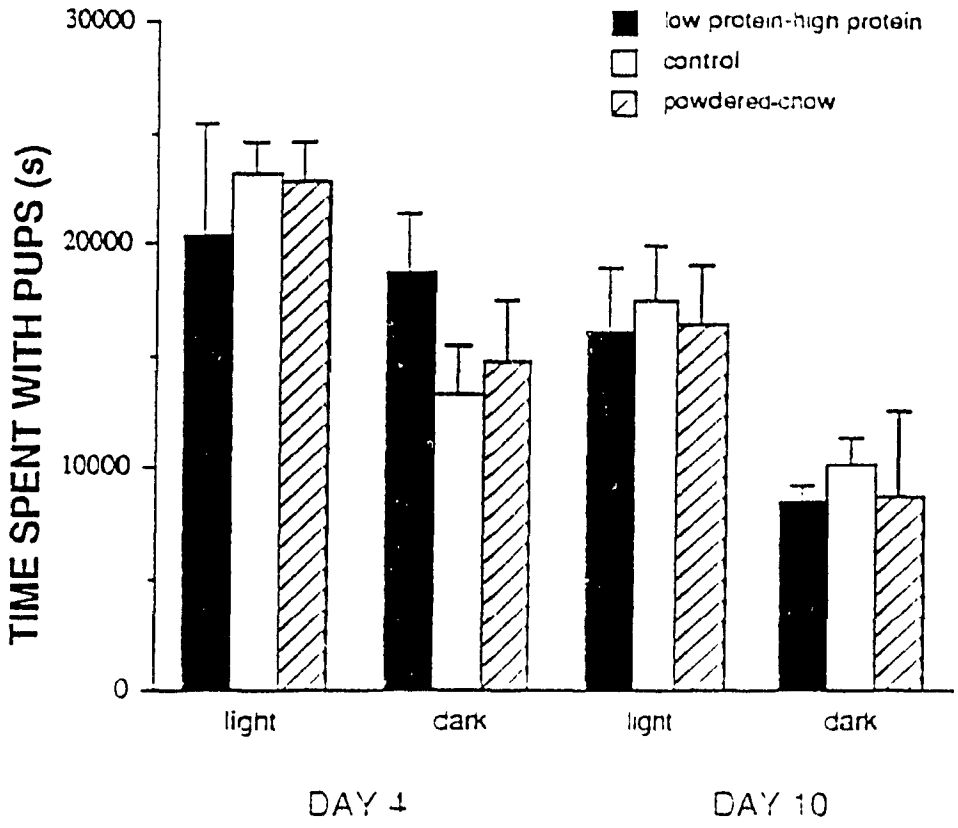

Figure 13. The time (in s) spent with pups by low protein-high protein, control diet, and powdered-chow groups in the light/dark cycle on Day 4 and Day 10 of lactation. *SEMs* are shown as vertical bars.

Time Spent with Pups

Low protein-High Protein and Semi-purified Control Diet Groups. Every time the dam gathered her pups and stayed with them was defined as Time Spent With Pups. These bouts were not analyzed to check for milk ejections (Woodside & Jans, 1988), that is, these were not necessarily nursing bouts; but, it is assumed that some of these contacts with the pups involved feeding of the litter. The data were calculated in seconds and analyzed using a three-way Split-plot ANOVA (Between. Groups (2) x Within: Cycle (2), Days (2); see Appendix D10).

Dams spent more time with their pups during the light hours than during the dark period (Cycle: $F(1,6) = 10.43$, $p < .05$) and more time was spent with the pups on Day 4 than on Day 10 (Days: $F(1,6) = 19.17$, $p < .01$; see Figure 13). There was no difference between the low protein- high protein and semi-purified diet groups in the amount of time spent with the pups (Groups: $F(1,6) = .006$, $p > .05$). None of the interactions was found to be significant (Groups x Cycle: $F(1,6) = .906$, $p > .05$; Groups x Days: $F(1,6) = 1.19$, $p > .05$; Cycle x Days: $F(1,6) = .141$, $p > .05$; Groups x Cycle x Days: $F(1,6) = .935$, $p > .05$).

Powdered-chow Group. As was the case for the low protein-high protein and semi-purified control groups, it appears that the powdered-chow dams spent more time with their pups in the light than in the dark part of the cycle and on Day 4 than on Day 10.

Discussion

This experiment investigated caloric intake, protein intake, meal patterns, and time spent with the young versus time spent eating, across the light/dark cycle of lactation. The effects of different types of diet on these measures was also examined.

Caloric intake was greater during the dark part of the cycle than the light hours, and was shown to increase across the days of lactation. These data are consistent with the findings of Strubbe and Gorissen (1980), as well as of Munday and Williamson (1983) who found that although food intake was greater in the light period for lactating than for nonlactating rats, the dark part of the cycle was still the period of greater food intake. Furthermore, Experiment 4 demonstrates that the dark period is not only a time where total food intake is elevated, it is also a time of concentrated protein feeding; the rats that were presented with the low and high protein diets ate more high protein diet in the dark part of the cycle than they did in the light period. All groups ate more grams of protein in the dark than in the light part of the cycle.

