Protein Intake in the Rat:
A Function of Reproductive State
and Properties of the Diet

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ABSTRACT

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Protein intake in the female rat during pregnancy and lactation was investigated. In experiment 1, three lactating groups and three nonlactating control counterparts were presented with one of three diet conditions for a period of 14 days. Two of the diet conditions allowed for a choice between either low (5%) protein - high (45%) protein, or protein (43%) - carbohydrate (0% protein) diets and the third condition consisted of 22% protein (stock chow) placed in two jars. Lactating females selected a greater percentage of protein than nonlactating females; however, the percentages selected differed depending upon the diets presented. Rats presented with the protein - carbohydrate diets chose percentages less than that of the stock chow; whereas, rats presented with the low protein - high protein diets selected percentages greater than that of the stock chow. These differences were attributed to differences in palatability between diets. Lactating rats given stock chow increased total food intake such that they ate the same number of grams of protein as the lactating rats given the low protein - high protein diet choice. In experiment 2 a similar procedure was

employed, except that the period of study was extended to include pregnancy. Two groups (impregnated and nonimpregnated) were given the low protein - high protein diets, and one group of impregnated rats were fed stock chow. During pregnancy the impregnated groups chose similar amounts of protein; but, during lactation the stock chow group ate a greater amount. These studies showed that the female rat can regulate protein intake, and that protein intake was increased during pregnancy and lactation. Further research will attempt to clarify the mechanisms underlying this pattern of diet selection.

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I would also like to thank my parents for understanding all the late hours and weekends spent at the lab. Dear mom and dad: I'll see you after the Ph.D.

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Animals have been classified as either specialists or generalists on the basis of their food selection behavior. Specialists, are dependent upon a particular food or class of foods, and their food selection behavior is said to be genetically determined. Generalists, on the other hand, eat a wide variety of foods, and for the most part, it has been supposed that their food selection behavior is governed by learning mechanisms. It is to this latter group that the rat belongs (Rozin, 1976). Since the rat chooses from and eats many different types of substances, it should be able to discern which foodstuffs are harmful, and which are beneficial to its survival. Indeed a number of studies have documented the rat's apparent ability to select a diet appropriate for its organismic state (for reviews see Overman, 1976, and Rozin, 1976). For example, rats that suffer from an inability to retain sodium because of adrenalectomy will show an increased appetite for sodium (Nachman, 1962), thiamine deficient rats will eat more of a diet containing thiamine (Rozin and Rodgers, 1966), and during pregnancy and lactation, rats will select great amounts of protein, fat and calcium (Richter and Barelare, 1938).

Perhaps the most impressive evidence for the rat's ability to select foodstuffs in a way that is beneficial to its well-being, can be found in the cafeteria selection studies that Richter reported in the 1930's. These early studies of diet selection in the rat conducted by Richter and others (e.g. Kon, 1931; Pilgram and Patton, 1947), employed a cafeteria selection procedure where each diet component was presented in a separate container, in order to assess whether the rat was capable

of selecting sufficient amounts of various nutrients to maintain normal growth and reproductive success.

Richter and Barelare (1938) found that as well as being able to select a diet that maintained normal growth, the female rat changes her dietary self-selection pattern as a function of reproductive state. They presented 11 dietary elements, three dry (powdered) and eight liquid, to 10 female rats for an initial period of one month. All rats maintained a 4-5 day estrous cycle and selected a diet which resulted in normal growth. The females were then mated, and the diet selection procedure continued from day 1 of pregnancy through to day 35 postpartum, with pups weaned on day 25. Pregnancies resulted in a normal litter size and dams nursed their young. When intake of the dietary components was compared across estrous cycling, pregnancy, and lactation periods, three specific diet elements were found to be of importance during the latter two reproductive states. Protein (casein), fat (olive oil), and calcium (2.4% solution calcium lactate) were selected in greater amounts during pregnancy and even greater amounts during lactation than were selected during the pre-mating period. Richter and Barelare (1938) suggested that by increasing intake of these specific nutrients, rat dams were able to meet the nutritional demands of their litters. It is not clear, however, whether the dam first becomes deficient in some nutrient and consequently increases intake or whether her intake changes occur before a deficiency develops.

It is interesting that when compared with animals fed a stock chow, animals presented with the self-selection regime consumed less calories

during the pre-mating, lactation, and post-weaning periods. This suggests that when presented with a stock chow rats must increase food and therefore caloric intake, in order to increase the intake of specific nutrients. On the other hand, the cafeteria fed rats are able to be selective, and need not ingest a large amount of calories (Richter and Barelare, 1938).

In some cases, a long-delay learning mechanism similar to that proposed in taste aversion studies, has been used to explain the food selection behavior of the rat. Rozin (1968) pointed out that the diet deficient animal can be compared to the poisoned animal, in that the inducement of a deficiency may be termed a slow poisoning effect. Rodgers and Rozin (1966) demonstrated that vitamin deficient rats, when presented with a diet that had induced the deficiency, and with a novel food, selected the latter. It has also been shown that rats will select the novel food whether or not it contained the needed vitamin, and whether or not the familiar food had been supplemented with this vitamin. It is clear that their apparent display of neophilia may simply have reflected their avoidance of the familiar food. Indeed, in a later study Rozin (1968) showed that given the choice between the familiar deficient diet, a novel diet and a familiar-preferred food, the rat selected the familiar-preferred diet. In this instance, neophobic behavior was evident. The familiar-unsafe food was abandonned, but no neophilic behavior had occurred. Thus, Rozin (1968) has proposed that, there will be a preference for a familiar-safe diet, but that the novel diet will be acceptable if there is a forced two choice test situation

between novel and unsafe foods.

Since the cafeteria selection procedure offers a choice of a variety of foodstuffs, deficiencies of, and subsequent preferences for, individual nutrients would have to have occurred in an extremely short space of time. Therefore, as Rozin and Kalat (1971) have suggested, it is difficult for a long-delay learning mechanism to account for such findings. There may be other factors besides learning that influence pattern of selection across reproductive states in the rat and the results of a number of studies suggest that this is, in fact, the case.

Although the rat is a generalist it can also display behavior characteristic of a specialist (Rozin, 1976). For example, there has been some question as to whether some specific hungers should be classified as "innate"; whereas, others seemed more appropriately defined as "learned" (Rozin and Kalat, 1971). The specific hunger for salt, for example, has generally been identified as "innate", or genetically hard-wired, and the animal's salt eating behavior is indicative of a specialist. Evidence in support of this can be found in Nachman's (1962) study where he presented a salt (NaCl) solution to the sodium deficient rat. He found an immediate, that is within 15 seconds, preference for the NaCl solution. In other words, the first taste of NaCl solution appeared to be sufficient for the rat to recognize a source that would satisfy its need for salt.

It has been suggested by Rozin and Kalat (1971) that it makes sense for salt to be so easily accepted. Salt is an extremely necessary element to the rat's survival, as it is so vital to fluid balance within

the organism, and is required in fairly large quantities. In contrast, specific hungers for elements which are not as immediately crucial to survival, could instead be governed by learning mechanisms, that is, the animal may be able to afford the delay in associating recovery with a new food. Again, however, it is not clear how such an interpretation can explain the results of studies of cafeteria selection in the rat.

Another factor that has been demonstrated to influence dietary self-selection in the female rat is that of hormonal status. It has been shown that when estradiol titers are high, as on the day of estrus, food intake will be decreased (Tartellin and Gorski, 1971; Wade, 1972, 1975). Wurtman and Baum (1980) extended these findings using a two choice diet selection paradigm and showed a reduction in carbohydrate intake at estrus, with a maintenance of protein intake at this time. Further, they were able to demonstrate that these changes could be elicited by administration of estradiol benzoate.

Results of a study by Sandberg, Stewart and Amit (1982) also suggest that high estradiol levels may be associated with reduced carbohydrate intake or at least caloric intake. They found that intake of sucrose and ethanol was decreased in the rat during the last week of pregnancy, whereas, intake of saccharin was maintained. They suggested that this decrease in carbohydrates might be related to the change in carbohydrate metabolism which occurs during the last trimester of pregnancy.

Moreover, Sandberg and Stewart (1982) were able to induce similar changes in patterns of intake by administering estradiol benzoate to

non-pregnant ovariectomized females. These studies indicate that estrogen, and possibly other hormones may play an important role in influencing diet selection in the rat. The mode of hormonal action, however, is not clear. One possibility is that the hormone rapidly creates an apparent deficiency in the animal by changing enzyme levels in a manner analogous to that described by Wade (1976) to explain estrogenic effects on food intake. Given these recent data it seems probable that the pattern of diet selection across reproductive states observed by Richter and Barelare (1938) in the female rat reflects some hormonal influence independent of the nutritional demands of the litter, and it would clearly be of interest to investigate this possibility. Before this can be done, however, it is necessary to develop a paradigm that yields reliable baseline data and, unfortunately, to this point such a paradigm does not seem to be available.

There has, for example, been difficulty in replicating the results obtained by Richter and Barelare (1938). Tribe (1955) also employed a cafeteria selection procedure, but reported problems with self-selection of a normal diet and hence reproductive outcome. Fifteen female rats were presented with seven dietary components. After an initial six week period, two rats failed to select an adequate diet and died. The remaining rats were then mated, one male per female, and three females failed to become impregnated. It is not reported, however, whether vaginal smears were taken to assess whether the females had been cycling normally before being introduced to the cafeteria diet.

For those rats who were cycling, and could therefore be

impregnated, dietary selection was followed throughout pregnancy and lactation. Litter size was normal, but pup weight at birth was slightly substandard. Pup weight at weaning was lower than that of pups reared by dams fed a commercial diet. Tribe (1955) reported that the self-selecting dams chose a diet inferior to a standard stock chow, in terms of percentages of nutrients as diet selected. Nevertheless, protein (casein) intake was increased in the last week of lactation.

The use of cafeteria selection procedures to investigate protein intake may be complicated by palatability factors. Casein, the most widely used source of protein, is unpalatable to rats. Once in the mouth and mixed with saliva, casein becomes gummy and paste-like. It is, thus, difficult to examine a preference for protein, when the foodstuff itself is not a preferred substance. Therefore, because of palatability factors and in order to examine the importance of a single nutrient separately, recent research has instead tended to employ a test between two diet mixtures, where the nutrient under investigation is present in one diet, and either absent or available in a small quantity in the other. Such two choice tests have been employed in an investigation of diet selection in active and nonactive rats (Collier, Leshner and Squibb, 1969) and in selection in cold temperatures (Leshner, Collier and Squibb, 1971). This procedure has also been used to examine diet selection over various reproductive periods in the female rat (Leshner, Siegel, and Collier, 1972).

When presented with a two choice test between a protein (45% protein) and a carbohydrate (0% protein) diet fraction, impregnated

animals selected a higher percentage of diet as protein, than did nonimpregnated controls (Leshner et al, 1972). Percentage protein intake was increased during the last week of pregnancy and days 5-10 of lactation. One puzzling aspect of their findings was that neither group, impregnated or nonimpregnated, selected a percentage of diet as protein equal to amounts found in commercial diets (22% protein). In addition, data were not recorded past day 10 of lactation, and so the period of peak milk production (days 14-16; Babicky, Ostadolova, Parizek, Kolar, and Bibr, 1970) was not included.

The findings reported by Leshner et al (1972) are similar to those obtained by Richter and Barelare (1938), since in both studies it was shown that protein intake increased over pregnancy and lactation.

Just as it was previously reported that there have been conflicting results obtained when cafeteria selection procedures were employed, two choice tests have also produced varying results. Prior to impregnation of the experimental group, in the Leshner et al (1972) study, data were recorded for all females, and vaginal smears taken in order to examine diet selection during the estrous cycle. Leshner et al (1972) did not find that self-selecting female rats altered their pattern of selection over the estrous cycle.

Wurtman and Baum (1980), however, as noted earlier, did report a change in pattern of selection over the estrous cycle. They presented a different two choice test to cycling female rats, from whom vaginal smears were taken daily. Leshner, et al's (1972) diets were criticized by Wurtman and Baum (1980) as rats were forced to eat from the protein

fraction in order to obtain any protein at all, since the carbohydrate fraction was protein-free. Thus, Wurtman and Baum (1980) presented a choice between a low protein (5% protein) and a high protein (45% protein) diet mixture, each containing equal amounts of carbohydrate (40% carbohydrate). It was found that food intake was decreased at estrus, and thus carbohydrate intake was also reduced; but, protein intake was maintained at levels similar to those selected during the other days of the cycle. The net result,therefore, was an increased percentage protein intake at estrus. This paradigm also yielded overall, a higher protein intake than that of Leshner et al (1972).

Since the Leshner et al (1972) and Wurtman and Baum (1980) diets produced different patterns of selection in the cycling female rat, the experiments described below were carried out in order to investigate the effects of the different diet fraction choices used in those studies on protein selection during pregnancy and lactation. It seemed probable that the proportion of diet as protein that the female rat selects is, in part, dependent upon the taste and texture properties of the specific diets presented, and that this factor might influence pattern of selection across reproductive states.

The overall objectives of these studies then, was to further examine the influence of reproductive state on protein intake, as well as to establish an experimental procedure that would yield reliable baseline data, against which the effects of hormonal manipulations could be assessed.

