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Diet-induced hyperthermia: Time course of response and influences on mother-litter contact and pup development in the rat

Frank Joshua Ellison

A Thesis

in

The Department

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ABSTRACT

Diet-induced hyperthermia: Time course of response and influences on mother-litter contact and pup development in the rat.

Frank Joshua Ellison

Dietary influences on body temperature in lactating and non-lactating female rats were examined. In Experiment 1, two groups of lactating females (n=8) were presented with one of two nutritionally adequate and similar commercially available diets (Purina Lab Chow (LC) or AIN-79 Semipurified diet (SP)) for 14 days postpartum. Dams fed the SP diet had consistently higher core temperatures at both 0830 and 2030 h than did those fed the lab chow diet even though gram food and caloric intakes were similar between the two conditions. LC and SP pup body weight gain was also similar. In Experiment 2, diet-induced hyperthermia was used to alter patterns of mother-litter contact while nutritional differences were minimized to determine if such changes could alter the course of pup development. Two groups of lactating females (n=8) were presented with one of the two diets and contact time was continuously monitored for 14 days postpartum. Compared to females fed the lab chow, females fed the semipurified diet and their pups had higher core and skin temperatures which contributed to a reduction in nest time during the light portion of the light/dark cycle while the frequency of nest visits was similar. It was shown that the

reduction in SP nest time resulted from shorter nest bout durations. Reduced nest time did not affect the growth or development of the SP pups although they were better able to thermoregulate than LC offspring. Reasons which may have enabled the SP young to grow as well as their LC counterparts are discussed. Experiment 3 examined the time course of the hyperthermic response as well as any transient hyperphagia in non-lactating females (n=11) when after 10 days the lab chow was switched to the semipurified diet and when original dietary status was reinstated. Presentation of semipurified diet produced an immediate but transient caloric hyperphagia as well as immediate hyperthermia which lasted until days 11-15 of the lab chow reintroduction phase. Potential mechanisms contributing to these effects are discussed.

Acknowledgements

A special debt of graditude is owed to my thesis supervisor, Dr. Barbara Woodside. Throughout this undertaking she has constantly given her full support and encouragement and has given of herself more than one could hope to receive. Most of all, I thank her for her guidance and patience, especially for having to have read several earlier drafts of this thesis.

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having to leave in the middle of whatever it was that we were doing so that I might collect my data.

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Rat young are dependent upon their mother as a source of heat and nourishment. Initially, the lactating female rat spends approximately 80% of the day with her young (Grota and Ader, 1969) during which time she provides warmth and food to her offspring. Meeting the needs of her young for warmth and food places demands on the dam for both time and energy. A number of studies (Fleming, 1976; Kennedy, 1953; Leon, Fischette, Chee and Woodside, 1983; Richter and Barelare, 1938; Woodside, Wilson, Chee and Leon, 1981) have examined how the dam meets these demands as well as the way in which dietary and thermal factors influence the interaction between rat dams and their litters.

Kennedy (1953) has reported that the female rat consumes more digestable energy per day during lactation than at any other time in her lifespan, showing a three-fold increase over the levels of intake of non-lactating females (Kennedy, 1953; Fleming, 1976). Moreover, the increase in food intake during lactation in the rat is directly proportional to the size of the suckling litter (Ota and Yokoyama, 1973). Even when lactating rats are presented with diets diluted with a non-nutritive substance they are able to compensate for the decrease in caloric density by increasing the quantity of food ingested (Peterson and Baumgart, 1971; Leon and Woodside, 1983).

The female rat is not only able to increase her overall food intake to meet the demands of lactation, she also shows a change in her intake of certain macronutrients during this period. Richter and Barelare (1938) assessed self-selection patterns of the female rat across pregnancy and lactation when given a variety of foodstuffs including protein, fat, carbohydrate, and calcium. Their results indicated that

during pregnancy, females selected greater amounts of protein, fat and calcium than during estrous cycling and even greater amounts during lactation.

While Richter and Barelare's (1938) study has proved difficult to replicate (see Tribe, 1955) other researchers using different procedures (i.e. two-choice tests) have been able to confirm that rats do indeed increase their protein (Cohen, 1983; Leshner, Collier and Siegel, 1972) and calcium (Millelire and Woodside, 1985) intake.

Depriving the female of either food or a specific macronutrient has been shown to have negative consequences on both her and her offspring. For example, imposing food restriction has resulted in reduced body weight of the dam relative to control females allowed ad libitum access to the same diet (Leon et al., 1983; Woodside, Wilson, Chee and Leon, 1981). The reduction in the dam's weight during these restrictive conditions is apparently an indication that maternal corporal energy reserves have been mobilized enabling the dam to buffer the consequences of a poor maternal diet on the offspring (Leon and Woodside, 1983). Moreover, food restriction affects not only dam weight gain but also pup growth, which is impaired (Altman, Das, Sudarshan and Anderson, 1971; Altman and McCrady, 1972; Chow and Lee, 1964; Ottinger and Tanabe, 1969). Further, relative to those pups whose mothers were given ad libitum access to the same diet, the young of malnourished dams show decreased spontaneous locomotor activity (Altman and McCrady, 1972) and a decreased ability to perform a learning task (Ottinger and Tanabe, 1969). Similarly, the presentation of a low protein diet has resulted in the reduced body weight of the dam (Lynch, 1976; Massaro, Levitsky and Barnes, 1972) as well as a reduction in offspring body weights

(Forbes, Tracy, Resnick, and Morgane, 1977; Massaro, Levitsky and Barnes, 1974), an elevation in brain:body weight ratio until after weaning (Forbes, Resnick and Morgane, 1977), reduced exploratory behaviour (Weiner, Robinson and Levine, 1983) and a decreased proportion of time spent feeding, drinking and climbing (Massaro, Levitsky and Barnes, 1974; Hall, Leahy and Robertson, 1979).