The differences in caloric and protein intake across the various diet groups are interesting to note. The low protein - high protein dams, who could increase protein intake without having to increase total food or caloric intake, ate fewer calories than did the powdered-chow group. The powdered-chow group, faced with a diet of a lower caloric density than the semi-purified control group, overall selected more calories than the latter group; this implies that the powdered-chow group ate a much larger amount of total food than did the semi-purified controls. The differences between

the two control groups was accounted for by caloric intake in the dark period; the powdered-chow group ate far more calories than did the control group, whereas, in the light part of the cycle, the powdered-chow rats ate a similar number of calories to the semi-purified control diet group.

Interestingly, the increase in food intake exhibited by the powdered-chow group allowed them to match grams of protein eaten with the low protein-high protein dams, and both of these groups ate more protein than did the semi-purified control group. These data are consistent with those of Cohen & Woodside (1989) in which rats that were presented with a control diet, that is powdered-chow, were shown to increase total food intake to match the total protein intake of dams allowed to self-select protein.

It is interesting to note that the semi-purifed control diet group did not match the powdered-chow group in grams of protein eaten. These diets differ in terms of texture and physical properties. It has also been demonstrated (Ellison, 1985) that rats that eat powdered chow defecate more than do rats that eat semi-purified diets. If the latter diet is passed less quickly, the rat may be more sated on such a diet and may therefore be encouraged to eat less total food. Similarly, the powdered-chow dams may be less sated than the semi-purified control dams and more able to increase food intake. Given that the percentage protein was already similar in both of these diets, if one group ate more total food, the groups would necessarily be dissimilar in grams of protein eaten. It is therefore, possible, that differences in gut motility and or number of fecal boli produced would account for differences in protein

intake across these control groups.

As the powdered-chow group consumed more food than the low protein-high protein and control diet groups, it would be expected that this would result in differences in meal patterns across groups. The powdered-chow group ate longer meals and spent more total time eating than the low protein-high protein group, but was similar to the semi-purified control diet group on these measures. Meal patterns were not only affected by the type of diet, but were also shown to differ across the light/dark cycle. Consistent with previous research, there were more meals, longer meals, and more total time spent eating observed in the dark part of the cycle than the light (Strubbe & Gorissen, 1980).

There were no differences in meal frequency, meal duration, or total time eating from Week 1 to Week 2, when Day 4 and Day 10 were compared for sub-groups of each of the diet groups. Given the increase in food intake observed from the first to the second week of lactation, the dams must have had to eat more quickly during each meal in the dark part of the cycle in the second week, in order to account for this increase in food intake. In addition, meal size, that is, the amount of food eaten in each meal, would likely have had to increase. In contrast to these results, Strubbe and Gorissen (1980) reported an increase in meal frequency across the weeks of lactation. There are, however, some methodological differences between their study and the experiment described in this chapter. The litter size used by Strubbe and Gorissen (1980) was similar, but not identical to, that used in the experiment described in this chapter; in the latter case litter size was 8 pups, and Strubbe and

Gorissen (1980) used 10-pup litters. In addition, Strubbe and Gorissen (1980) did not use semi-purified or powdered diets; instead, the rats were presented with food pellets. It is possible that the physical properties of the food might have an effect on meal patterns. Similarly, the different types of diet might be metabolized at different rates, which, in turn, could affect meal patterns.

Meal patterns may also be affected by the need of the dam to engage in other behaviors. Lactating female rats need to balance the hours of the day between caring for themselves and for their young, and such demands on time might result in the dam eating more quickly. Lactating dams spend much of their time nursing and the results of this experiment seem to support those reported in the literature (e.g., Munday & Willamson, 1983; Woodside & Jans, 1988); dams spent more time with pups during the light period and the first week, than in the dark part of the cycle and second week, respectively. Unfortunately, it is difficult to assess the impact that being presented with various diets might have on time spent with the pups, as the data in this experiment were analyzed for only a few rats per group. It would be interesting to pursue this question further to determine whether powdered-chow rats behave differently from rats presented with semi-purified diets. By the second week's dark period, where the dams from all diet groups are consuming large quantities of food, it may be that maternal contact time would be affected.

There are two factors that might affect time spent eating, and in turn, time spent with the litter. First, the texture of the

powdered chow necessitates that more time be spent at the food jar. Similarly, as low protein-high protein dams can selectively increase protein intake they could eat a protein rich diet in relatively less time than do dams in either of the control diet groups, and could, therefore, spend more time with their young. Although the powdered chow group ate similar amounts of protein to the low protein-high protein dams, the pups nursed by the latter group weighed more at the end of the experiment. The effect of pup growth might be due to variations in the quality of the milk produced given the different types of diets, or may be due to differences in maternal-litter contact time. This question needs to be explored using a larger group of animals, and in a testing paradigm in which mother-litter contact and milk could be compared across all three diet groups.