In experiment 1 the Leshner et al (1972) and Wurtman and Baum

(1980) diets were presented to both lactating and nonlactating rats and compared to controls presented with a stock chow. It was expected that lactating females would eat a higher amount of protein than their nonlactating female counterparts, and that in the case of the diet choice rats, percentage protein intake would also be affected in this fashion. It was further expected that the rats presented with the Wurtman and Baum (1980) diets would select a higher percentage of protein, than those presented with the Leshner et al (1972) diets.

Experiment 2 investigated protein selection over both pregnancy and lactation, where an impregnated group presented with the Wurtman and Baum diets was compared to a nonimpregnated control counterpart, and an impregnated control group presented with a stock chow. It was hypothesized that impregnated animals would increase protein intake during pregnancy and lactation as compared to nonimpregnated females.

Since in both experiments control groups were presented with a stock chow, it was possible to record normative data not only for food intake, but for pup growth as well. Therefore, in experiment 1 pup growth, and in experiment 2 litter size and pup growth, were recorded in order to evaluate the possible effects of diet on reproductive outcome.

Experiment 1

The properties of the diets presented to the female rat may influence patterns of dietary selection, more specifically, the percentage of protein selected. In the experiment described below, percentage protein intake during lactation was examined by comparing the the special diets of Leshner et al (1972) and Wurtman and Baum (1980), with powdered stock chow. Whereas Leshner et al (1972) reported protein intake until day 10 of lactation; thus, falling short of the period of peak milk production, this experiment recorded data until day 14 of the lactation period. The growth rates of the pups reared by mothers maintained on the different diets, as well as maternal weight change were measured throughout the lactation period.

Method

Subjects. Forty-eight female Wistar rats, originally obtained from Charles River Breeding Farms, St. Constant, Quebec, served as subjects. The lactating groups consisted of primiparous females mated in our laboratory. The females in the nonlactating groups had previously given birth to one litter. Their pups had been removed at least 48 hours prior to group assignment.

Apparatus. All rats were housed individually in polypropylene clear cages (38 x 33 x 17 cm) with zinc-plated wire bar lids. Wood chip bedding was provided. Food was provided ad lib in glass jars (4.5cm in diameter and 7cm high) which were glued with epoxy to aluminum sheets (1 mm x 8cm x 16.9cm) bent in an S-shape, so that the top lip curved over to fasten onto the side wall of the cage under the cage lid. General purpose wire was also attached around the jar and fastened behind the metal plate. A Sartorius scale was used daily to weigh the food jars. An Ohaus triple beam balance (2610g capacity) was used to determine the dams' and, where applicable, the pups' weight. Water was available ad lib in 100 ml capacity polyethylene graduated cylinders. An automatic timer controlled the light cycle at 12hr light/ 12hr dark with lights on at 0800 hrs and off at 2000 hrs. Room temperature was maintained at 21 ^{o}C.

Diets. The diet choices presented were as follows: 1) Low protein (LP) (5% protein) and high protein (HP) (45% protein) diets described by Wurtman and Baum (1980), 2) Protein (P) (43% protein) and carbohydrate (C) (0% protein) diets described by Leshner et al (1972),

and 3) Charles River powdered chow (CR) (22% protein). The LP-HP diets described below were prepared in our laboratory, and contained the following ingredients: With the exception of fat, all ingredients were obtained from ICN Nutritional Biochemicals, Cleveland, Ohio:

	LP	HP
Protein (Casein:Purified high nitrogen)	5%	45%
Carbohydrate (Dextrin)	40%	40%
Nonnutritive Bulk (Aplhacel)	22%	—
Fat (Vegetable Shortening)	33%	15%
plus the following per 1 kgm of diet mixture		
Vitamins (Vitamin diet fortification mixture)	2.2%	2.2%
Minerals (Rogers-Harper salt mixture)	4%	4%
Agar	3.5%	3.5%
Water	1000ml	1000ml
Caloric value (less 10% moisture)	4.32kcal/g	4.32kcal/g

The P-C diets were prepared in our laboratory according to the specifications outlined by Leshner, Collier and Squibb (1971). The soybean oil meal was obtained from Maple Leaf Mills, Ltd., and had 2%

less protein than the meal used by Leshner et al (1971). All other ingredients came from ICN. The composition of the diets was as follows:

	P	C
Soybean oil meal (48% protein)	90%	—
Carbohydrate (Dextrin)	—	90%
Fat (Corn oil)	5%	5%
Vitamins (Vitamin diet fortification mixture)	1%	1%
Minerals (Rogers-Harper salt mixture)	4%	4%
Dl-Methionine	2.7g/kg	—
Water	1000ml/kg	1000ml/kg
Caloric value (less 10% moisture)	2.95kcal/g	3.64kcal/g

The CR diet contained 22% protein, a minimum of 4% fat and a maximum of 5% fiber. The caloric value of the CR diet was 4.16 kcal/gm.

The LP-HP, and P-C diets were stored in air tight plastic containers and refrigerated. The CR diet was stored in a sealed plastic bin at room temperature.

Procedure. Twenty-four female rats were group mated in our

laboratory and transferred to plastic cages two days prior to the expected date of parturition. In order to ensure equal suckling stimulation to the dams, all litters were culled to 8 pups within one day of birth. Lactating females were assigned to one of the three following groups (n=8 per group): Lactating LP-HP diets, lactating P-C diets, or lactating CR diets. Twenty-four nonlactating female rats were divided equally amongst three groups and served as control counterparts to the lactating groups. On the day of group assignment the weight range and mean weight per group were as follows: Lactating LP-HP : 204.3g-304.3g ($\bar{X}$=271.59g); lactating P-C : 214.2g-282.8g ($\bar{X}$=249.38g); lactating CR : 250.5g-328.8g ($\bar{X}$=282.39g); nonlactating LP-HP : 250.7g-366.1g ($\bar{X}$=296.04g); nonlactating P-C : 249.4g-346.2g ($\bar{X}$=289.42g); nonlactating CR : 238.5g-284.7g ($\bar{X}$=258.82g).

All rats were presented with two food jars per cage; for the CR groups, the CR diet was placed in both jars. The jars were located on the left and right sides of the front of the cage. The positions of the jars were alternated daily in order to control for position preferences. The food was emptied and the jars cleaned with hot water every other day. Fresh food was added daily.

Food intake, water intake, female and, where applicable, litter weight, were recorded daily for 14 days. The amounts of LP-HP and P-C diet fractions eaten were divided by two, since water accounted for half the weight of the fractions, and since the CR diet did not have such a high water content. Percentage protein selected, protein intake (in grams) and caloric intake were also calculated daily.

Food intake, percentage protein selected, protein intake (in grams) and caloric intake were analyzed using a three-way split-plot ANOVA with two independent measures, diet and reproductive state, and one repeated measure, days. For each of these analyses, the Tukey (α =.05) post hoc test was employed in order to determine whether daily differences between groups were statistically significant.

Diet fraction choice of the LP-HP females was analyzed separately from that of the P-C females. For each, a three-way ANOVA with one independent measure (reproductive state) and two repeated measures (diet fraction and days) was used.

Proportional pup growth and percentage change in maternal weight were analyzed using a one-way ANOVA. A Tukey (α =.05) test was used for post hoc comparisons between groups.

Summary tables of all ANOVAs can be found in Appendix A.

Results

Food Intake

Mean daily food intake for lactating and nonlactating groups is shown in Figure 1. Lactating females ate more than their nonlactating counterparts, as evidenced by a significant main effect for reproductive state ($F(1,42) = 293.12$, $p < .01$). There was a significant main effect for days ($F(13,546) = 36.21$, $p < .01$), due apparently to the large increase in food intake in lactating animals. The significant reproductive state x days interaction ($F(13,546) = 57.45$, $p < .01$) reflects this difference between states. Furthermore, every post hoc comparison between lactating and nonlactating groups was significant. Although there was, as well, a significant main effect for diets ($F(2,42) = 11.10$, $p < .01$) apparently due to the fact that in both reproductive conditions the least amount of food was eaten by the LP-HP animals, more interesting is the significant diet x reproductive state interaction ($F(2,42) = 8.86$, $p < .01$). Among nonlactating females, diet did not affect food intake. Lactating females, on the other hand, appeared sensitive to diet. The diet x reproductive state x days interaction was not significant. Tukey post hoc tetsts revealed that among lactating animals, the CR group ate significantly more than the LP-HP group from days 3 to 14, with the exception of day 4. The CR and P-C groups differed significantly on days 10, 11, and 14 only. With the exception of day 9, there were no significant differences between P-C and LP-HP groups.

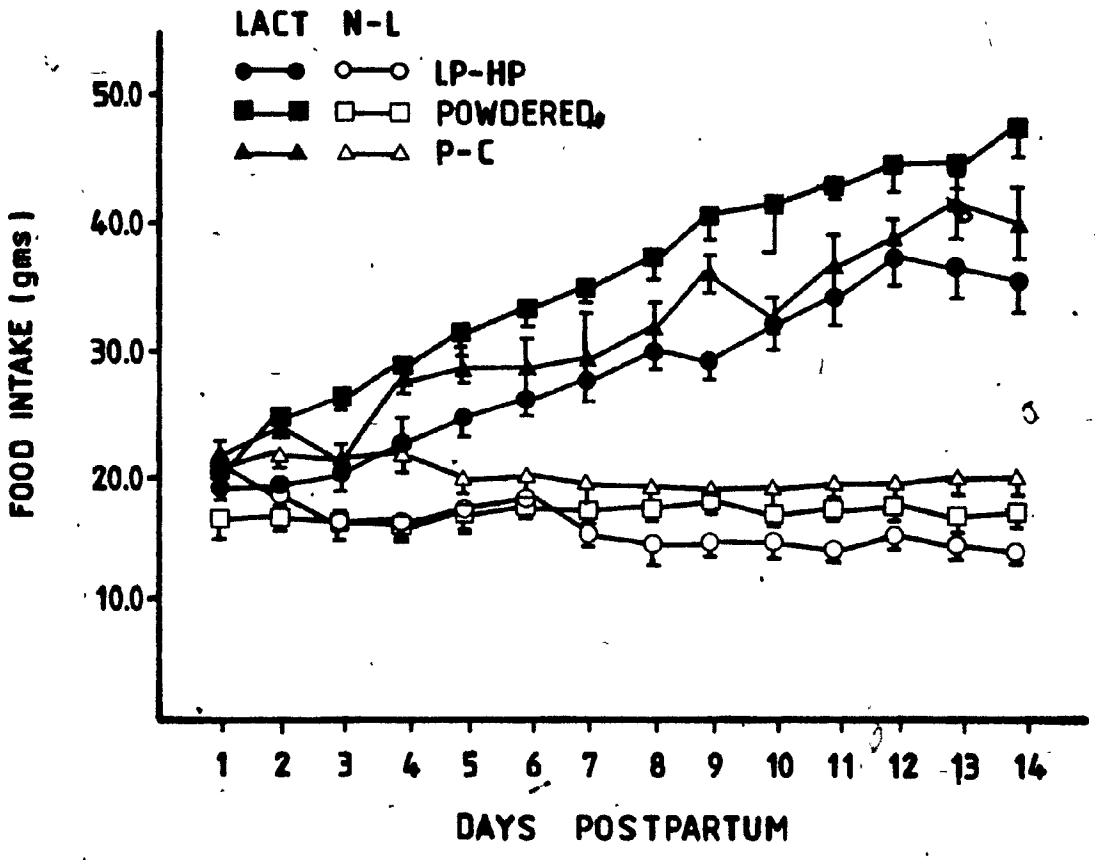

Figure 1. Mean daily food intake of LP-HP, P-C, and CR Lactating and Nonlactating Groups. SEMs are shown.

Diet Fraction Choice

Figure 2 depicts the amount of food eaten from each diet fraction within the LP-HP and P-C diet choice tests; thus, serving as an illustration of fraction preferences. Figure 2a shows that among lactating LP-HP females, LP intake was stable; whereas, HP intake increased dramatically over days postpartum. In contrast, among nonlactating LP-HP females (see Figure 2b), food intake was split evenly between the LP and HP fractions. Indeed, there were significant main effects for both reproductive state ($F(1,14)=90.63$, $p < .001$) and diet fraction ($F(1,14)=8.02$, $p < .01$), as well as, a significant interaction effect of reproductive state x diet fraction ($F(1,14)=11.11$, $p < .01$). There was a significant main effect for days ($F(13,182)=7.72$, $p < .001$), and significant interaction effects for reproductive state x days ($F(13,182)=20.27$, $p < .001$) and reproductive state x diet fraction x days ($F(13,182)=2.05$, $p < .05$).