Although the food rationing and protein restriction studies indicate that limiting nutrient availability to the dam adversely affects the development of the offspring, these outcomes may reflect not only a direct effect on milk production but also the result of some change in maternal behaviour in response to the diet manipulations. Plaut (1970), among others (Condo and Carllini, 1974; Meir and Shutzman, 1968), cautions that studies investigating the outcome of early environmental manipulations may not be examining only the consequences of the manipulation per se. Rather, what is observed may not be just the result of such early manipulations but changes in the mother rat's responsiveness to and interaction with her offspring may ulimately result as well. Specifically, Plaut suggests that by imposing an early nutritional deprivation state on the female, the young may also be deprived of the mother and consequently, a source of thermal, social, sensory and nutritional factors she brings with her. As a result, the differences in quality and quantity of maternal care received by the pups may lead to differences in the subsequent development of the offspring (Barnett and Burn, 1967; Meir and Shutzman, 1968).

Dietary manipulations which have been shown to delay the development of the rat progeny have been reported to change patterns of

mother-young interactions, but the findings have been inconsistent. Smart and Preece (1973), for example, undernourished mother rats during pregnancy and lactation by presenting them with 50% of the ad lib ration that control females consumed during comparable reproductive stages. Throughout 20 days of lactation the proportion of food-rationed females in their nests with the young at 0900 and 1300 h decreased, but at 1700 h there was a reverse in this trend; a greater proportion of food rationed females were in their nests with the young than were the ad lib controls. Leon et al (1983) however, found that compared to control females, food restricted dams spent more time with their young during the light portion of the light/dark cycle.

These discrepant findings are not unique. Frankova (1981) reported that dams fed a low protein diet spent less time with their litters than dams fed a diet with adequate protein whereas others have shown that lactating female rats presented with a low protein diet have increased contact with their offspring (Massaro, Levitsky and Barnes, 1972; Weiner, Fitzpatrick, Levin, Smotherman and Levine, 1977; Hall, Leahy and Robertson, 1979; Lynch, 1976). Such differences in the observed mother-young interactions among the various studies may be attributed to the different methods of assessment of this behaviour. In all but the Leon et al (1983) study, the proportion of time that the dam spent with her young was not continuously monitored. Rather, only brief, periodic observations of, for example, dam nest attendance, were made. Moreover, not all studies recorded this behaviour at the same time of day or on the same day postpartum. Only Leon et al (1983) circumvented these potential problems by continuously monitoring daily patterns of dam nest attendance for fourteen days postpartum.

One factor which might contribute to the alteration in mother-young contact is that the malnourished female may be monitoring the status of the pups and changing her behaviour as a result. For example, the dam may be compensating for the reduced energy supplied by increasing the time spent with the pups. The increased warmth supplied to the pups might reduce the amount of energy the young would need to expend to maintain their own body temperature. Another possibility is that the thermal status of the malnourished dam, and indirectly that of the pups, may mediate any observed changes in the time that the dam spends with the young for it has been shown that fuel restriction results in body temperature depression of both non-lactating and lactating rats (Leon et al, 1983; Westerter, 1977).

Recent investigations (Jans and Leon, 1983; Leon et al., 1978; Leon, Fischette, Chee and Woodside, 1981; Woodside and Leon, 1980) have demonstrated that patterns of mother-young interactions in the Norway rat are, in part, thermally mediated in that nest bout termination may often result from an acute rise in maternal temperature. According to the thermal model, when the dam is in contact with the litter during nursing bouts, the consequent mother-litter huddle occludes a portion of the dam's ventral surface resulting in a reduction of the surface area-to-volume ratio of the unit. This reduction decreases the efficiency by which the dam dissipates heat to the environment (Leon, Croskerry, and Smith, 1978), which can lead to an acute elevation of maternal body temperature while nursing. One way by which the dam can effectively diminish the acute hyperthermia is to remove herself from the suckling litter. Indeed, the curtailment of nursing bouts is

correlated with an acute rise in maternal ventral, core and brain temperature (Leon et al, 1978; Woodside et al, 1980).

This model may well explain the increased time that undernourished females spend with their young. As noted earlier, either the dam, her offspring, or both suffer from a loss of body weight as a consequence of the dietary manipulations, resulting in an increase in the surface area for heat dissipation relative to the decreased mass of the heat producing tissue. This provides for greater efficieny of heat dissipation, thus delaying the rate of rise of maternal temperature (see Woodside et al., 1980; Leon et al., 1978; Leon and Woodside, 1983) thereby allowing the dam to prolong contacts with the litter. Further, the reduction in body temperature associated with food restriction (Leon et al, 1983) would clearly render the dam less vulnerable to the acute hyperthermia associated with huddling, thus producing an elevation in mother-young contact time.