General Discussion

In this thesis, different facets of the pattern of protein selection seen in lactating rats were examined. The first chapter investigated a possible role for placentophagia in the decrease in relative protein intake immediately postpartum. The results of Experiment 1 suggest that placentophagia did not affect protein choice at this time, nor did it lead to differences in food intake later in the lactation period. Therefore, although Blank and Friesen (1980) observed differences in hormonal levels between groups of animals that were allowed to eat the placentae and those that were not, such differences if they do exist apparently do not affect protein self-selection. This suggests that although placentophagia is an ingestive behavior, it does not seem to be regulated like food intake. This is in keeping with the work of Kristal and his associates (e.g., Abbott et al.,1991; Kristal, 1980), who have noted that eating the placentae is related to an enhancement of opioid effects.

In the second chapter of this thesis, the relative contributions of milk delivery and hormonal status to the increase in protein intake shown across the first two weeks of lactation was investigated. Litter size was shown to be directly related to an increase in protein intake in the lactating rat, but even when no milk was delivered to the offspring, suckling stimulation, which directly affects hormone levels, specifically prolactin, was a sufficient stimulus to produce an increase in protein intake, although there is a potential confound in these data.

Taken together, the results of Experiment 2 and 3 demonstrate

that even rats nursing small litters, as well as rats that are suckled but do not suffer a protein deficit as they do not produce or deliver milk, will increase protein intake across the weeks of lactation. It can be stated that these dams display a preference for protein because tne increase in food intake from Week 1 to Week 2 of lactation reflects an increase in high protein diet intake; however, the amount of protein ingested, as measured by grams of protein eaten, is less than that consumed by milk-delivering dams and dams who are nursing relatively large litters. Therefore, hormonal status, influenced by suckling stimulation, will influence protein self-selection; but, the dam seems to eat large quantities of protein only when there is the additional component of a drain on her nutritional stores and/or a delivery of nutrients to her offspring. It may be that suckling-induced changes in prolactin levels, or DA and 5-HT, may serve as a signal to trigger the initiation of protein consumption, but that the nutritional status of the dam will then affect the continuation of feeding, perhaps by inhibiting satiety mechanisms. It would, therefore, be interesting to examine meal patterns, including meal size, in the groups of rats investigated in both experiments of Chapter Two of this thesis.

It may be that prolactin is not the only hormone influencing feeding behavior in lactation. Another hormone that should be considered is estrogen. In the lactating rat, estrogen levels are very low compared to the peak observed in the last few days prior to parturition (Shaik, 1971), and estrogen levels do not rise until late in the lactation period, at approximately Days 15 to 20 (Smith & Neill, 1977; Taya & Greenwald, 1982).

In the nonlactating female rat, it has been shown that estrogen not only affects total food intake (Wade, 1976), and possibly macronutrient self-selection (Wurtman & Baum, 1980); but, meal patterns as well. Blaustein and Wade (1976) reported that when estrogen is high, meal size is decreased. Sieck, Nance, & Groski (1978) demonstrated that ovariectomized rats treated with estradiol benzoate decreased meal duration over the 24 h period, and decreased meal size in the dark part of the cycle. Blaustein and Wade (1976) and Sieck et al. (1978) presented different interpretations regarding the mechanisms involved in the effects of estrogen on meal patterns. The former suggested that estrogen encourages the termination of a meal through a short-term satiety mechanism, whereas, Sieck et al. (1978) suggested a longer process involving body weight set-point. Regardless of how the effect is being mediated, estrogen does appear to affect food intake and meal patterns.

As the data collected in this thesis were gathered until Day 16, it is assumed that estrogen was relatively low during this period of investigation. It would follow, therefore, that low levels of estrogen would be associated with high levels of food intake, which was observed, and might also be expected to affect meal duration and meal size. Although meal size was never assessed in this thesis, in the last chapter meal duration was found to not change from the first to the second week of lactation. Although in contrast to Strubbe and Gorissen's (1980) findings, this would be consistent with the fact that estrogen levels are relatively unchanged across the first two weeks of lactation (Smith & Neill,

1977; Taya & Greenwald, 1982).

The focus of the last chapter of this thesis was around the patterns of intake across the light/dark cycle and the effects of various diets on the selection patterns. Protein intake was found to be higher in the dark period and in Week 2 of lactation. Differences across diet groups were observed; dams who were able to self-select protein ate fewer calories than did the powdered-chow rats. Meal patterns were different in the light and dark parts of the cycle in that meal frequency, meal duration, and total time spent eating were all greater in the dark period. Here too, diet was a factor; powdered-chow rats ate longer meals and spent more time eating than did rats presented with the low protein-high protein diets or control diet. There was no change in any of these meal parameters from Day 4 to Day 10 of lactation.

Given that there was an increase in food and protein intake across the weeks of lactation, but no change in meal frequency or duration, the dams must have had to eat their meals more quickly. This is probably especially true in the dark period of the second week of lactation where hyperphagia is its most pronounced. The dark appears to be a time of concentrated feeding, whereas, the light period is a time for nursing and caring for the young (Munday and Williamson, 1983).