Both lactating P-C and nonlactating P-C groups selected a greater amount from the C fraction than from the P diet fraction, as indicated by a significant main effect for diet fraction ($F(1,14) = 200.43$, $p < .001$). There were significant main effects for both reproductive state ($F(1,14) = 46.17$, $p < .001$) and days ($F(13,182) = 8.58$, $p < .001$), and a significant interaction effect for reproductive state x days ($F(13,182) = 14.94$, $p < .001$), since lactating P-C females increased their intake from both the P and C food jars over the days postpartum (see Figure 2c), while nonlactating P-C females exhibited a stable pattern of intake of these diet fractions (see Figure 2d). Neither the

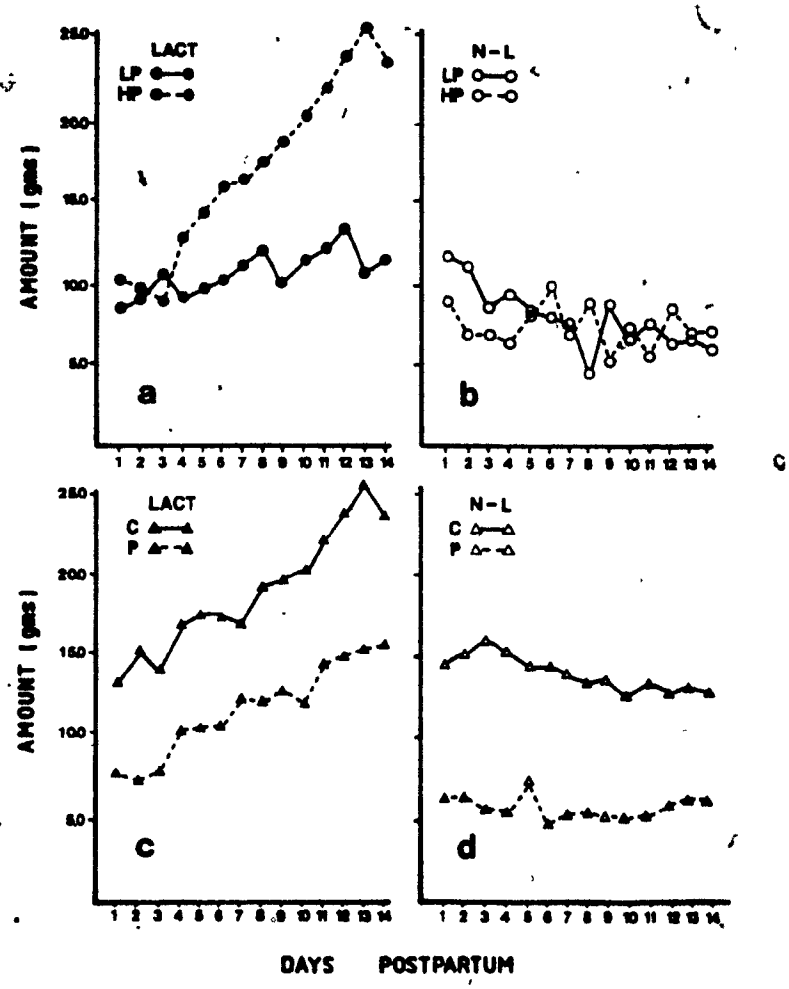

Figure 2. Mean daily amount of diet fraction selected by LP-HP and P-C Lactating and Nonlactating groups.

reproductive state x diet fraction, nor diet fraction x days, nor reproductive state x diet fraction x days interactions were found to be significant.

Percentage Protein Intake

Mean percentage protein intake over days postpartum is indicated in Figure 3. A horizontal line has been drawn at 22%, the percentage of protein in the GR diet. Lactating females selected a higher percentage of protein than nonlactating females, as indicated by a significant main effect for reproductive state ($F(1,28) = 16.57$, $p < .01$). There was also a significant main effect of diet ($F(1,28) = 131.84$, $p < .01$); LP-HP groups selected a greater percentage protein than the P-C groups. There was a significant main effect for days ($F(13,364) = 2.44$, $p < .05$). None of the interaction effects were found to be significant.

Protein Intake

Mean protein intake (in grams) for all groups over the days postpartum is indicated in Figure 4. Analysis of variance indicated that all three main effects and their interactions were significant (see Appendix A). It can be seen that protein intake increased over days for lactating groups, whereas, it remained fairly stable for nonlactating groups. Tukey post hoc tests revealed that from days 4 to 14 LP-HP lactating females ate a significantly greater amount of protein than the lactating P-C group. From days 2 to 14, excluding day 4, the lactating CR group ate a greater amount of protein than the lactating P-C group. With the exception of day 13, the lactating LP-HP and CR groups did not differ significantly. There were no significant differences among the

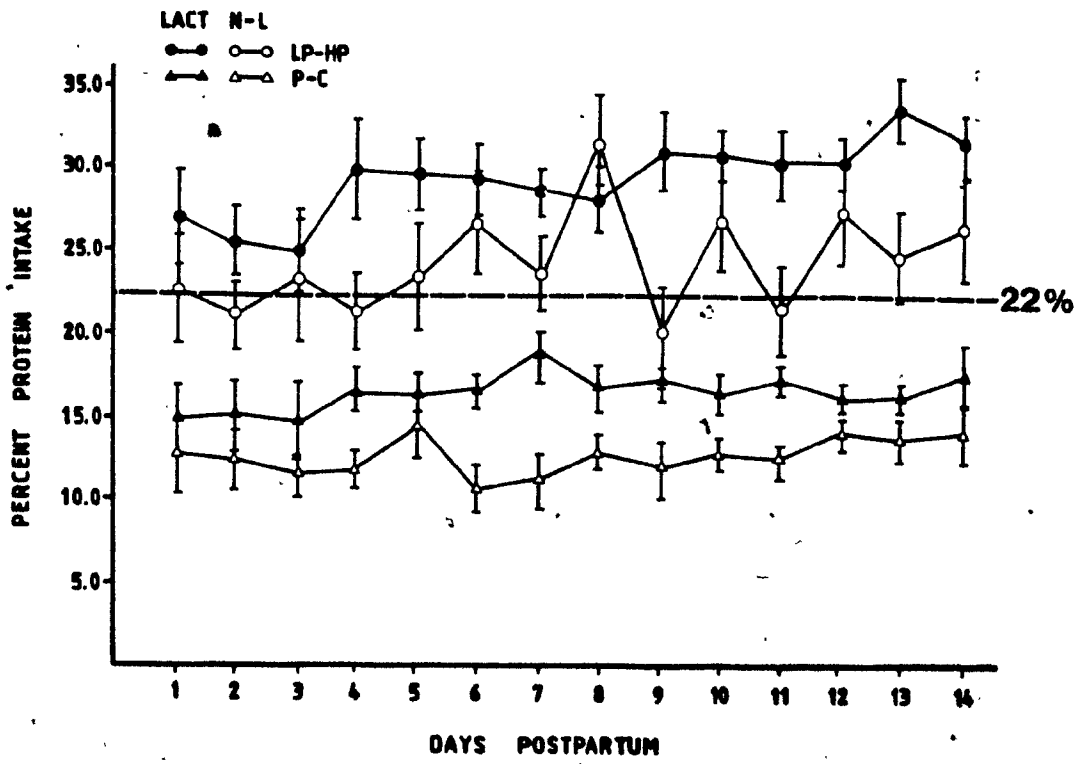

Figure 3. Mean percentage protein intake of LP-HP and P-C Lactating and Nonlactating Groups. SEMs are shown.

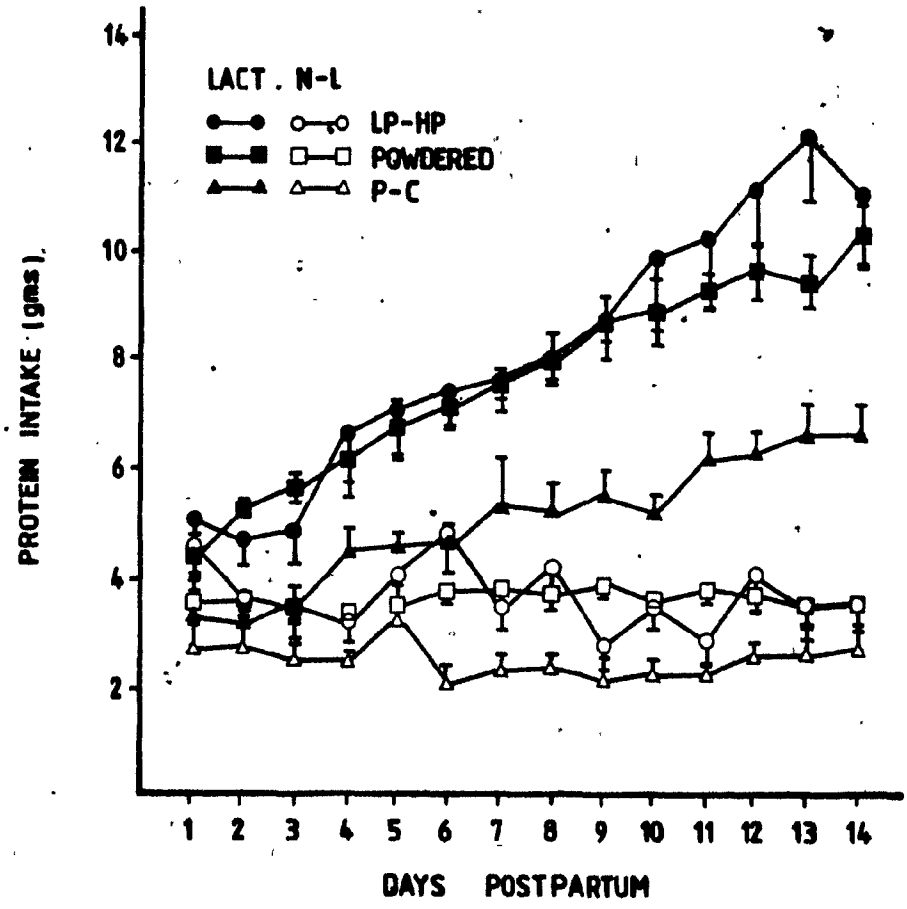

Figure 4. Mean protein intake of LP-HP, P-C and CR Lactating and Nonlactating groups. SEMs are shown.

nonlactating groups, with the exception of day 6 for the LP-HP and P-C groups.

Caloric Intake

Mean caloric intake for all groups over the days postpartum is indicated in Figure 5. All main effects and the two way interactions were statistically significant; only the diet x reproductive state x days interaction was not (see Appendix A).

Caloric intake for the lactating groups increased sharply over days. Tukey post hoc tests revealed that the CR group selected the greatest amount of calories and was significantly different from the P-C group on days 3 to 14, and from the LP-HP group on days 7 to 11, and day 14. The LP-HP group selected a greater amount of calories than the P-C group, but the differences in intake reached statistical significance only on days 6, 10 and 12. Caloric intake for the nonlactating groups remained fairly stable, with no significant differences among the latter groups.

Mother Weight

A one-way ANOVA carried out on day 1 body weight among lactating LP-HP, CR and P-C animals, revealed no significant differences. Percentage change in lactating dam weight was analyzed using a one-way ANOVA. No significant differences were found (see Appendix A).

Pup Growth

On day 1 postpartum there was no significant difference among groups for pup weight. Proportional pup growth of pups reared by dams presented with the LP-HP, P-C, and CR diets, is indicated in Figure 6.

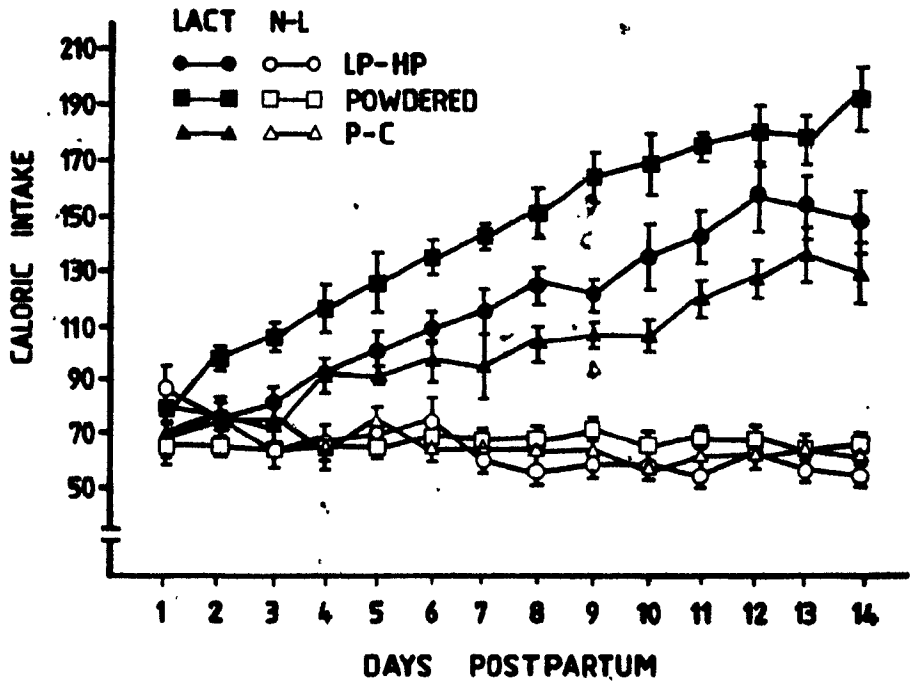

Figure 5. Mean daily caloric intake of LP-HP, P-C and CR Lactating and Nonlactating groups. SEMs are shown.