Indeed, imposing a malnourished state on the dam alters her body temperature (Léon et al, 1983) and this, in turn, can affect the time that she spends in contact with her young. Because these two factors are exerting their influences simultaneously, it is difficult to determine the extent to which each of the factors, nutritional status or patterns of mother-young contact, is influencing the development of the offspring. One situation which would help to separate the contribution of these two factors on pup growth would be to alter mother-young contact by manipulating dam body temperature while her nutritional status is maintained.

While malnourishing the rat by restricting food availability depresses body temperature, dietary over-consumption seems to produce an

opposite effect. Rothwell and Stock (1983), for example, have demonstrated that when rats are presented with a cafeteria diet of highly palatable food items, they become hyperphagic and can increase metabolizable energy intake from 40 to 120% above that of rats offered only a stock lab chow diet. Interestingly, increased body temperature has been associated with this dietary manipulation (e.g. Rothwell and Stock, 1979; Rothwell, Stock and Stribling, 1982).

Under such dietary conditions it may be speculated that both the dam and her offspring would be receiving a diet adequate to support growth. Hyperphagia, however, produces hyperthermia which may reduce contact between the dam and her young. Again, as in the malnourishment studies, a similar problem of interpretation would still exist: it would not be clear whether the resulting course of pup development could be attributed to nutritional overconsumption or whether it is the result of altered patterns of mother-young contact.

Heroux (1969) and Heroux, Johnson and Flattery (1971) have also indicated diet-related thermal effects in the rat. These researchers have shown that when rats presented with a commercially available thyroxine-free semipurified diet[1] are exposed to prolonged periods of cold stress, they are better able to regulate body temperature, as well as show greater survival rates, than do animals under the same conditions fed only a stock lab chow. Unlike Rothwell's studies, this

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1. The major distinction to be made between the stock lab chow and a semipurified diet is that the lab chow is composed of a variety of unrefined ingredients (e.g. protein source could be soybean meal) whereas a semipurified diet, as the name implies, is composed of refined ingredients (e.g. protein source could be in the form of casein).

dietary influence on body temperature has not been attributed to hyperphagia. Rather, Heroux has speculated that these observed results may be attributable to greater utilization of the semipurified diet since those animals showing better temperature regulation also showed decreased fecal output and fecal-bound energy relative to stock chow fed animals. Indeed, this may be possible since purified diets, at least, are more permeable to the intestinal mucosa than are stock lab diets (Shaw and Greep, 1949).

Presenting the lactating rat with a semipurified diet might help to disentangle the impact that patterns of mother-young contact and nutritional factors may have on influencing pup growth. In the Rothwell and Stock studies animals become hyperthermic on a cafeteria diet but their nutritional status may be difficult to assess. Because presenting the dam with a nutritionally adequate semipurified diet may alter its temperature status and potentially mother-young contact while minimizing nutritional differences from animals fed a stock chow, one might anticipate that the only aspect of the young rat's early environment which would be changed would be contact with the dam.

In the present set of experiments several consequences of semipurified and stock lab chow diet consumption were examined. In Experiment 1, the ability of a nutritionally adequate semipurified diet to increase the core temperature of the lactating female rat was examined. As in Heroux's studies, two groups of dams were presented with either an AIN-76 semipurified diet mixture or a stock Purina laboratory chow to determine the potential hyperthermic response to the semipurified diet. Given that this diet could indeed produce an elevation in the body temperature of the lactating female rat,

Experiment 2 examined the diet x thermal interaction on maternal behaviour and pup development. If semipurified diet consumption resulted in an elevation of dam body temperature, then according to the thermal model of mother-young interactions, we would have the potential to alter patterns of contact between the mother and her offspring. Specifically, mothers consuming this diet should demonstrate a decrease in the time spent with her offspring. This situation would potentially afford the ability to determine whether nutritional or maternal factors, or an interaction of the two, more strongly influences the course of rat offspring development. In Experiment 3 the hyperthermic phenomenon itself was investigated, specifically, the time course of its onset as well as time taken to return to baseline when original diet status is resumed.

Experiment 1

A number of studies have shown that diets can influence the temperature status of the rat (Heroux, 1969; Heroux et al, 1971; Rothwell and Stock, 1979; Swick and Gribskov 1983; Leon et al, 1983). The results of pilot investigations in this laboratory have suggested that dams consuming a nutritionally adequate commercially available semipurified diet mixture have elevated body temperatures relative to those dams consuming a stock laboratory chow. In this experiment an attempt was made to replicate our initial temperature findings for rats presented with a semipurified diet during the first two weeks of lactation. In addition, the growth rate of the offspring reared by mothers maintained on either the semipurified diet or the stock lab chow as well as the weight gained by dams was monitored during this period.

Method

Subjects Sixteen virgin female Wistar rats, obtained from Charles River Breeding Farm, St. Constant, Quebec, were mated in our laboratory. Two to three days prior to parturition each mother and her litter was assigned to either a standard Purina Lab Chow (LC) group or to an AIN-76 Semipurified Diet Mixture (SP) group (ICN, Nutritional Biochemicals, Cleveland, Ohio). Each diet group contained eight mothers and their litters of eight pups. Subjects were housed in a 12 h light/12 h dark cycle room with lights on at 0800 and lights off at 2000 h.