It is interesting to note that there are other circumstances, besides lactation, where rats will display rapid eating. One substance which has been shown to result in an increased speed of eating is amphetamine, specifically low doses of amphetamine. Wise, Fothui, and Colle (1989) demonstrated that when d-

amphetamine was placed in the nucleus accumbens of male rats, the rats ate meals more quickly. Furthermore, Wise et al. (1989) have suggested that this effect was likely due to a dopaminergic mechanism specific to the nucleus accumbens and not mediated via the hypothalamus because of the rapid onset of the effect. This is interesting in that in male rats, prolactin administration has been shown to result in greatly elevated levels of DA in the nucleus accumbens (Gonzalez-Mora, Guadalupe, & Mas, 1990). It is possible that, in lactation, where prolactin levels are high and where the dam may have to increase her speed of eating, the meal patterns of the dam are affected by DA activity separate from any feeding and satiety effects associated with the hypothalamus. Although there is evidence of feedback between prolactin and DA and 5-HT in the hypothalamus, it has not yet been determined whether the mechanisms affecting feeding behavior in lactation are specific to the hypothalamus or are affected by other areas of the brain.

Taken together the results of the experiments presented in this thesis suggest that it is necessary to conduct more exploration into the effects of brain chemistry and hormonal status of the lactating dam on feeding behavior, including total food intake, protein self-selection, and meals patterns.

As has been stated earlier, there is a wealth of evidence to suggest that both hormones and other chemical messengers, such as, DA and 5-HT, may affect either food and/or macronutrient intake. Meal size has been shown to be affected by changes in 5-HT. In free-feeding male rats, tryptophan will lead to a decrease in meal size and total food intake, and has been shown to decrease meal

frequency in the dark cycle. Moreover, these effects are found in the first four hours of the dark part of the cycle, immediately after administration of tryptophan. As Leibowitz (Stanley, et al., 1989) and Yokogoshi and Wurtman (1986) have made clear, however, it is difficult to assess the effects of light/dark cycle patterns of neurotransmitters, such as 5-HT, when mixed diets are presented.

In order to assess the effects of 5-HT on macronutrient self-selection and meal patterns in the lactating dam, each macronutrient must be presented in a separate container. Dial and Avery (1991) did follow this procedure. They presented protein, carbohydrate, and fat in separate containers, where each macronutrient was combined with minerals and vitamins, and examined feeding during pregnancy and lactation. They reported that protein intake and carbohydrate intake were greater during lactation than during pregnancy. There are, however, methodological problems with this research as litter sizes were not culled until Day 14, so that dams nursing 4 pups were included with dams nursing 14 pups. Furthermore, they did not present diet intake for days or weeks of the reproductive episodes under investigation; instead, they reported total intake of each macronutrient during the entire pregnancy period and total intake during lactation.

In a similar investigation, Woodside (personal communication, September, 1992) presented diets similar to those employed by Dial and Avery (1991) to dams during pregnancy and lactation; in the latter period litter sizes were culled to eight pups. Woodside found that, during the last week of pregnancy, protein was selected in larger amounts than were carbohydrate or fat. From the first to

the last week of pregnancy, carbohydrate intake decreased, whereas, fat intake remained fairly stable throughout the pregnancy period. These data highlight the possible role of estrogen in influencing diet intake. The decrease in carbohydrate intake with the preference for protein intake in the last week is consistent with the effects of estrogen on macronutrient choice (see Cohen & Woodside, 1989; Sandberg & Stewart, 1982). When estrogen levels are high, the observed decrease in caloric intake reflects a decrease in carbohydrate intake with a sparing of percentage protein intake (Wurtman & Baum, 1980).

Questions have been raised concerning the ease with which results from experiments that differ in terms of diet presentation methodology can be compared. For example, some experiments have used a two-choice test and others have presented each macronutrient in a separate container. It will be recalled that two-choice tests allow the experimenter to present rats with a choice between two isocaloric diets but two macronutrients are manipulated at the same time (i.e., if the diets are isocarbohydrate, both percentage fat and percentage protein must be different between the diets). On the other hand, when the macronutrients are presented in three separate bowls, this problem is eliminated but because protein and carbohydrate have a lower caloric weight than fat, the three diets must differ in terms of caloric density. Comparing the results presented in this thesis with the findings reported by Woodside where the macronutrients were presented individually, it is interesting to note that the pattern of macronutrient intake is quite similar. In both cases protein is a

preferred nutrient during lactation, and protein intake increases during the lactation period.

It now would be interesting to use the presentation of macronutrients in separate containers to examine intake in lactation over the light/dark cycle, and to separate the cycle into blocks so that intake early in the dark part of the cycle could be compared with intake later in the dark period. It is yet to be determined whether the lactating dam selects carbohydrate and protein meals in a fashion similar to males, where carbohydrate is preferred early in the dark hours and protein is preferred at the end (Larue-Achiogotis et al., 1991; Tempel & Leibowitz, 1990).

Moreover, the presence or absence of DA and/or 5-HT could be manipulated through the adminstration of substances that would selectively either block or enhance a particular messenger. It would also be important to compare central and peripheral administration of hormones and other messengers.