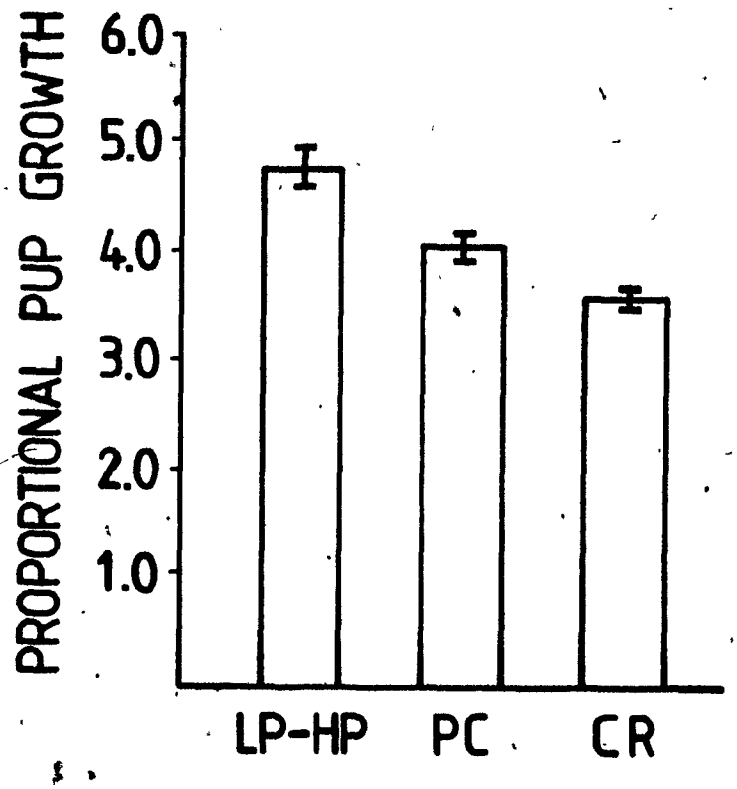

Figure 6. Proportional pup growth of pups reared by dams presented with the LP-HP, P-C or CR diets.

A significant difference between groups was found ($F(2, 21) = 16.37$, $p < .01$). Tukey post hoc tests revealed that the LP-HP group, which exhibited the greatest proportional pup growth was significantly different from the other two groups. The P-C group appeared to have had a greater proportional pup growth than the CR group; but, Tukey tests indicated that the difference between groups fell just short of significance (HSD = .52; Lactating P-C - Lactating CR = .50).

Discussion

Lactating females selected a diet containing a greater percentage of protein than did nonlactating females. This finding is consistent with earlier studies which reported that self-selecting lactating dams will choose a diet rich in protein (e.g., Richter and Barelare, 1938; Leshner et al, 1972).

In the present study, it was also shown that specific properties of diets given in the two choice tests did indeed affect the percentage of protein selected. Those dams presented with the P-C diets chose percentages of protein similar to those reported by Leshner et al (1972), that is, less than 22% protein ($\bar{X}$ = 16.41%), which is the percentage of protein found in a standard laboratory chow. On the other hand, lactating dams allowed to select from the LP-HP diets consistently selected greater than 22%, and as high as 33.59%, protein ($\bar{X}$ = 29.29%). These findings extend those of Wurtman and Baum (1980) that when nonlactating females were presented with the LP-HP diets, they too selected a higher percentage of protein, than reported by Leshner et al (1972) who had employed the P-C diets.

Because the HP diet-fraction contained a similar percentage of protein as the P diet fraction (HP = 45%; P= 43.2% protein) and because vitamin and mineral constituents were similar in the two diets, it would seem that the lower percentage protein intake exhibited by the P-C animals was due to the relative unpalatabilty of the P diet fraction, and was not based on nutrient factors. Although fat content did differ across diet fractions, the amount of fat available in each of the diet

fractions was always greater than the amount present in the standard chow. As long as the rat was able to obtain a more than adequate amount of fat regardless of which fraction was selected, it would appear that differing fat contents would not have affected diet choice.

Support for the proposed palatability factor can be found by examining the amounts of food selected from the P and C diet fractions. It will be recalled that both lactating and nonlactating P-C dams preferred the C diet fraction, whereas in the LP-HP groups, the nonlactating animals ate equally from both diets and maintained a percent protein equivalent to that found in laboratory chow. While it is true that lactating dams increased their intake from the P fraction over days, they also increased the amounts selected from the C diet fraction, and at all times consumed more C than P fraction. Thus, although they did increase the amount of protein eaten during lactation, they never achieved even the 22% protein of the standard chow, a result that would appear to indicate low palatability.

It should also be noted that the P-C diet test presented the rat with a choice between a protein-free and a high protein fraction. In addition, the P fraction was virtually carbohydrate-free. It might be argued that the rats ate so much of the C fraction because the P fraction was virtually carbohydrate free and they had to maintain a certain level of carbohydrate intake; however, if they had simply chosen half of their total food intake from each bowl, they would have eaten a greater amount of carbohydrates than the LP-HP females (i.e. 45%; LP-HP diets contained 40%) and would also have selected 22% protein. It is

clear that both protein and carbohydrate requirements could have been satisfied in this fashion, although this did not occur.

In this experiment, as in many earlier studies, (e.g. Fleming, 1976; Ota and Yokoyama, 1967) lactating females ate more than their nonlactating female counterparts regardless of diet. Interestingly however, the total amount of food eaten by the dams differed depending on which diets had been presented to them. The greatest amount of food was eaten by the lactating CR females, and there was no difference in the amount of food eaten by the LP-HP and P-C dams.

As a rule, the amount of food eaten in turn affects the amount of calories consumed. Thus, the CR lactating dams, were found to have a higher caloric intake than the LP-HP group, even though the stock chow had a slightly lower caloric value than the LP-HP diets. In addition, the caloric intake of the lactating P-C dams was less than the caloric intake of the CR females. This difference in caloric intake between animals presented with the two choice tests and control females fed a stock chow, is reminiscent of the findings of Richter and Barelare (1938), who reported that rats on a cafeteria diet took in fewer calories than rats on a stock diet. Thus, when faced with the opportunity to select for nutrients, it appears that total food intake and calories can be spared.

The diets with lowest caloric values were the P-C diets. In addition the diets within this two choice test were not isocaloric, although according to Leshner et al (1972) the P-C diets which they employed were isocaloric. It will be recalled that, the P-C diets used

in the present experiment differ from the Leshner et al (1972) diets only slightly. The difference lies in that the soybean oil meal used in this experiment consisted of 48% protein; whereas, Leshner et al (1972) used a 50% protein meal. The caloric value for the former meal was 3.15 kcal/gm. In order for Leshner's diets to have been isocaloric, the caloric value of their meal would have to have been 4 kcal/gm, the value that would have been assigned had the meal contained 100% protein. We can only assume that their meal had a high enough fat content in order to compensate for this discrepancy. However, the finding that the percentages of protein obtained here closely parallel those reported by Leshner et al (1972) attest to the similarity between their diets and the ones used in the present experiment. Despite the fact that the C diet fraction of the P-C diets was higher in calories than the P fraction, had the P-C rats consumed a high enough amount of C diet, they could have increased their caloric intake to match the caloric intake of the CR group. Since this was not accomplished, this lends further support for the proposal that caloric intake was not being regulated.

If calories are not of primary importance, then one might question why the CR lactating animals exhibited such a high caloric intake. It is here suggested that, this was the only way in which these dams were able to regulate their intake of protein. It will be recalled that, while animals presented with the LP-HP diets chose a higher percentage of protein than was available to the animals on the stock diet (i.e., 22%), interestingly, the CR control group was able to compensate in terms of <u>grams of protein</u> consumed by increasing its total food intake,

and hence caloric intake. This led to a net result of a pattern of protein intake similar to that of females presented with the LP-HP choice test. Again, these data suggest that protein intake is being controlled in some way during lactation at the expense of caloric intake. Similarly, lactating females presented with a diluted purina diet, have been found to increase their food intake such that the grams of purina (i.e., the diet base before dilution) consumed matched that of lactating controls (Leon and Woodside, 1983). Thus, it has been shown that maternal food intake can be regulated to compensate for a diet deficiency, and that nutritional intake is being regulated.

Despite differences between the lactating CR and lactating P-C and LP-HP females, in terms of caloric intake, there were no differences in percentage mother weight change over the postpartum period. The diets presented to the females did, however, lead to differences in terms of pup growth. The LP-HP pups exhibited a greater growth than the P-C or CR litters. It is unclear, at present, how the diets affected reproductive outcome, and this issue will be addressed further in experiment 2.

In summary, it is proposed that, in the present experiment, protein intake is being regulated and that food intake, and finally caloric intake were affected as a result of this. The experiment which follows was conducted in an extension of the investigation of protein intake in the female rat, and thus, included the reproductive period of pregnancy as well as that of lactation. Pup growth of litters reared by dams presented with either the LP-HP or CR diets allowed for a further

investigation of the effects of the diets on offspring.

Experiment 2

There are two important factors which differentiate the reproductive states of pregnancy and lactation in the female rat. The first factor is the energetic demands of maternal care directly related to the stage of development of the young and the second is that of the hormonal status of the female.

During pregnancy, the demands placed on the dam are not great until the last trimester, when the foetuses begin to grow at a rapid rate (Stotsenburg, 1915). Until this time, one might say that the cost of pregnancy is relatively cheap as compared to lactation, where energy demands are greatly increased (Brody, Riggs, Kaufman, and Herring, 1938). During the latter state, the dam faces high metabolic demands as she produces milk to nourish her young, as well as, exhibiting such behaviors as retrieval, grooming and warming her young (Wiesner and Sheard, 1933). In sum, during most of pregnancy, the female rat is relatively inactive, with the exception of nest building behaviours which can be seen immediately prior to parturition. In contrast, the lactation period places many and varied demands on the mother.

Another factor which differentiates these two reproductive episodes is hormonal status. The description of hormonal status during pregnancy which follows, applies to the rat with a 23 day gestation period (for an overview see Rosenblatt and Siegel, 1981). In the rat, progesterone is fairly dominant throughout most of pregnancy. Plasma progesterone levels consistently rise from day 2 post impregnation and reach a plateau for days 15 through 19, after which point there is a

dramatic decrease (Pepe and Rothchild, 1974). Estradiol, which has been quite low throughout pregnancy, rises sharply (on about day 20) as progesterone begins its decline (Shaikh, 1971). This rise in estradiol in turn leads to a very sharp increase in prolactin on day 22 and an even more dramatic increase on the day of parturition (Morishige, Pepe and Rothchild, 1973). Estradiol is also high on this day.

In contrast, estradiol's pre-partum rise, is not maintained during lactation (Smith and Neill, 1977; Taya and Greenwald, 1982). Serum estradiol is extremely low throughout most of lactation, rising only very late in the reproductive period, around days 15 through 20 postpartum. Prolactin levels are, on the other hand, maintained and further increased till about day 5 postpartum, after which time they drop slightly, but are kept at a fairly high level till sometime between days 15 and 20 of lactation (Amenomori, Chen, and Meites, 1970). Prolactin is necessary for milk production and is thus 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 and Eik-Nes, 1967; Smith and Neill, 1977; Woodside, Leon, Attard, Feder, Siegel, and Fischette, 1981).

Given these differences between pregnancy and lactation in the female rat, one might expect that her pattern of diet selection and protein intake might also differ across these states. Indeed, it will be recalled that although Richter and Barelare (1938) and Leshner et al (1972) employed different procedures, both studies found that protein

intake was greater during lactation than it was during pregnancy. The above studies, however, have not been without problems. Richter and Barelare's (1938) findings have been difficult to replicate, because later studies have found that casein seems to be unpalatable when presented on its own. In addition, it will be recalled from the results of experiment 1 that it appeared that there might be palatability problems with the diets used by Leshner et al (1972), which the LP-HP diet fractions appeared to overcome. Moreover the latter diets yielded better pup growth. The present experiment, therefore, investigated the pattern of protein selection in the female rat across both pregnancy and lactation, using the LP-HP diets described by Wurtman and Baum (1980).

In Experiment 1 it was demonstrated that lactating females presented with the CR control diet increased their food intake, such that their protein intake (in grams) was similar to the LP-HP lactating females. To further investigate this phenomenon an impregnated group presented with the CR diet was included in order to examine whether a similar compensatory pattern of food intake would occur during pregnancy. The presence of this group also enabled a further opportunity to compare pup growth of young reared by dams presented with either the LP-HP or CR diets. In Experiment 2, pup growth was examined in greater depth than in Experiment 1, with the additional measures of day of eye opening, weaning weight, and weight of the litter on day 40 postpartum being recorded.

Method

Subjects. Twenty-four virgin female Wistar rats, weighing between 225-230g, obtained from Charles River Breeding Farms, St. Constant, Quebec, served as subjects.

Apparatus. Same as for experiment 1.

Diets. The LP-HP and CR diets described in Experiment 1 were employed.

Procedure. All rats were housed individually in plastic cages with two food jars per cage. Presentation of food, rotation of jars, and measurement of food and water intake, and body weight were as described in Experiment 1. Sixteen rats were presented with the LP-HP choice test and eight rats were presented with the CR diet in two jars. Vaginal smears were taken daily with moistened cotton swabs and slides assessed in order to determine the days of the estrous cycle for each female, for a period of 12 days. After such time, eight LP-HP and the CR rats were mated, one male per female, with males introduced on the evening that the female was to come into estrus, and then removed the following morning. Vaginal smears were taken and presence of spermatazoa taken to be an indication that impregnation had occurred. If impregnation did not occur, vaginal smears were continued and the male presented the next time the female was to come into estrus. (No female was allowed more than three mating days altogether). The morning on which impregnation had been determined was recorded as day 1 of pregnancy. These females comprised either the impregnated LP-HP (ILP-HP) or impregnated CR (ICR) groups, depending on which diet had been presented to them. Only five

females in the CR group became impregnated, the remaining three rats were thus dropped from the study. Data: food and water intake, dam and litter weight, were recorded throughout pregnancy and through 16 days of lactation, with the day following parturition designated as day 1 of lactation. As in experiment 1 the amounts of LP and HP fractions eaten were divided by two. Litter size and weight on day 0, the day of parturition were recorded. All litters were then culled to 8 pups as for Experiment 1. On day 16 postpartum, all lactating dams were placed on Charles River hard chow.