Apparatus and Procedure Virgin females (225-250 g) were group mated with a Wistar stud male. Vaginal smears were taken between 0830 and 0930 h each day and the presence of spermatazoa was taken as an indication of pregnancy. Two to three days prior to parturition, each female was placed in a polycarbonate cage (38 X 33 X 17 cm; Fisher Scientific Limited, model no. 01-260-50E) with woodchip bedding and introduced to their respective diets which were available ad libitum. (See Table 1 for a list of diet constituents). Both diets were presented in glass jars affixed to aluminum supports which in turn were secured to the side of the cage. Water was also available ad libitum and was presented in 100 mL graduated cylinders fitted with rubber stoppers and metal sipper tubes. On the day of parturition (Day 0), all litters were arbitrarily adjusted to eight pups by addition or elimination to ensure that equal suckling stimulation was received by the dams. All recordings began on Day 1 postpartum and were continued until Day 14 postpartum. During this period, dam colonic temperature was recorded twice daily between 0830 and 0930 h and between 2030 and 2130 h using a Yellow Springs (YSI) Telethermometer (model no. 43TA) with

Table 1

Dietary Constituents +

	Lab Chow	Semipurified
Protein	22.5%	(casein) 20.0%
Fat	5.0%	5.0%
Carbohydrate	51.1% *	(sucrose) 50.0% (corn starch) 15.0%
Fiber	3.8%	5.0%
Vitamins	no more than 1.0%	1.0%
Minerals	no more than 3.5%	3.5%
Moisture	10.0% **	5.0-8.0% **
Energy Value	3.39 kcal/gram	3.85 kcal/gram

+ See Appendix A for a detailed analysis of constituents

* Metabolically available carbohydrate

** Moisture contents already considered when proportions of dietary constituents had been determined

an attached YSI temperature probe (model no. 423) which was inserted 4 cm into the dam's rectum. In addition, mother weight, food and water intake as well as litter weight were recorded daily between 0830 and 0930 h. Mother weight was recorded using an Ohaus triple beam balance (2610 g capacity). Food jars and litters were weighed on a Sartorius electronic balance (model no. 1206 MP).

Since some data were lost due to food spillage or water leakage, all data were condensed to facilitate similar statistical analyses by finding the mean value for each subject on all measures between days 2-4, 5-7, 8-10 and 11-14, thus yielding 4 blocks of days. Data in graphs, however, are presented on a daily basis.

Results

Temperature Figure 1 represents mean daily colonic temperature at 0830 and 2030 h for dams in both the lab chow (LC) and semipurified (SP) diet groups during the first 14 days postpartum. A three-way analysis of variance (Diet x Time of Day x Days) was performed. SP females had higher core temperatures than LC females (Diet; $F(1,14)=29.81$, $p<.001$) and core temperatures were higher at night than during the day ($F(1,14)=23.20$, $p<.001$). All other main effects and interactions were not statistically significant (Days, $F(3,42)=.731$; Diet x Time of Day, $F(1,14)=.025$; Diet x Days, $F(3,42)=1.55$; Time of Day x Days, $F(3,42)=.44$; Diet x Time of Day x Days, $F(3,42)=.15$; p's $>.05$).

Food Intake Figure 2 shows mean daily food intake for dams in the LC and SP diet groups for the first 14 days postpartum. A two-way analysis of variance (Diet x Days) revealed no difference in the quantity of food ingested by the dams between the two diet groups (Diet; $F(1,14)=2.24$, $p>.05$). There was, however, a significant main effect of Days ($F(3,42)=37.98$, $p<.001$) indicating an overall increase in total food consumption over days postpartum. The Diet x Days interaction was not statistically significant suggesting that patterns of food intake were similar for both diet groups.

Caloric Intake The mean daily caloric intake for dams in both diet groups are presented in Figure 3. Data were subjected to a two-way (Diet X Days) analysis of variance. The results from the analysis indicated no difference in caloric consumption between the two diet groups (Diet effect; $F(1,14)=.414$, $p>.05$) yet there was an increase over blocks of days (significant main effect for Days; $F(3,42)=39.92$,