It is further possible that these mechanisms are not responsible or are not solely responsible for food intake and macronutrient patterns observed in the lactating female rat. Clearly, there are many differences between lactating females and males, the latter being the usual subject of choice in feeding experiments. It may be that the taste and olfactory changes that accompany a change in reproductive status could influence diet choice. Indeed, odor, taste, and texture cues have often been discussed as possibly affecting the intake of protein even in the male rat.

Heinrichs, Deutsch, and Moore (1990) have demonstrated that

protein-deprived rats were able to use odor cues of some proteins to determine which diets to ingest, and they further suggest that olfactory cues can be used irrespective of taste or texture indicators. Ashley (1986) has suggested that taste receptors may be influenced by receptors found in the liver or intestine that are sensitive to amino acids. Such a mechanism, or an olfactory mechanism, would, as Heinrichs et al. (1990) have suggested, allow for a much faster signal than would a mechanism that involves changing concentrations of amino acids in the bloodstream and across the blood-brain barrier leading to a change in neurotransmitter status.

Although Gibson and Booth (1986) have reported that protein appetite is learned in rats that are in protein deficit, others have suggested that there is a specific appetite for protein that does not involve any conditioning (DiBattista, 1991; Deutsch, Moore, & Heinrichs, 1989). DiBattista (1991) presented male rats with macronutrients in separate containers, and after allowing the rats a period to become familiar with the diets, placed them on a regimen where the protein diet was made available during only one hour of the day. A substantial preference for the protein diet was demonstrated following the restriction period. In the female rat, lactation is a period where protein need is enhanced and this, in turn, could trigger a specific appetite for protein. This might be accomplished through the effects of sensory cues and/or brain chemistry. It is not yet clear which mechanisms allow the female rat to regulate protein intake during lactation. Further research should serve to investigate taste, odor, and texture cues, as well as,

more central mechanisms.

Throughout this thesis and in previous work (e.g., Cohen & Woodside, 1989; Leshner, et al., 1972) it has been demonstrated that lactating rats selectively increased protein intake. These data give rise to questions of what function might be served by such a change in ingestive behavior. In the experiment described in Chapter Three of this thesis, the data appear to show no differences in dam weight or pup growth as a function of the ability to self-select protein, suggesting that as long as there is some minimum level of protein in the diet female rats can compensate either behaviorally or metabolically so as to obtain the protein requirements of lactation. The data from this chapter show, however, that being able to choose to ingest a protein rich diet saves the female time. As foraging is costly in terms of risks of predation this ability might well produce benefits. Perhaps above all these data illustrate the capacity of the lactating female to discriminate protein density; a trait that is of little use in the laboratory when a single composite diet is typically presented, but, may be very useful in the wild where animals come across a variety of foodstuffs that vary considerably in their protein content.

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Appendix A1

Dam weight

Source	SS	DF	MS	F	p
Groups	2269.549	2	1134.774	1.549	.241
Error	11715.087	16	732.192		

Appendix A2

Number of pups at birth

Source	SS	DF	MS	F	p
Groups	19.016	2	9.508	.744	
Error	204.333	16	12.770		

Appendix A3

Weight of pups (g) at birth

Source	SS	DF	MS	F	p
Groups	1.121	2	.560	1.682	.216
Error	5.331	16	.333		

Appendix A4

Intake of the low protein (LP) and high protein (HP) diets for five days pre-partum and the day of parturition (DOP) for Placentae-Removed (Removed), Disturbed, and Undisturbed groups

			Days Pre-partum					
			-5	-4	-3	-2	-1	DOP
Group	Diet							
Removed	LP	M	9.15	6.67	5.76	5.74	4.34	3.03
		SEM	2.34	1.31	1.66	1.11	0.72	0.72
	HP	M	9.57	10.47	10.66	11 02	6.20	4.26
		SEM	1.27	1.10	1.16	2.02	1.82	1.31
Disturbed	LP	M	5.96	6.12	5.71	3.66	3.54	1.56
		SEM	0.91	0.97	0.68	1.11	1.31	0.27
	HP	M	10.53	9.39	9.32	8.22	6.73	4.75
		SEM	1.04	0.78	1.03	1 82	2.28	2.12
Undisturbed	LP	M	7.16	5.71	7.06	4.61	4.89	5.80
		SEM	1.80	1.51	1.05	1.27	1.00	0.68
	HP	M	15.27	17.35	14.25	15.32	9 89	7 51
		SEM	1.51	1.39	1.31	1.58	1.03	1.34

Appendix A5

Intake of the low protein and high protein diets pre-partum

Source	SS	DF	MS	F	p
Between					
Groups	218.798	2	109.399	17.050	<.001
Error	102.657	16	6.416		
Within					
Diets	802.524	1	802.524	18.375	<.001
Diets x Groups	191.977	2	95.988	2.197	.142
Error	698.782	16	43.673		
Days	10.757	2	5.378	1.630	.210
Groups x Days	4.337	4	1.084	.328	
Error	105.589	32	3.299		
Diet x Days	16.463	2	8.231	1.194	.316
Groups x Diets x Days	60.041	4	15.010	2.177	.093
Error	220.615	32	6.894		