The remaining eight unmated LP-HP females, served as a nonimpregnated control counterpart (NLP-HP) to the ILP-HP group. After the initial 12 day estrous cycling period, vaginal smears were discontinued and data were recorded for 39 days.

In addition to daily pup weight, pups were assessed in the following ways: 1) day of eye opening, which was defined as the first day on which all the pups had at least one eye open (Galler, 1980), 2) weight at weaning on the morning of day 25 postpartum, and 3) weight on day 40 postpartum. All litters were maintained on the hard stock chow after weaning.

Daily means of food intake, change in percentage protein selected, protein intake (in grams) and caloric intake were calculated in weekly blocks and analyzed using two-way ANOVAs with one independent measure, either reproductive state or diets, and one repeated measure, weeks. Analyses were conducted for weeks post impregnation (week 1= days 1-7; week 2= days 8-14; week 3= days 9-21) and weeks postpartum (week 1= days

1-8; week 2= days 9-16). Separate analysis were conducted to compare the data from the two LP-HP groups (reproductive state x weeks (or days)) and to compare the data from the two impregnated groups, ILP-HP --- ICR (diet x weeks (or days)). Summary tables of all ANOVAs can be found in Appendix B. The Scheffé post hoc test (α = .05) was employed in order to determine whether differences in weeks post impregnation between ILP-HP and ICR groups in terms of protein intake and caloric intake were statistically significant.

Mother weight and pup growth data were analyzed using the Student's t-test for independent groups.

Results

Food Intake

Mean daily food intake expressed in weekly blocks over pregnancy and lactation is indicated in Figure 7.

Pregnancy

There was no significant difference in food intake between the ILP-HP group and its control counterpart, NLP-HP; but, the ICR group ate significantly more than the ILP-HP group, as indicated by a significant main effect for diet ($F(1,11) = 10.76$, $p < .01$). There was no significant main effect for weeks, nor diet x weeks, nor reproductive state x weeks interactions.

Lactation

The ILP-HP group ate more than the NLP-HP group ($F(1,14) = 190.19$, $p < .01$). There was a significant main effect for weeks ($F(1,14) = 156.87$, $p < .01$) as well as a significant reproductive state x weeks interaction ($F(1,14) = 118.48$, $p < .01$), as food intake for the ILP-HP group increased over weeks, whereas the NLP-HP group ate similar amounts in the time periods corresponding to weeks 1 and 2 of lactation.

The greatest amount of food was eaten by the ICR group which ate a significantly greater amount than the ILP-HP group, as indicated by a significant main effect for diet ($F(1,11) = 155.23$, $p < .01$). The ICR group consumed more food in week 2 than in week 1 of lactation. There was a significant main effect for weeks ($F(1,11) = 463.30$, $p < .01$) as well as a significant diet x weeks interaction ($F(1,11) = 27.66$, $p < .01$).

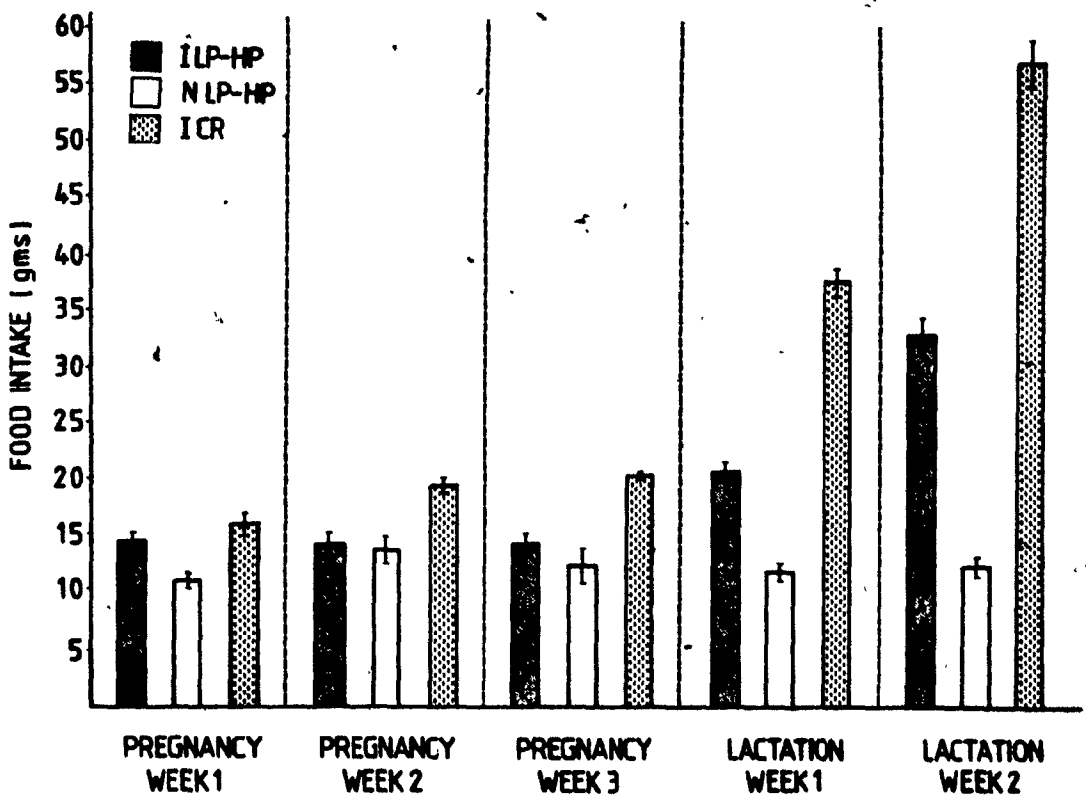

Figure 7. Mean daily food intake of ILP-HP, NLP-HP and ICR groups expressed in weekly blocks over pregnancy and lactation. SEMs are shown.

Diet Fraction Choice

The amount of food selected from each diet fraction within the LP-HP choice test, for the ILP-HP and NLP-HP groups over both pregnancy and lactation is depicted in Figure 8.

Pregnancy

Although there was no significant main effect for reproductive state, there was a significant effect for diet fraction ($F(1,14) = 9.84$, $p < .01$) and a significant diet fraction x reproductive state interaction ($F = 4.91$, $p < .05$), where more HP than LP diet was eaten by the impregnated group. There was no significant days effect and none of the other interactions were found to be significant (see Appendix B).

Lactation

There were significant main effects for reproductive state ($F(1,14) = 209.15$, $p < .001$), diet fraction ($F(1,14) = 31.18$, $p < .001$) and days ($F(15,210) = 23.59$, $p < .001$), apparently due to the dramatic increase in HP fraction over lactation, for the ILP-HP group, which increased LP fraction to a lesser degree, coupled with the NLP-HP group having exhibited a stable pattern of intake of both fractions, selecting similar amounts of each fraction. All two-way and the three-way interactions were also found to be significant (see Appendix B).

Percentage Protein Intake

Mean weekly percentage protein intake for the ILP-HP and NLP-HP groups can be found in Appendix C. Means of the eight day pre-impregnation period show that the NLP-HP group selected a greater percentage of protein than the ILP-HP group. Because of this inequality

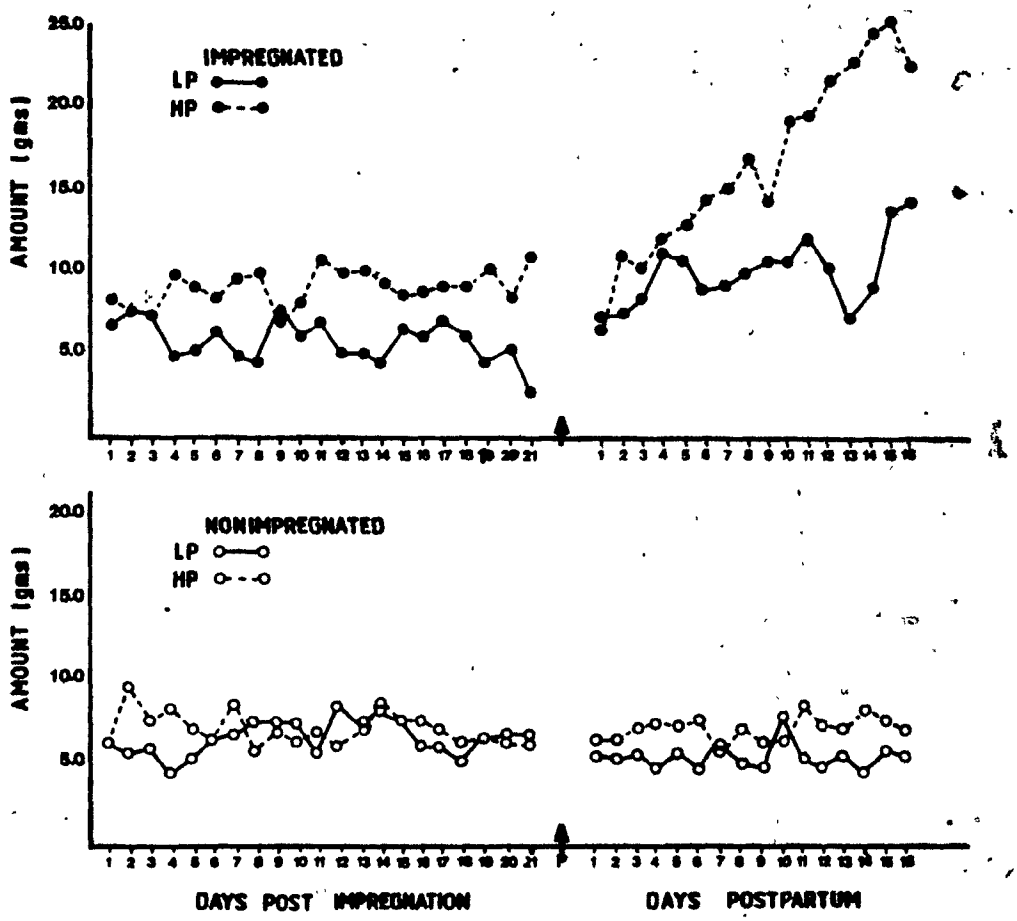

Figure 8. Mean daily amounts of diet fraction selected by ILP-HP and NLP-HP groups for days post impregnation and days postpartum.

prior to the pregnancy and lactation periods, percentage protein intake was analyzed in terms of a change in percentage protein intake, with the eight days of pre-impregnation data serving as a baseline comparison.

Daily change in percentage protein intake expressed in weekly blocks over pregnancy and lactation is indicated in Figure 9.

Pregnancy

The ILP-HP group exhibited a great change in percentage protein intake, whereas the NLP-HP group chose percentages remarkably similar to baseline. The difference between groups was significant, as indicated by a significant main effect for reproductive state ($F(1,14) = 31.97$, $p < .01$). There was no significant weeks effect, but there was a significant reproductive state x weeks interaction ($F(2,28) = 4.24$, $p < .05$), reflecting an increase in percentage protein intake for the impregnated group but not for the nonimpregnated group.

Lactation

As in pregnancy, the NLP-HP group showed a neglible amount of change from baseline in percentage protein intake, and the ILP-HP group increased change in percentage protein intake; thus, there was a significant main effect for reproductive state ($F(1,14) = 9.55$, $p < .01$). The ILP-HP group exhibited a greater change in week 2 as compared to week 1, and there was a significant main effect for weeks ($F(1,14) = 7.80$, $p < .05$), as well as a significant reproductive state x weeks interaction ($F(1,14) = 5.70$, $p < .05$).

Protein Intake

Mean daily grams of protein intake expressed in weekly blocks over

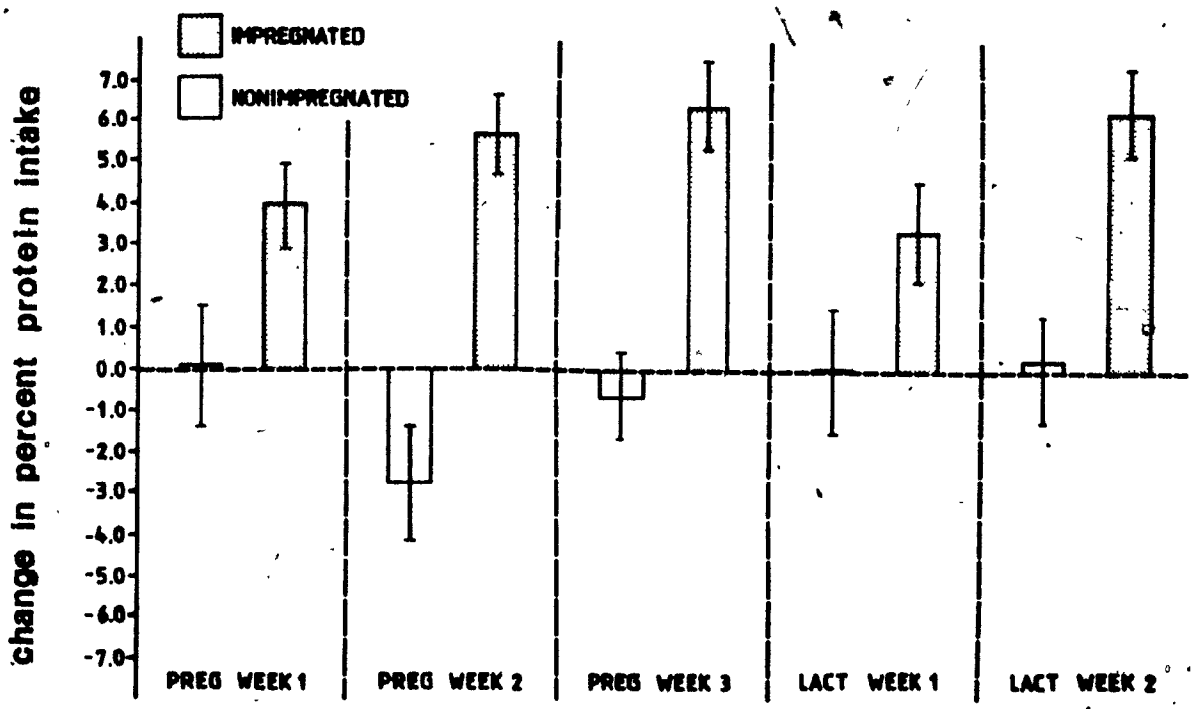

Figure 9. Daily change in percentage protein intake of ILP-HP and NLP-HP groups expressed in weekly blocks over pregnancy and lactation. SEMs are shown.

pregnancy and lactation is indicated in Figure 10.