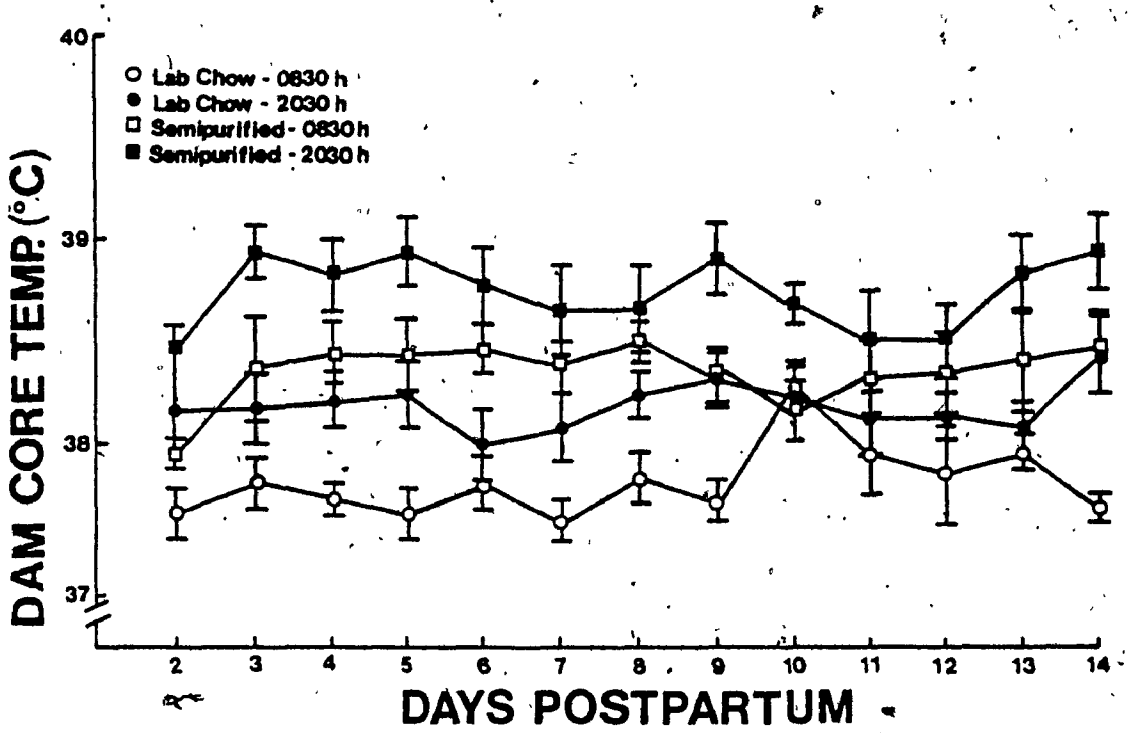

Figure 1. Mean daily LC and Sp dam core temperature at 0830 and 2030 h during the first two weeks postpartum. SEM's are shown.

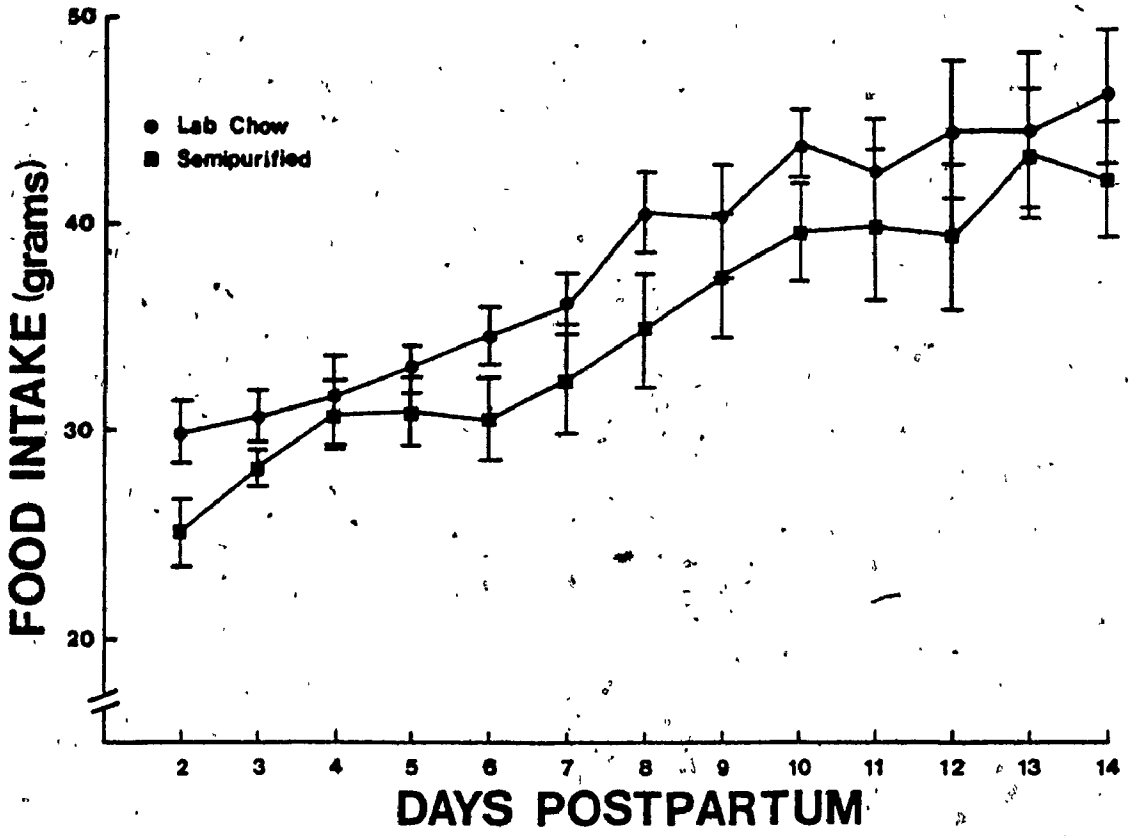

Figure 2. Mean daily LC and SP dam total food intake during the first two weeks postpartum. SEM's are shown.