Appendix A6

Percentage protein intake three days prior to parturition

Source	SS	DF	MS	F	p
Between					
Groups	109.477	2	54.738	.584	
Error	1497.335	16	93.583		
Within					
Days	73.98	2	36.99	2.139	.132
Groups x Days	96.546	4	24.136	1.395	.257
Error	553.292	32	17.290		

Appendix A7

Percentage protein intake days 1-2 postpartum

Source	SS	DF	MS	F	p
Between					
Groups	371.976	2	185.988	1.221	.321
Error	2435.246	16	152.202		
Within					
Days	125.667	1	125.667	4.595	.045
Groups x Days	76.792	2	38.396	1.404	.273
Error	437.542	16	27.346		

Appendix A8

Intake of the low protein and high protein diets three days prior to parturition

Source	SS	DF	MS	F	p
Between					
Groups	192.236	2	96.118	5.506	.015
Error	279.280	16	17.455		
Within					
Diets	751.480	1	751.480	21.285	<.001
Groups x Diets	88.224	2	44.112	1.249	.313
Error	564.865	16	35.304		
Days	168.43	12	84.215	17.728	<.001
Groups x Days	20.402	4	5.100	1.073	.386
Error	152.009	32	4.750		
Diets x Days	58.147	2	29.073	5.487	.009
Groups x Diets X Days	19.540	4	4.885	.921	
Error	169.554	32	5.298		

Appendix A9

Calculations of percentage protein intake for two groups with disparate total food intake

The following two groups selected the same percentage protein intake, but would be found to be significantly different from one another if intake of the low protein and high protein diets was assessed as this measure's main effect for group would take total food intake into account:

	Group 1	Group 2
Low protein	25 g	100 g
High protein	25 g	100 g
Total food intake	50 g	200 g

Percentage Protein

$$\frac{.05(25) + .45(25)}{50\ g} \times 100 = 25\%$$

$$\frac{.05(100) + .45(100)}{200\ g} \times 100 = 25\%$$

Although these groups have selected an equivalent percentage of their diet as protein, one group has clearly eaten more protein in grams and more total food. Percentage protein intake is not a good measure to determine protein intake when total food intake is different across groups.

Appendix A10

Intake of the low protein and high protein diets days 1-2 postpartum

Source	SS	DF	MS	F	p
Between					
Groups	25.438	2	12.719	1.356	.285
Error	150.049	16	9.378		
Within					
Diets	53.033	1	53.033	.911	
Groups x Diets	159.709	2	79.854	1.372	.281
Error	930.876	16	58.179		
Days	104.985	1	104.985	23.140	.001
Groups x Days	14.472	2	7.236	1.594	.232
Error	72.591	16	4.536		
Diets x Days	30.071	1	30.071	2.916	.104
Groups x Diets x Days	54.003	2	27.001	2.618	.102
Error	164.976	16	10.311		

Appendix A11

Intake of the low protein and high protein diets difference scores days 1-2 postpartum

Source	SS	DF	MS	F	p
Between					
Groups	128.725	2	64.362	3.031	.0765
Error	339.780	16	21.236		
Within					
Diets	105.112	1	105.112	23.144	.0002
Groups x Diets	14.484	2	7.242	1.595	.2336
Error	72.667	16	4.542		
Days	239.901	1	239.901	5.856	.0278
Groups x Days	202.030	2	101.015	2.466	.1166
Error	655.447	16	40.965		
Diets x Days	30.137	1	30.137	2.916	.1070
Groups x Diets x Days	54.119	2	27.059	2.618	.1038
Error	165.359	16	10.335		

Appendix A12

Intake of the low and high protein diets difference scores - 16 days

Source	SS	DF	MS	F	p
Between					
Groups	2236.005	2	1118.002	5.281	.0173
Error	3386.935	16	211.683		
Within					
Diets	12154.554	1	12154.554	279.201	.0000
Groups x Diets	30.705	2	15.352	.352	.7083
Error	696.683	16	43.561		
Days	21319.822	15	1421.321	24.006	.0000
Groups x Days	2014.351	30	67.145	1.134	.2957
Error	14209.557	240	59.206		
Diets x Days	7570.203	15	504.680	21.080	.0000
Groups x Diets x Days	846.845	30	28.228	1.179	.2471
Error	5745.957	240	23.941		

Appendix B1

Intake of the low protein and high protein diets expressed in weekly blocks

Source	SS	DF	MS	F	p
Between					
Groups	1100.474	1	1100.474	70.148	<.001
Error	235.315	15	15.687		
Within					
Diets	1807.421	1	1807.421	32.832	<.001
Groups x Diets	845.098	1	845.098	15.351	.001
Error	825.750	15	55.050		
Weeks	609.550	1	609.550	140.667	<.001
Groups x Weeks	69.968	1	69.968	16.146	.001
Error	64.999	15	4.333		
Diets x Weeks	379.028	1	379.028	30.827	<.001
Groups x Diets X Weeks	99.262	1	99.262	8.073	.012
Error	184.427	15	12.295		