Pregnancy

There was a significant main effect for reproductive state ($F(1,14) = 8.47$, $p < .05$), with the ILP-HP group having, overall, eaten more protein as compared to its control counterpart. The main effect for weeks was not significant, nor was the reproductive state x weeks interaction. It can be seen that protein intake for the ILP-HP group is quite stable throughout pregnancy. There was, however, a slight increase from week 1 to weeks 2 and 3, for both impregnated groups. This may have contributed to a significant main effect for weeks ($F(2,22) = 4.16$, $p < .05$) when the two impregnated groups were compared. The ILP-HP and ICR groups ate similar amounts of protein over pregnancy, as there was no significant main effect for diet, nor a significant diet x weeks interaction.

Lactation

ILP-HP females ate more protein than NLP-HP animals during lactation; this is indicated by a significant main effect for reproductive state ($F(1,14) = 104.30$, $p < .01$). The NLP-HP group, did not show any change in protein intake from week 1 to week 2 of lactation; whereas, the ILP-HP group increased protein intake over lactation weeks. Thus, there was a significant main effect for weeks ($F(1,14) = 127.00$, $p < .01$) and a significant reproductive state x weeks interaction ($F(1,14) = 105.48$, $p < .01$). The greatest amount of protein was consumed by the ICR group, which ate significantly more than the ILP-HP group as indicated by a significant main effect for diet ($F(1,11)$

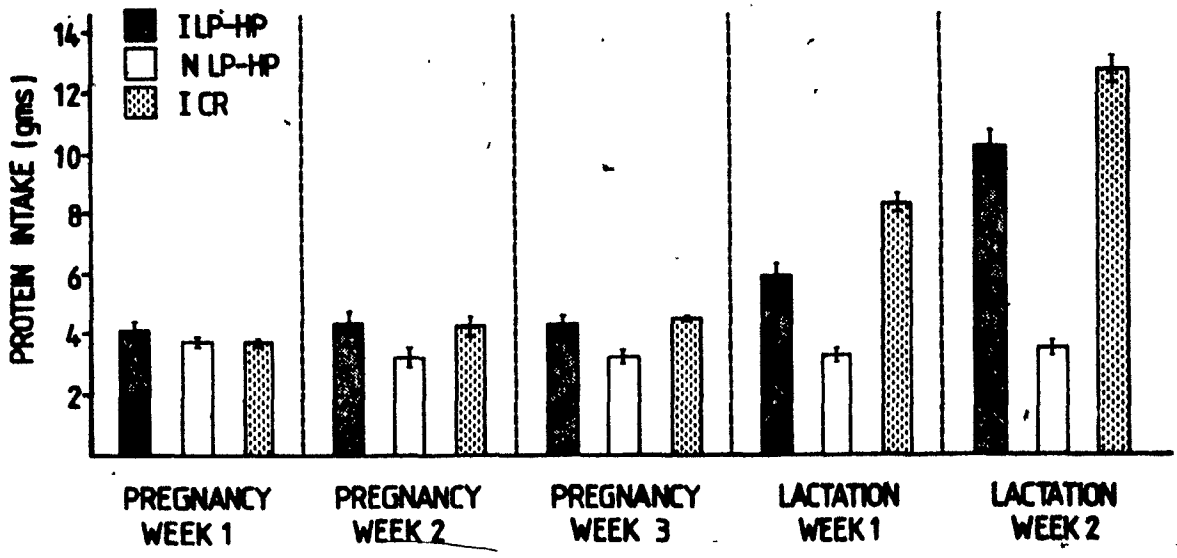

Figure 10. Mean daily protein intake of ILP-HP, NLP-HP and ICR groups expressed in weekly blocks over pregnancy and lactation. SEMs are shown.

= 18.10, $p < .01$). Both impregnated groups increased protein intake over time, and there was a significant weeks effect ($F(1,11) = 299.83$, $p < .01$). There was a nonsignificant groups x weeks interaction.

Caloric Intake

Mean daily caloric intake expressed in weekly blocks over pregnancy and lactation is indicated in Figure 11.

Pregnancy

Since there were no significant differences between ILP-HP and NLP-HP groups in terms of food intake, no statistical analyses were conducted to compare these two groups with respect to caloric intake. The ICR group consumed a greater amount of calories than the ILP-HP group, as shown by a significant main effect for diet ($F(1,11) = 14.89$, $p < .01$). There was a significant main effect for weeks ($F(2,22) = 8.71$, $p < .01$), as well as a significant diet x weeks interaction ($F(2,22) = 14.87$, $p < .01$). Scheffé post hoc tests revealed that the ILP-HP group ate similar amounts of calories during each week of pregnancy; however, the ICR group increased caloric intake from week 1 to weeks 2 and 3, with no significant difference between the latter two weeks.

Lactation

The ILP-HP group selected a greater amount of calories than its nonimpregnated control group, whose caloric intake did not change over lactation. Both impregnated groups increased caloric intake over time; but, the ICR group ate the greatest amount of calories and exhibited the greatest increase in intake. Thus, there were significant main effects

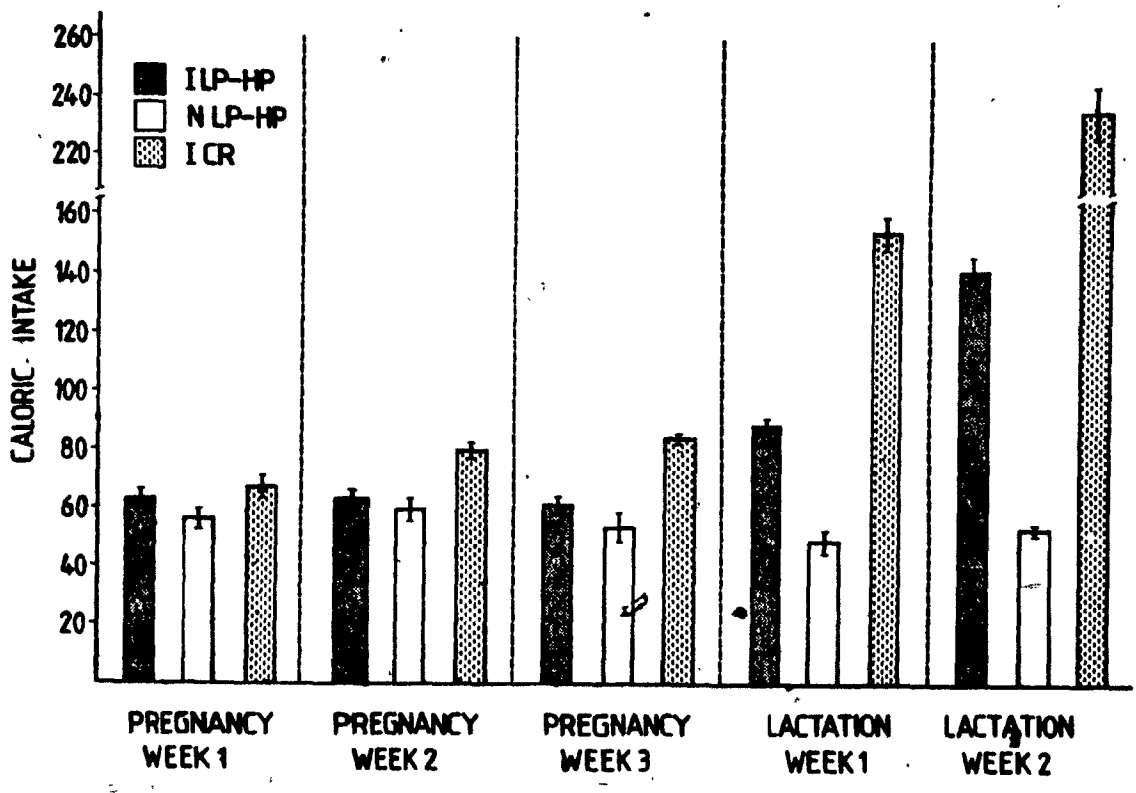

Figure 11. Mean daily caloric intake of ILP-HP, NLP-HP and ICR groups expressed in weekly blocks over pregnancy and lactation. SEMs are shown.

for diet ($F(1,11) = 133.32$, $p < .01$) and weeks ($F(1,11) = 425.37$, $p < .01$), as well as a significant diet x weeks interaction ($F(1,11) = 19.08$, $p < .01$).

Mother Weight

Pregnancy

Results of the t-tests for mother weight can be found in Appendix D. There was no significant difference between the ILP-HP and the ICR dams in terms of body weight on day 1 of pregnancy, nor for percentage weight change over pregnancy days.

Lactation

The ICR dams showed a significantly greater percentage weight change over lactation days, than the ILP-HP dams (see Appendix D).

Pup Growth

On day 0 postpartum, there was no significant difference between the ILP-HP and ICR groups for litter size or pup weight. There was no significant difference between groups for proportional pup growth over 16 days postpartum nor for day of eye opening occurred between days 15 and 16 postpartum. On day 25, at weaning, there was a significant difference for pup weight ($t = 3.14$, $p < .05$) with the pups reared by the ICR dams weighing more than those reared by the ILP-HP dams. On day 40 postpartum, there was no significant difference in pup weight between the ICR and ILP-HP groups.

Discussion

Impregnated females presented with the LP-HP diets selected a greater amount of protein and exhibited a greater change in percentage protein intake, than nonimpregnated controls, during both pregnancy and lactation. Impregnated CR females, as in experiment 1, increased food intake and thereby increased grams of protein intake during both reproductive episodes. The data obtained in this experiment thus confirm and extend the findings of experiment 1. Further, the general pattern of protein selection seen in this experiment is similar to that reported by Richter and Barelare (1938) and Leshner et al (1972).

The present findings differ from those obtained by Leshner et al (1972), however, in terms of the percentages of protein selected, which in the Leshner et al (1972) study never reached 22%. It will be recalled that the LP-HP diets have previously been shown, both by Wurtman and Baum (1980) in an investigation of protein selection in the nonlactating rat, and in experiment 1 above, to lead to the selection of greater than 22% protein. In experiment 2, this was also found to have occurred; during both pregnancy and lactation, percentages of protein greater than 22% were selected by the LP-HP groups.

The impregnated LP-HP group displayed an increased preference for protein as early as the end of the first week of pregnancy. In contrast however, Leshner et al (1972) who employed the P-C diets, reported that percentage protein intake was increased much later, that is, during the last week of pregnancy. This difference may be accounted for in terms of the greater palatability of the LP-HP diets. If the P fraction was,

as proposed in experiment 1, unpalatable, the dam might wait until the demand for protein was so great that intake of the P fraction had to be increased, regardless of palatability.

The opportunity to select a high protein diet did not appear to affect reproductive outcome in this experiment. Whereas, in Experiment 1, the pups reared by dams presented with the LP-HP diets exhibited a greater proportional pup growth than pups reared by the CR dams, in the present experiment there was no significant difference over lactation between pups reared by these different diet group dams. This probably reflects the much greater food intake during lactation of the CR dams in experiment 2 compared to the same group in experiment 1. The mechanism mediating this difference is unclear. It may be that the CR pups in Experiment 2 are providing more suckling stimulation to the dams in the ICR group, leading to an overcompensation in terms of food intake, and that this eliminated the differences in proportional pup growth. Alternatively, the dams in experiment 2 may have received the same degree of suckling stimulation as those in experiment 1 and showed a greater intake because of some other factor. Further research is necessary in order to examine the relationship between pups and dam in more detail. It is proposed that not only nest bout frequency and duration be investigated, but that the quality of the milk delivered to the young should be analyzed as well.

The only time at which there was a difference between groups in terms of pup weight was on day 25, at the time of weaning, when the ICR pups weighed more than the ILP-HP pups. Since the ILP-HP dams were

placed on CR hard chow after day 16, it is possible that they may have had difficulty in adapting to the change in diet and that this factor may have caused a temporary difference in pup weight between groups. By day 40, there was again no difference between groups. Similarly, in this laboratory, it has been observed that when females who have been presented with the LP-HP diets are placed on hard stock chow and then later impregnated, they seem to have difficulty in adjusting to the normal protein diet, and in some cases the dams have failed to produce a litter (Cohen and Woodside, unpublished observations). Further research employing a properly controlled experiment will soon be underway in order to attempt to replicate this finding, and to discern the amount of time the dam requires on the stock chow before she may be able to adequately deal with the increased protein demands of pregnancy.