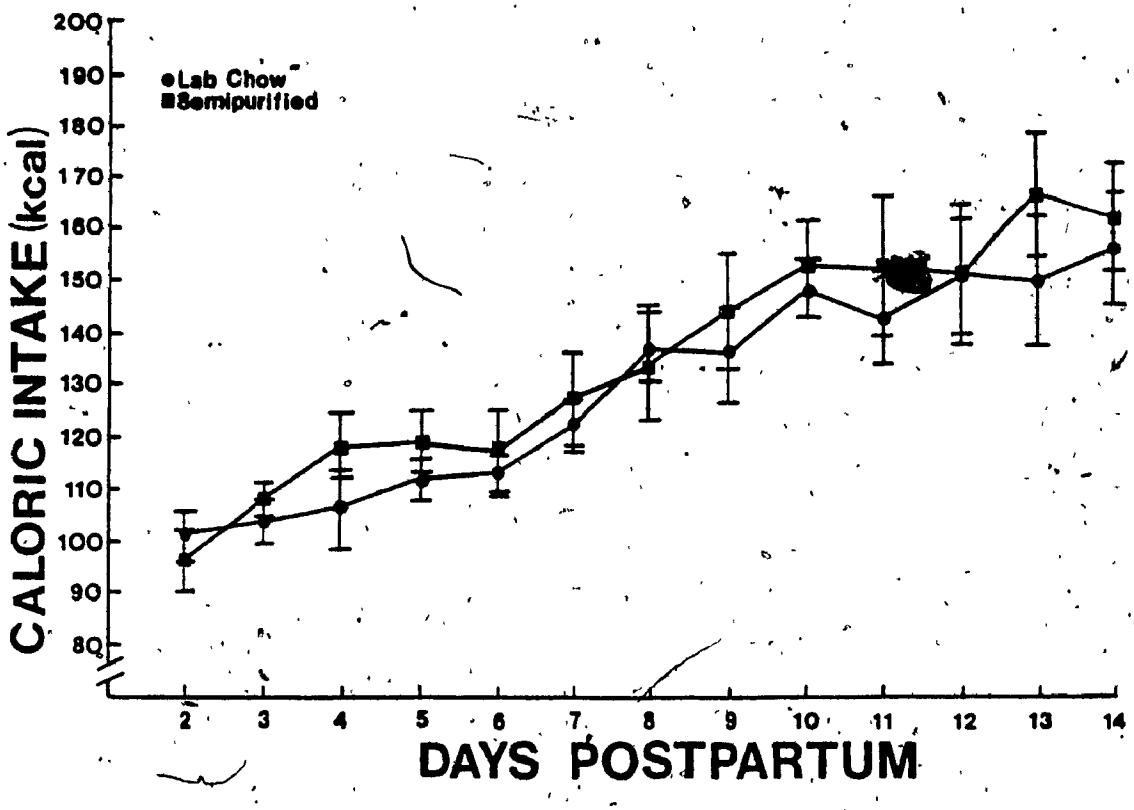

Figure 3. Mean daily LC and SP dam total caloric intake during the first two weeks postpartum. SEM's are shown.

$p < .05$). As with food intake, the Diet x Days interaction was not statistically significant ($F(3,42)=.10$, $p>.05$).

Water Intake Mean daily water intake for LC and SP dams are shown in Figure 4. Data were analysed by a two-way (Diet x Days) analysis of variance. The analysis revealed a significant Diet effect ($F(1,14)=38.01$, $p<.001$) due to the greater quantities of fluid intake demonstrated by the LC dams. The significant main effect for Days ($F(3,42)=13.56$, $p<.001$) indicates increases in water consumption over days and that this pattern was similar for both diet groups since the Diet x Days interaction was not statistically significant.

Mother Weight A two-tailed independent t-test performed on Day 1 body weight between the LC and SP animals revealed no difference on this parameter at this time. A two-tailed independent t-test performed on percentage weight change between Day 1 and Day 14 postpartum was significant ($t(14)=2.76$, $p<.05$) indicating a greater percentage increase for LC dams relative to SP dams over the first two weeks of lactation. Day 1 dam weight and percentage weight change for LC and SP dams are indicated in Figures 5 and 6, respectively.

Pup Growth Mean individual daily pup growth for pups reared by dams presented with the LC and SP diets is shown in Figure 7. A two-way analysis of variance (Diet x Days) revealed a nonsignificant main effect for Diet ($F(1,14)=.007$, $p>.05$) but the effect of Days was significant ($F(3,42)=12.45$, $p<.001$). The nonsignificant Diet x Days interaction indicates that both groups of pups maintained similar patterns of growth over the 14 days postpartum.