Appendix B2

Gram protein intake in weekly blocks

Source	SS	DF	MS	F	p
Between					
Groups	400.480	1	400.480	60.984	<.001
Error	98.504	15	6.566		
Within					
Weeks	201.262	1	201.262	146.357	<.001
Groups x Weeks	33.059	1	33.059	24.040	<.001
Error	20.627	15	1.375		

Appendix B3

Percentage Protein

Source	SS	DF	MS	F	p
Between					
Groups	456.651	1	456.651	9.424	.007
Error	726.830	15	48.455		
Within					
Weeks	24.600	1	24.600	.842	
Groups x Weeks	.055	1	.055	.001	
Error	437.946	15	29.196		

Appendix B4

Pup weights (g)

Source	SS	DF	MS	F	p
Between					
Groups	5048.861	1	5048.861	30.320	<.001
Error	2497.786	15	166.519		
Within					
Days	159830.555	4	39957.638	789.985	<.001
Groups x Days	3032.306	4	758.076	14.987	<.001
Error	3034.813	60	50.580		

Appendix C1

Average body weight on Day1 and Day 16 in galactophore-cut, sham-operated, and nonlactating groups

		Day 1	Day 16
Galactophore-cut	*M*	307.78	367.09
	SEM	7.18	11.76
Sham-operated	*M*	297.84	334.90
	SEM	5.46	5.54
Nonlactating	*M*	309.02	332.80
	SEM	5.63	9.12

Appendix C2

Dam weight

Source	SS	DF	MS	F	p
Groups	590.575	2	295.287	8.496	.001
Error	834.128	24	34.755		

Appendix C3

Intake of the low protein and high protein diets in weekly blocks

Source	SS	DF	MS	F	p
Between					
Groups	3152.673	2	1576.336	45.312	<.001
Error	834.919	24	34.788		
Within					
Diets	723.283	1	723.283	23.879	<.001
Groups x Diets	887.376	2	443.688	14.648	<.001
Error	726.933	24	30.288		
Weeks	175.542	1	175.542	160.501	<.001
Groups x Weeks	325.238	2	162.619	148.685	<.001
Error	26.249	24	1..093		
Diets x Weeks	259.377	1	259.377	48.040	<.001
Groups x Diets x Weeks	285.215	2	142.607	26.412	<.001
Error	129.579	24	5.399		

Appendix C4

Gram protein intake in weekly blocks

Source	SS	DF	MS	F	p
Between					
Groups	797.195	2	398.597	59.835	<.001
Error	159.876	24	6.661		
Within					
Weeks	87.096	1	87.096	134.172	<.001
Groups x Weeks	125.899	2	62.949	96.974	<.001
Error	15.579	24	.649		

Appendix C5

Percentage protein

Source	SS	DF	MS	F	p
Between					
Groups	340.796	2	170.398	3.80	.036
Error	1076.031	24	44.834		
Within					
Weeks	105.560	1	105.560	19.367	<.001
Groups x Weeks	90.308	2	45.154	8.284	.002
Error	130.807	24	5.450		

Appendix D1

Average body weight on Day 1 and Day 16 in low protein-high protein, control, and powdered-chow groups

		Day 1	Day 16
low protein-high protein	*M*	291.64	324.18
	SEM	5.03	2.32
control	*M*	288.74	332.70
	SEM	8.44	8.14
powdered-chow	*M*	306.24	333.49
	SEM	8.24	7.60

Appendix D2

Dam weight

Source	SS	DF	MS	F	p
Groups	193.047	2	96.523	2.457	.103
Error	1060.276	27	39.269		

Appendix D3

Litter Weight

Source	SS	DF	MS	F	p
Between					
Groups	10444.739	2	5222.369	5.168	.012
Error	28294.276	28	1010.509		
Within					
Days	994925.609	1	994925.609	1090.862	<.001
Groups x Days	11094.162	2	5547.081	6.081	.006
Error	25537.508	28	912.053		

Appendix D4

Caloric intake

Source	SS	DF	MS	F	p
Between Groups	19930.60	2	9965.15	5.84	0.0076
Error	47753.38	28	1705.48		
Within Cycle	371084.33	1	371084.33	212.97	0.00
Groups x Cycle	64.330.95	2	32165.47	18.46	0.00
Error	48787.15	28	1742.40		
Days	334878.37	15	22325.22	115.42	0.00*
Groups x Days	17591.83	30	586.39	3.03	0.00†
Error	81241.38	420	193.43††		
Cycle x Days	19015.30	15	1267.69	6.56	0.00*
Cycle x Days x Groups	8994.83	30	299.83	1.55	0.03¥
Error	81128.40	420	193.16¥¥		