Perhaps the most interesting finding of experiment 2 though, was that although in both pregnancy and lactation diet selection changed such that protein intake was increased, the overall pattern of food intake between these two states was very different. These different behaviors are evident when comparing both impregnated and nonimpregnated LP-HP groups, and when comparing impregnated groups given either CR or LP-HP diets.

During pregnancy, there was no difference in total food intake between impregnated and nonimpregnated LP-HP groups. The difference between the latter groups in terms of protein selection, was clearly a result of differing diet fraction choices. The impregnated group showed a preference for the HP fraction, whereas the nonimpregnated group

selected similar amounts of each fraction. Thus, the impregnated LP-HP group was able to increase grams of protein intake and to increase the percentage of protein selected without an accompanied increase in food intake. However, during lactation, an increase in protein intake and change in percentage protein intake in the impregnated LP-HP group was accompanied by a large increase in food and thus, caloric intake.

Even though overall, protein intake was greater during lactation as compared to pregnancy, there was a reduction in protein intake from the last week of pregnancy to the first week of lactation. Indeed, it can be seen that diet fraction choice was altered over these times. Whereas at the end of pregnancy, the ILP-HP females ate more HP than LP fraction, at the onset of the lactation period, the ILP-HP group selected similar amounts of each fraction and after a few days sharply increased HP intake while increasing LP intake as well, though at a more gradual pace.

On the day of parturition, the female eats the placentae which have a very high protein content. It would be fascinating if this new rich source of protein could provide the dam with sufficient protein, such that she need not eat large quantities of the HP fraction early in the lactation period. It might also be possible that this effect may last for the first few days of lactation. An experiment which could determine the effects of placentaphagia on dietary protein intake in the rat, would entail housing the rats in wire hanging cages, that have wide mesh floors. In this fashion, upon delivery of the litter, the pups would fall through the bottom of the cage and the dam would be unable to

eat the placentae. Her diet selection intake could then be compared to that of the dam who was able to benefit from this added source of protein.

It is interesting to note that the impregnated CR group also displayed a change in pattern of selection across these two reproductive states. The impregnated CR group increased food intake during pregnancy just enough for grams of protein intake to equal that of the LP-HP group. However, during lactation, the CR group increased food intake to such a high degree that it surpassed the impregnated LP-HP group in terms of grams of protein eaten. In fact, food intake, and thus caloric intake, were shown to be almost double the amounts consumed by the impregnated LP-HP group. It is not surprising, therefore, that the only differences in body weight between impregnated groups was in terms of percentage weight change over lactation---a reflection of the high amounts of calories ingested by the ICR group.

Two factors might explain these differences in eating between lactation and pregnancy. The first is simply that the demands of each stage of reproduction are very different. Foetal growth is considerable only during the last trimester of pregnancy (Stotsenberg, 1915), but as Brody et al (1938) have demonstrated the metabolic demands of pregnancy even at this time are only half of those of lactation. It is possible that given the opportunity to select high levels of protein the pregnant female can cope with simple caloric demands by a decrease in activity. Lactation on the other hand, may necessitate not only an increase in protein intake, but also caloric intake. In sum, pregnancy

may make heavy demands on the female in terms of protein, whereas, lactation demands both protein and calories.

A second possibility is that the differences between pregnancy and lactation reflect different levels of hormones during these periods.

The high percentage of protein intake unaccompanied by an increase in food intake, exhibited by the ILP-HP group at the end of pregnancy, may have been affected by the hormonal status of the female rat, and perhaps reflects the high levels of estradiol present at this time. Such an analysis is compatable with the findings of Wurtman and Baum (1980), who reported that on the day of estrus, when estradiol titers are high, nonlactating rats presented with the LP-HP diets, will show an increase in percentage protein intake, with food intake and carbohydrate intake decreased at this time. Sandberg et al (1982) have shown that the rat will decrease sucrose and ethanol consumption at the end of pregnancy, in otherwords they will shy away from carbohydrates and calories at this time. There is a difference, however, between the results of the present experiment and those obtained by Sandberg et al (1982), and these differences can be directly attributed to the type of diet choice offered to the rat. The LP-HP animals did not have the opportunity to separate carbohydrate or caloric intake, from protein intake, since the LP and HP fractions contained an equivalent percentage of carbohydrate and had equivalent caloric values.

Since estradiol is low during lactation, it would follow from the work of Wade (1975), Wurtman and Baum (1980) and others, that there should be an increase in food intake, and indeed in the present

experiment food intake does increase during the first week of lactation as compared to the last week of pregnancy, for impregnated animals. From what has already been presented of the findings of Wurtman and Baum (1980), it would also follow that, when estradiol is low, and therefore, food intake is increased, percentage protein intake would be decreased. In the present experiment, this has also been shown to be the case during the first week of lactation as compared to the third week of pregnancy. However, estradiol is still low during the second week of lactation, and although food intake is increased at this time, percentage protein intake was increased as well. This may reflect a possible interaction between the role of hormonal status, and the increased energy and metabolic demands of lactation, or the action of another hormone.

One possible candidate here is prolactin, elevated levels of which are maintained in the dam during lactation by exteroceptive stimuli from the pups. The effect of prolactin on food intake in general, has been examined in a number of studies and some conflicting results have been obtained. Fleming (1976) starting on day 18 postpartum, administered injections of prolactin to females who had been suckled during the first week postpartum only. She found that prolactin injected females did not eat more food than saline injected controls. However, Leon (1974) has demonstrated that prolactin injected virgins will exhibit an increase in food intake. As Fleming (1976) points out this effect may not have been due to prolactin itself; rather, to the effects of prolactin on progesterone secretion from the adrenals. Wade (1976) has

reported that progesterone will antagonize the effects of estradiol on food intake, thus, leading to a net result of an increase in food intake. Fleming (1976), on the other hand, has found that a combination of prolactin, hydrocortisone acetate and oxcytocin administered to virgins did not increase food intake. She has proposed that hydrocortisone acetate may have acted to counteract any effects which prolactin may have had on food intake. It appears, therefore, that the effects of prolactin on food intake are dependent upon the reproductive state of the female, and interaction with other hormones in the system. It is not clear at present, how prolactin might affect the selection of protein in the female rat.

In summary, the results of this experiment, in general, confirm those of Richter and Barelare (1938) and Leshner et al (1972), in that the female rat does increase food and protein intake over pregnancy and lactation. The percentages of protein selected, however, are dependent upon the properties of, and the palatability of the diets presented to the rat. Moreover females presented with a stock chow can compensate for a standard percentage of protein by increasing total food intake.

Summary and General Discussion

It is clear from the results of the experiments described above that the female rat can regulate protein intake. The lactating females in experiment 1, and impregnated females in experiment 2, given access to the low and high protein diets, adjusted their intake of the diet fractions to increase both the percentage of protein and the grams of protein eaten. It was also shown that the pattern of selection was different across the reproductive states of pregnancy and lactation, in that during the latter, there was a greater intake of total food and of the low protein fraction. The mechanisms mediating these effects are not clear. Let us first consider the mechanism of long-delay learning.

It was stated in the introduction that a long-delay learning mechanism could not account for the ability of Richter and Barelare's (1938) rats to self-select a diet which resulted in normal growth. Similarly, the results of the present experiments do not, as well, seem to be compatable with long-delay learning. The LP-HP females adapted rather quickly to the new diets, especially in experiment 1, where the diet fractions were presented on day 1 postpartum. Despite the absence of an introductory period the selection of diet fractions displayed by the LP-HP groups in experiment 1, were remarkably similar to the results obtained with the ILP-HP group in experiment 2 over lactation, even though the latter group had been feeding on the LP-HP diets since 12 days prior to impregnation.

It would appear, therefore, that the rat has the ability to determine almost immediately, how to maintain an adequate nutritional

intake. In this way, it can be seen that the distinction between innate and learned specific hungers may not be as clear as some have proposed (Rozin and Kalat, 1971). Indeed, Frumkin (1975) has shown that the specific hunger for calcium shares some similarities with the so-called innate hunger for salt. He showed that adrenalectomized and parathyroidectomized rats equally failed to form an aversion to the object of their respective specific hungers, salt and calcium. In the present studies, the specific hunger for protein seems, as well, to be at least partially predetermined, in that the rapid adjustments that the rat makes in terms of protein intake during times of reproductive stress are similar to the behavior of a specialist.

One strategy of gaining information about the mechanisms mediating this diet selection behavior would be to do a much finer analysis of the behaviors emitted by the animal when it is first presented withe the diet choice. Rozin and Kalat (1971) have stated that, when faced with a two choice test situation, the rat will eat primarily from one bowl for a particular length of time, for example a few hours, before selecting from the second bowl. This would allow the animal the opportunity to determine whether the food was associated with harmful or beneficial physiological consequences. If such a trial procedure were used in the present experiments, it might be argued that long-delay learning can account for some of the results. However, although this would be applicable to the LP-HP females in experiment 1, there is a problem in terms of the LP-HP females in experiment 2, since during the pre-impregnation period there is presumably no great demand or specific

hunger for protein; therefore, there would be no recovery from protein deficiency or beneficial effects to be associated with intake of the HP fraction. In other words, relative to an animal who, for example is in the latter stages of pregnancy, or to an animal who is deficient in protein, these nonimpregnated females who had previously been fed a normal protein stock chow, could have afforded a delay in determining what amounts of each fraction needed to be selected that would result in a balanced nutritional intake. It would, nevertheless, be interesting to conduct research in which the rat's behavior is closely observed, in order to assess how the different fractions are selected. A study of meal patterns, that is, meal size, duration, and intermeal interval, may also prove interesting.

The central question remains and that is what cues, internal or external, the animal is using on which to base its diet selection. There are a number of possible candidates, for example, gustatory or visual cues, and it is possible that hormones interact with one or a number of these cues to change behavior. It has been suggested that since hormonal status fluctuates during pregnancy and lactation, coinciding with changes in the pattern of protein intake, that it may play a major role in influencing dietary selection. A series of experiments are presently underway, in order to clarify the importance of hormonal status during lactation. Nipple-sealed rat dams share a common hormonal status with intact suckled females, by virtue of the suckling stimulation received which affects hormonal balance. For example, this stimulation signals the release of prolactin, the major

circulating hormone during this reproductive episode. The nipple-sealed dam, does not, however, deliver milk to her young, and therefore, does not lose nutrients, such as protein, to them. Fleming (1976) has demonstrated that nipple-sealed dams exhibit an greater food intake than nonlactating females. It would be interesting if protein intake is also affected in this fashion. If the nipple-sealed rat dam selects a greater amount of protein, by showing a preference for the HP fraction; whereas, nonsuckled females do not exhibit a preference, it will be demonstrated that hormonal status influences protein selection in the lactating rat.

In addition to raising a number of questions concerning the mechanisms that might underly the pattern of diet selection in the female rat, these studies have also helped to clarify some methodological issues.

The properties of the diets presented did affect the percentage of protein selected. The lower percentages of protein and grams of protein ingested by the P-C lactating dams in experiment 1, and the higher percentages of protein eaten by the ILP-HP group during pregnancy, as compared to the results obtained by Leshner et al (1972), indicate that the P-C diets are not as palatable as the LP-HP diets. Because of this factor, and since the P-C diet involves a choice between a protein-free and a high protein fraction, it is here suggested that these diets are not suitable for an investigation of protein intake in the female rat.

The results of these experiments also emphasize the importance of examining grams of protein intake, and not simply percentage protein

intake. It will be recalled that CR females can increase grams of protein eaten by increasing their total food intake. Studies which claim that rats are being protein deprived due to the presentation of a diet which has a substandard percentage of protein, and that fail to consider the amount of protein selected, may be operating under a false assumption. If the diet used has a percentage of protein as low as 5 or 8%, the dam may not be capable of increasing total food intake to a high enough degree to compensate (Ellison and Woodside, unpublished observations); but, if the percentage is around 12-15%, the dam may be able to take in a sufficient amount of protein, such that she will not in fact be protein deficient. Thus, it is suggested that grams of protein eaten should always be considered in studies of this kind.

In experiment 1 lactating LP-HP and CR females selected equivalent amounts of grams of protein; however, the LP-HP pups grew at a better rate. In experiment 2, the impregnated CR group ate more protein, food and calories than the impregnated LP-HP group; yet, there were no differences in pup growth, except on day 25, after all rats had been fed stock chow for a period of nine days. It appears, therefore, that higher protein, food and caloric intake does not necessarily result in increased pup growth. Neither, it appears do lactating females eat to capacity for, in principle, the LP-HP dams could have eaten amounts similar to control females. As Leon and Woodside (1983) have suggested, the female rat does not necessarily utilize all available mechanisms in order to produce pups that grow at a faster rate; rather, pup growth is the result of a variety of factors impinging on the dam. It would be

interesting to observe the relationship between dam and pups in more detail, in order to assess the amount of time spent with the litter. For example, as was proposed in the discussion to experiment 2, nesting behavior could be recorded, and the quality of the milk delivered to the young could be analyzed.

In addition to protein, Richter and Barelare (1938) found that intake of fat and calcium were also increased during pregnancy and lactation. Thus, studies similar to the experiments described in this thesis, will investigate the selection of these dietary elements and the effects on reproductive outcome. At present, female rats are being given the choice between a calcium-free diet and a solution of calcium of lactate. Data is being recorded from pre-impregnation days through to pregnancy and lactation, as was described in experiment 2 above. It will be interesting to discover whether the patterns of calcium intake will parallel the pattern of protein intake.