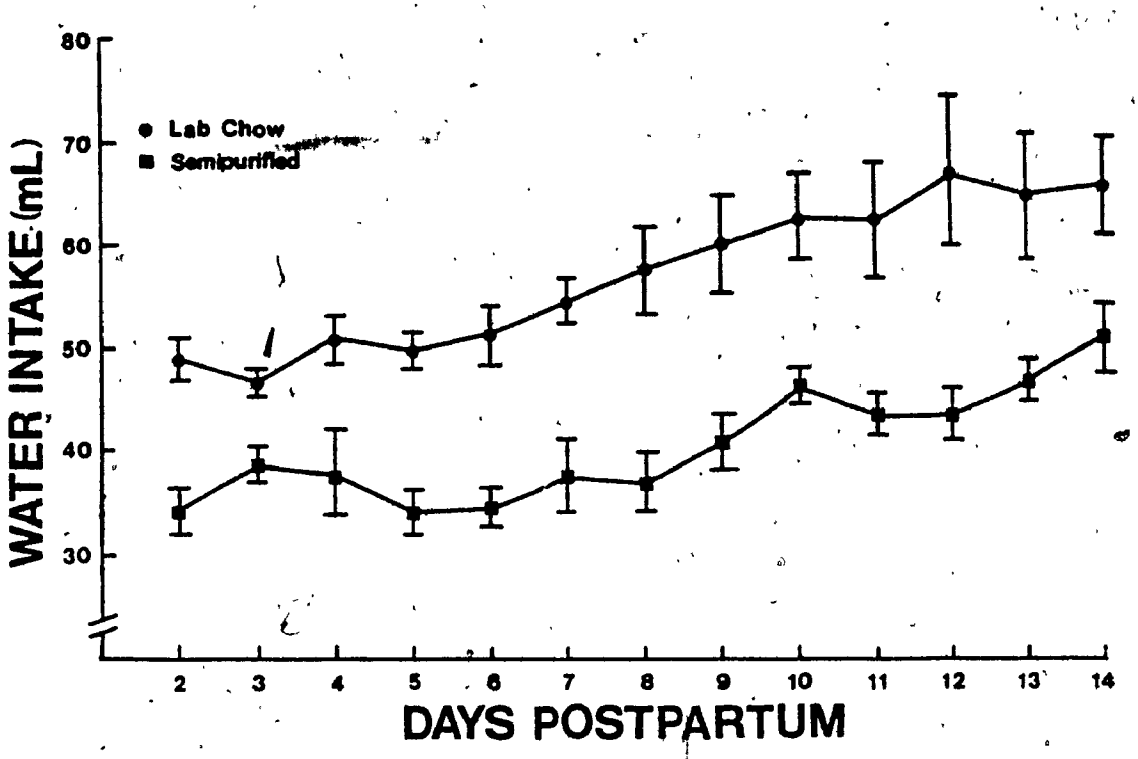

Figure 4. Mean daily LC and SP dam total water intake during the first two weeks postpartum. SEM's are shown.

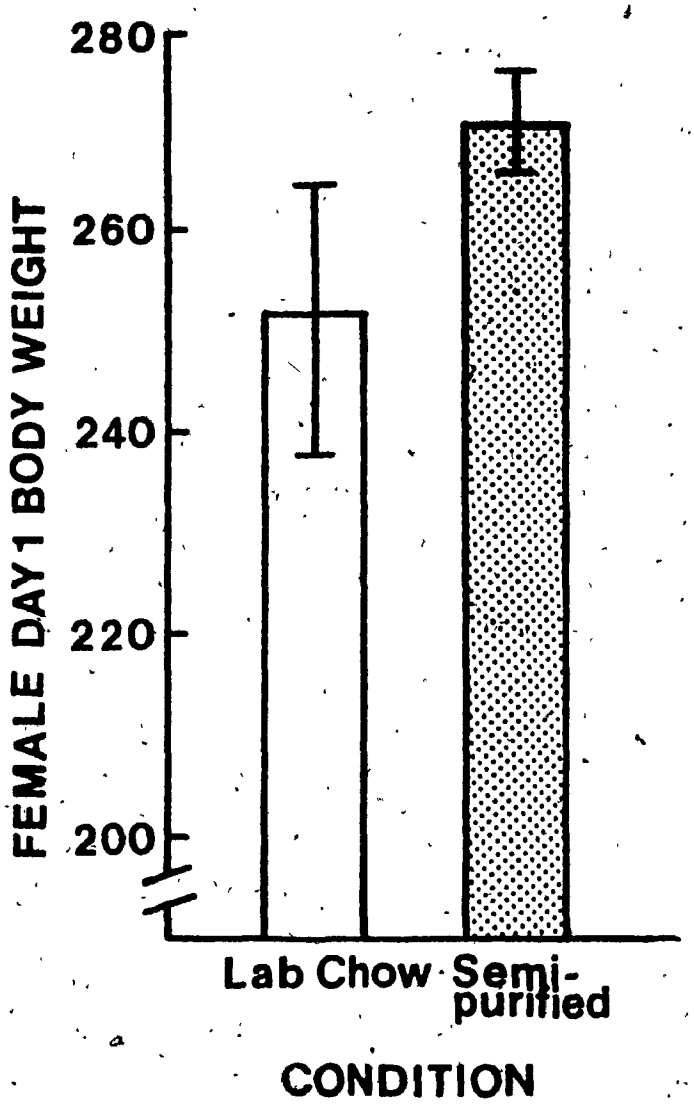

Figure 5. Mean Day 1 LC and SP dam body weight. SEM's are shown.

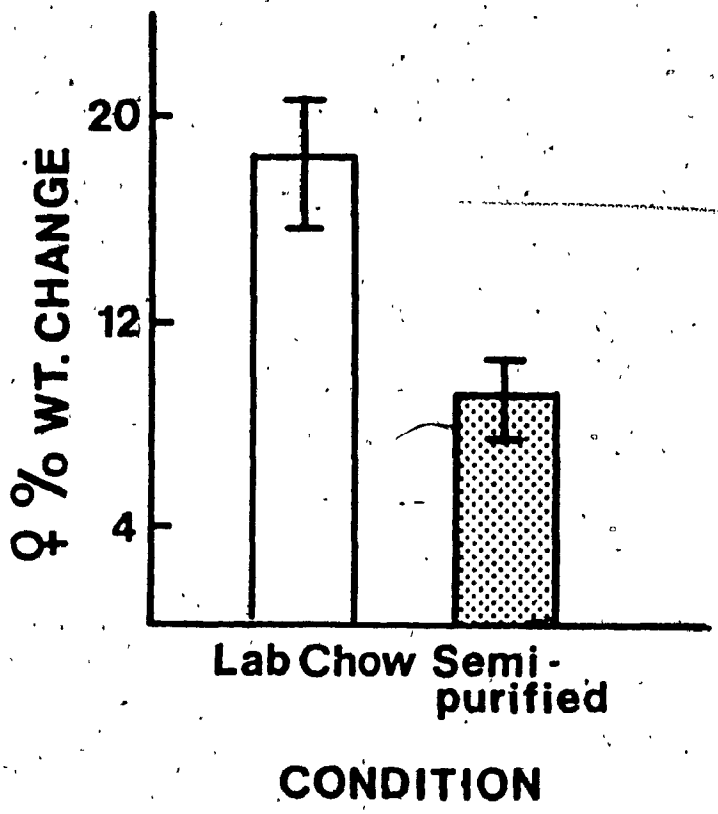

Figure 6. Mean LC and SP percentage weight gain over first two weeks postpartum. SEM's are shown.