	Greenhouse Geisser	Huynh Feldt
*	0.0000	0.0000
†	0.0007	0.0001
¥	0.0338	0.0921

Epsilon Factors for Degrees of Freedom Adjustment

	Greenhouse Geisser	Huynh Feldt
††	0.3989	0.5556
¥¥	0.4864	0.7218

Appendix D5

Gram protein intake

Source	SS	DF	MS	F	p
Between Groups	374099	2	187.50	17.91	0.00
Error	293.18	28	10.47		
Cycle	16916.60	1	1696.60	155.81	0.00
Groups x Cycle	296.12	2	148.06	13.60	0.0001
Error	304.89	28	10.889		
Days	1700.32	15	113.35	98.61	0.00*
Groups x Days	112.46	30	3.75	3.26	0.00†
Error	482.78	420	1.15**		
Cycle x Days	66.93	15	4.46	3.88	0.00¥
Cycle x Days x Groups	35.97	30	1.20	1.04	0.4065+
Error	482.64	420	1.15††		

	Greenhouse Geisser	Huynh Feldt
*	0.0000	0.0000
†	0.0002	0.0000
¥	0.0005	0.0000
+	0.4122	0.4108

Epsilon Factors for Degrees of Freedom Adjustment

	Greenhouse Geisser	Huynh Feldt
**	0.4167	0.5879
††	0.4728	0.6947

Appendix D6

Intake of the low protein and high protein diets

Source	SS	DF	MS	F	p
Cycle	2608.68	1	2608.68	48.98	0.0002
Error	372.83	7	53.26		
Diets	9369.95	1	9369.95	94.50	0.0000
Error	694.11	7	99.16		
Cycle x Diets	471.05	1	471.05	10.70	0.0136
Error	308.11	7	44.02		
Days	2021.22	15	134.75	28.82	0.0000*
Error	490.90	105	4.68**		
Cycle x Days	124.02	15	8.27	1.77	0.0491†
Error	491.02	105	4.68††		
Diets x Days	1355.51	15	90.37	8.44	0.0000¥
Error	1123.66	105	10.70¥¥		
Cycle x Diets x Days	85.33	15	5.69	0.58	0.8876+
Error	1037.52	105	9.88++		

	Greenhouse Geisser	Huynh Feldt
*	0.0000	0.0000
†	0.1676	0.0944
¥	0.0001	0.0000
+	0.6973	0.8639

Epsilon Factors for Degrees of Freedom Adjustment

	Greenhouse Geisser	Huynh Feldt
**	0.2643	0.6525
††	0.2524	0.5867
¥¥	0.2863	0.7974
++	0.2926	0.8452

Appendix D7

Meal frequency

Source	SS	DF	MS	F	p
Between					
Groups	7.730	2	3.865	.954	
Error	40.487	10	4.048		
Within					
Cycle	158.250	1	158.250	36.899	<.001
Groups x Cycle	21.001	2	10.500	2.448	.135
Error	42.887	10	4.288		
Days	6.450	1	6.450	1.260	.287
Groups x Days	13.437	2	6.718	1.312	.312
Error	51.187	10	5.118		
Cycle x Days	2.143	1	2.143	.682	
Groups x Cycle x Days	4.501	2	2.250	.717	
Error	31.387	10	3.138		

Appendix D8

Meal duration

Source	SS	DF	MS	F	p
Between					
Groups	1403269.544	2	701634.772	5.456	.024
Error	1285925.984	10			
Within					
Cycle	2797818.440	1	2797818.440	33.793	<.001
Groups x Cycle	902045.683	2	451022.841	5.447	.024
Error	827917.447	10	82791.744		
Days	97.140	1	97.140	.001	
Groups x Days	257374.059	2	128687.029	1.540	.260
Error	835096.814	10	83509.681		
Cycle x Days	4105.739	1	4105.739	.074	
Groups x Cycle x Days	197844.902	2	98922.451	1.785	.216
Error	5539787.617	10	55397.861		

Appendix D9

Total time eating

Source	SS	DF	MS	F	p
Between Groups	78285057.843	2	39142528.921	5 892	02
Error	66430377.000	10	6643037.699		
Within Cycle	540655836.50	1	540655836.50	156 252	<.001
Groups x Cycle	28565503.875	2	14282751.937	4.127	.048
Error	34601385.000	10	3460138.500		
Days	3807334.285	1	3807334.285	.973	
Groups x Days	1156080.016	2	578040.008	.147	
Error	39098036.00	10	3909803.599		
Cycle x Days	20601.361	1	20601.361	010	
Groups x Cycle x Days	5044817.195	2	2522408.597	1.323	309
Error	19057413.250	10	1905741.325		

Appendix D10

Time spent with pups

Source	SS	DF	MS	F	p
Between					
Groups	158190.00	1	15819.00	.006	
Error	158134292.00	6	26355715.335		
Within					
Cycle	352597282.00	1	352597282.00	10.427	.017
Groups x Cycle	30646628.00	1	30646628.00	.906	
Error	202890990.00	6	33815165.00		
Days	274330168.00	1	274330168.00	19.166	.005
Groups x Days	17046968.00	1	17046968.00	1.191	.317
Error	85878419.00	6	14313069.832		
Cycle x Days	5412056.00	1	5412056.00	.141	
Groups x Cycle x Days	35823878.00	1	35823878.00	.935	
Error	229834857	6	38305809.50		