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Food Intake

Source	SS	df	MS	F
Between Subjects	41119.06	47		
Diet	2435.52	2	1217.76	11.10+
Reproductive State	32135.52	1	32135.52	293.12+
Diet x Reproductive State	1943.49	2	971.75	8.86+
Between Subjects x Diet x Reproductive State	4604.52	42	109.63	
Within	23707.70	624		
Days	6042.68	13	464.82	36.21+
Diet x Days	869.60	26	33.45	2.61+
Reproductive State x Days	9587.30	13	737.48	57.45+
Diet x Reproductive State x Days	199.07	26	7.66	0.60
Error	7009.05	546	12.84	

+ $p < .01$

Diet Fraction LP-HP

Source	SS	df	MS	F
Between Blocks/ Subjects				
Reproductive State	4133.43	1	4133.429	90.63*
Error	638.52	14	45.61	
Within Blocks/ Subjects				
Diet Fraction	954.72	1	954.72	8.02+
Reprodcutive State x Diet Fraction	1322.06	1	1322.06	11.11+
Error	1666.47	14	119.03	
Days	666.83	13	51.29	7.72*
Reproductive State x Days	1751.05	13	134.70	20.27*
Error	1209.14	182	6.64	
Diet Fraction x Days	973.09	13	74.85	4.02*
Reproducitve State x Diet Fraction x Days	495.40	13	38.11	2.05++
Error	3384.76	182	18.60	

* $p < .001$

\+ $p < .01$

++ $p < .05$

Diet Fraction P-C

Source	SS	df	MS	F
Between Blocks/ Subjects				
Reproductive State	3261.06	1	3261.06	46.17*
Error	988.76	14	70.62	
Within Blocks/ Subjects				
Diet Fraction	6418.30	1	6418.30	200.43*
Reproductive State x Diet Fraction	25.13	1	25.13	0.78
Error	448.31	14	32.02	
Days	805.61	13	61.97	8.58*
Reproductive State x Days	1403.24	13	107.94	14.94*
Error	1314.48	182	7.22	
Diet Fraction x Days	40.03	13	3.08	0.44
Reproductive State x Diet Fraction x Days	145.02	13	11.16	1.58
Error	1282.21	182	7.04	

* $p < .001$

Percentage Protein Intake

Source	SS	df	MS	F
Between Subjects	22593.44	31		
Diet	16863.15	1	16863.15	131.84+
Reproductive State	2118.71	1	2118.71	16.57+
Diet x Reproductive State	30.43	1	30.43	0.24
Error	3581.14	28	127.90	
Within Subjects	10391.17	416		
Days	741.46	13	57.04	2.44++
Diet x Days	288.06	13	22.16	0.95
Reproductive State x Days	465.46	13	35.80	1.53
Diet x Reproductive State x Days	377.38	13	29.03	1.24
Error	8518.80	364	23.40	

+ $p < .01$

++ $p < .05$

Protein Intake

Source	SS	df	MS	F
Between Subjects	3507.06	47		
Diet	622.16	2	311.08	28.19+
Reproductive State	2302.62	1	2302.62	208.69+
Diet x Reproductive State	118.87	2	58.44	5.39+
Between Subjects x Diet x Reproductive State	463.40	42	11.03	
Within Subjects	1944.06	624		
Days	484.63	13	37.28	26.25+
Diet x Days	71.08	26	2.73	1.92+
Reproductive State x Days	544.31	13	41.87	29.48+
Diet x Reproductive State x Days	68.68	26	2.64	1.86+
Error	775.36	546	1.42	

+ $p < .01$

Caloric Intake

Source	SS	df	MS	F
Between Subjects	684792.80	47		
Diet	54308.00	2	27153.99	16.40+
Reproductive State	511880.60	1	511880.60	308.30+
Diet x Reproductive State	48879.40	2	24439.70	14.72+
Between Subjects x Diet x Reproductive State	69724.80	42	1660.11	
Within Subjects	377131.40	624		
Days	94132.50	13	7240.96	35.80+
Diet x Days	16219.40	26	623.82	3.09+
Reproductive State x Days	150104.20	13	11546.48	57.11+
Diet x Reproductive State x Days	6280.63	26	241.56	1.19
Error	110394.60	546	202.19	

+ $p < .01$

Mother Weight

Day 1

Source	SS	df	MS	F
Between Subjects	4492.39	2	2246.19	2.42
Within Subjects	19516.44	21	929.35	
Total	24008.83	23		

Percentage Weight Change Days 1-14

Source	SS	df	MS	F
Between Subjects	105.14	2	52.57	0.73
Within Subjects	1515.06	21	72.15	
Total	1620.21	23		

Pup Growth

Day 1 Weight

Source	SS	df	MS	F
Between Subjects	0.40	2	0.20	0.49
Within Subjects	8.69	21	0.41	
Total	9.10	23		

Proportional Pup Growth

Source	SS	df	MS	F
Between Subjects	5.46	2	2.73	16.36+
Within Subjects	3.50	21	0.17	
Total	8.96	23		

+ $p < .01$

Appendix B

Food Intake - Pregnancy

ILP-HP - NLP-HP

Source	SS	df	MS	F
Between Subjects	211.86	15		
Reproductive State	21.64	1	21.64	1.59
Subjects x Reproductive State	190.21	14	13.59	
Within Subjects	63.92	32		
Weeks	3.01	2	1.50	0.72
Reproductive State x Weeks	2.53	2	1.26	0.60
Error	58.39	28	2.08	

Food Intake - Pregnancy

ILP-HP - ICR

Source	SS	df	MS	F
Between Subjects	260.37	12		
Diet	128.76	1	128.76	10.76+
Subjects x Diet	131.60	11	11.96	
Within Subjects	220.95	26		
Weeks	36.10	2	18.05	2.63
Diet x Weeks	34.13	2	17.06	2.49
Error	150.18	22	6.85	

+ $p < .01$

Food Intake - Lactation

ILP-HP - NLP-HP

Source	SS	df	MS	F
Between Subjects	1883.85	15		
Reproductive State	1754.69	1	1754.69	190.19+
Subjects x Reproductive State	129.16	14	9.22	
Within Subjects	634.51	16		
Weeks	344.01	1	344.01	156.87+
Reproductive State x Weeks	259.81	1	259.81	118.48+
Error	30.70	14	2.19	

+ $p < .01$

Food Intake - Lactation

ILP-HP - ICR

Source	SS	df	MS	F
Between Subjects	2823.25	12		
Diet	2634.16	1	2634.16	155.23+
Subjects x Diet	189.09	11	17.19	
Within Subjects	1625.11	13		
Weeks	1499.94	1	1499.94	463.30+
Diet x Weeks	89.56	1	89.56	27.66+
Error	35.61	11	3.24	

+ $p < .01$

Diet Fraction - Pregnancy

ILP-HP - NLP-HP

Source	SS	df	MS	F
Between Effect				
Reproductive State	84.18	1	84.18	1.88
Error	626.48	14	44.75	
Within Effect				
Diet Fraction	608.29	1	608.29	9.84+
Reproductive State x Diet Fraction	303.55	1	308.55	4.91++
Error	865.73	14	61.84	
Days	74.53	15	3.73	0.00
Reproductive State x Days	125.41	15	6.27	0.00
Error	1744.95	280	6.23	
Diet Fraction x Days	222.76	15	11.14	0.00
Reproductive State x Diet Fraction x Days	342.69	15	17.13	0.00
Error	3444.71	280	12.30	

\+ $p < .01$

++ $p < .05$

Diet Fraction - Lactation

ILP-HP - NLP-HP

Source	SS	df	MS	F
Between Blocks/ Subjects				
Reproductive State	7211.25	1	7211.25	209.15*
Error	482.69	14	34.48	
Within Blocks/ Subjects				
Diet Fraction	1980.56	1	1980.56	31.18*
Reproductive State x Diet Fraction	653.64	1	653.64	10.29+
Error	889.30	14	63.52	
Days	1985.91	15	132.39	23.59*
Reproductive State x Days	1630.82	15	108.72	19.37*
Error	1178.56	210	5.61	
Diet Fraction x Days	525.45	15	35.03	3.23*
Reproductive State x Diet Fraction x Days	515.23	15	34.35	3.17*
Error	2276.62	210	10.84	

* $p < .001$

\+ $p < .01$

Change in Percentage Protein Intake - Pregnancy

Source	SS	df	MS	F
Between Subjects	782.80	15		
Reproductive State	544.39	1	544.39	31.97+
Subjects x Reproductive State	238.41	14	17.03	
Within Subjects	215.21	32		
Weeks	7.89	2	3.95	0.64
Reproductive State x Weeks	48.16	2	24.08	4.24++
Error	159.16	28	5.68	

+ $p < .01$

++ $p < .05$

Change in Percentage Protein Intake - Lactation

Source	SS	df	MS	F
Between Subjects	432.17	15		
Reproductive State	175.27	1	175.27	9.55+
Subjects x Reproductive State	256.91	14	18.35	
Within Subjects	70.81	16		
Weeks	20.09	1	20.09	7.80++
Reproductive State x Weeks	14.67	1	14.67	5.70++
Error	36.05	14	2.57	

+ $p < .01$

++ $p < .05$

Protein Intake - Pregnancy

ILP-HP - NLP-HP

Source	SS	df	MS	F
Between Subjects	24.45	15		
Reproductive State	9.21	1	9.21	8.47++
Subjects x Reproductive State	15.23	14	1.09	
Within Subjects	9.81	32		
Weeks	0.01	2	0.01	0.05
Reproductive State x Weeks	1.05	2	0.52	1.68
Error	8.73	28	0.31	

++ $p < .05$

Protein Intake - Pregnancy

ILP-HP - ICR

Source	SS	df	MS	F
Between Subjects	12.18	12		
Diets	0.14	1	0.14	0.13
Subjects x Diets	12.04	11	1.09	
Within Subjects	8.01	26		
Weeks	2.00	2	1.00	4.16++
Diet x Weeks	0.72	2	0.36	1.50
Error	5.28	22	0.24	

+ $p < .05$

Protein Intake - Lactation

ILP-HP - NLP-HP

Source	SS	df	MS	F
Between Subjects	198.47	15		
Reproductive State	174.98	1	174.98	104.30*
Subjects x Reproductive State	23.49	14	1.68	
Within Subjects	76.13	16		
Weeks	39.23	1	39.23	127.00*
Reproductive State x Weeks	32.58	1	32.58	105.48*
Error	4.32	14	0.31	

* $p < .001$

Protein Intake - Lactation

ILP-HP - ICR

Source	SS	df	MS	F
Between Subjects	57.68	12		
Diets	35.88	1	35.88	18.10+
Subjects x Diets	21.80	11	1.98	
Within Subjects	123.82	13		
Weeks	119.41	1	119.41	299.83+
Diet x Weeks	0.03	1	0.03	0.08
Error	4.38	11	0.40	

+ $p < .001$

Caloric Intake - Pregnancy

ILP-HP - ICR

Source	SS	df	MS	F
Between Subjects	3623.75	12		
Diets	2084.37	1	2084.37	14.89+
Subjects x Diets	1539.42	11	139.95	
Within Subjects	1534.86	26		
Weeks	386.60	2	193.30	8.71+
Diet x Weeks	659.96	2	329.98	14.87+
Error	488.29	22	22.20	

+ $p < .01$

Caloric Intake - Lactation

ILP-HP - ICR

Source	SS	df	MS	F
Between Subjects	43739.98	12		
Diets	40406.17	1	40406.17	133.32+
Subjects x Diets	3333.82	11	303.07	
Within Subjects	29624.91	13		
Weeks	27668.28	1	27668.28	425.37+
Diet x Weeks	1241.14	1	1241.14	19.08+
Error	715.48	11	65.04	

+ $p < .01$

Appendix C

Mean Percentage Protein Intake

	ILP-HP		NLP-HP	
	$\bar{X}$	s.e.m.	$\bar{X}$	s.e.m.
Pre-Impregnation	24.17	0.68	28.07	1.27
Pregnancy Week1	28.11	1.07	28.22	0.96
Pregnancy Week2	29.79	1.28	25.23	1.04
Pregnancy Week3	30.77	1.18	27.37	0.60
Lactation Week1	27.16	0.77	28.03	0.71
Lactation Week2	30.45	0.37	27.86	0.65

Appendix D

Mother Weight Change over Lactation and Pregnancy

	ILP-HP		ICR		
	$\bar{X}$	s.e.m.	$\bar{X}$	s.e.m.	t
Day of Impregnation	234.58	5.90	230.98	3.35	0.53[a]
Percentage Weight Change over Pregnancy	44.01	2.86	46.98	2.63	0.76[a]
Percentage Weight Change over Lactation	13.86	1.40	13.46	1.66	3.52++

[a] $t > .05$

++ $t < .05$

Pup Growth

	ILP-HP		ICR		
	$\bar{X}$	s.e.m.	$\bar{X}$	s.e.m.	t
Litter size	11.00	0.84	9.75	1.03	0.94[a]
Weight at Parturition	6.00	0.14	5.88	0.13	0.64[a]
Day 16 Weight	5.48	0.21	4.96	0.26	1.56[a]
Day of Eye Opening	15.75	0.24	16.12	0.30	1.37[a]
Weight at Weaning	63.21	0.99	52.56	3.24	3.14++
Day 40 Weight	98.53	2.50	103.52	2.83	0.56[a]

[a] $t > .05$

++ $t < .05$