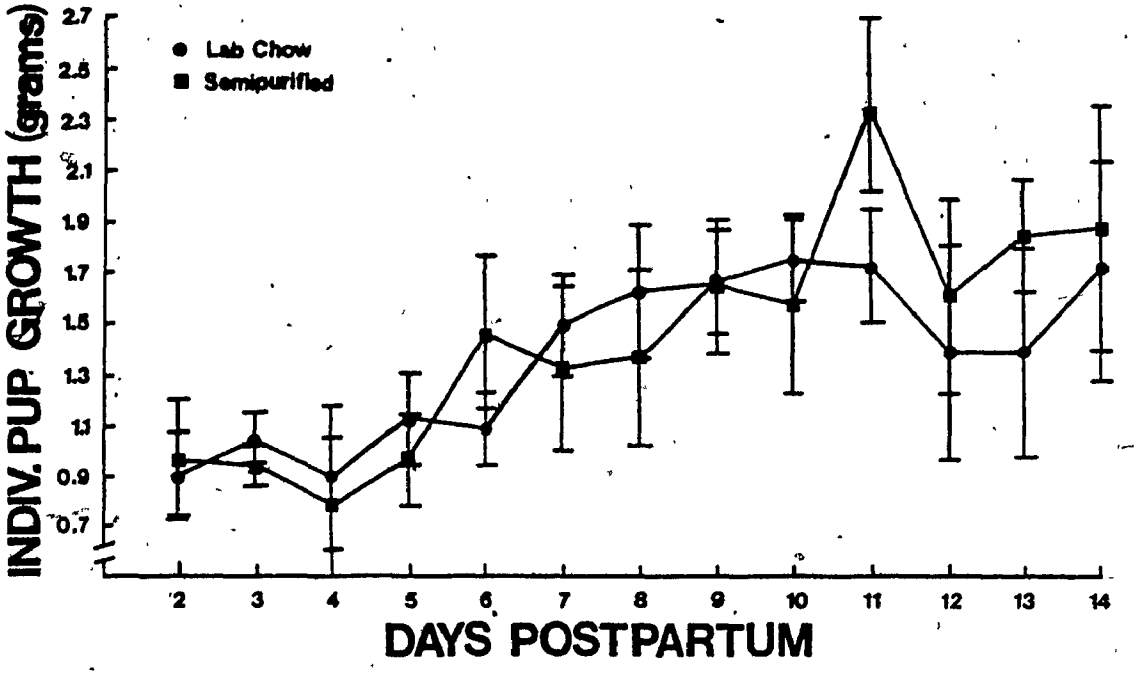

Figure 7. Mean daily LC and SP individual pup growth during first two weeks postpartum. SEM's are shown.

Discussion

The most interesting findings in this experiment were that dams that consumed the semipurified diet had higher core temperatures and gained less weight than did dams consuming the lab chow even though the animals consumed equivalent amounts of grams and calories from diets that were nutritionally similar. During the first two weeks postpartum, dams eating the semipurified diet had consistently higher core temperatures than those eating the lab chow at both 0830 and 2030 h. Furthermore, core temperature of SP dams was approximately .4 ^{o} C higher than that of LC dams both in the morning and at night.

Both LC and SP dams ate progressively more food over days, presumably because of the increased energy demands required to feed the growing pups. Furthermore, it also appears that dams in the two diet conditions were equally able to meet the increasing nutritional requirements of the growing pups since there was no difference between LC and SP offspring weight gain over the course of lactation.

LC dams consumed greater quantities of water than SP dams. It is not clear why this occurred since similar quantities of the two diets were consumed coupled with the fact that manufacturer specifications indicate similar moisture contents for both. One possibility may be that the lab chow became drier sooner than the semipurified diet since the lab chow was stored at room temperature whereas the semipurified diet was kept refrigerated in a sealed cellophane-lined bin which may have reduced moisture loss.

Another difference that was observed between the diet groups was maternal weight gain. Females in the semipurified diet group gained only about one half of the percent weight that dams consuming the stock

lab diet gained. This has not been a consistent finding in studies where diet-related thermal responses have been observed since there are reports of no difference (Swick and Gribskov, 1983), increases (Heroux et al, 1971) and decreases (Rothwell and Stock, 1979) in the rate of weight gain for animals consuming diets that produce hyperthermia.

Under the present dietary conditions, it might be reasonable to assume that dams, and perhaps pups, in both groups are equally well nourished because the constituents of the two diets are similar. Since dam body temperature was differentially affected, then, according to the thermal model, the potential to alter patterns of mother-young contact exists. Thus, while contact time may be indirectly manipulated as a result of diet-related differences in body temperature, the nutritional status of the dams in the two diet groups may be similar, affording the opportunity to separate the influences of nutritional factors and patterns of contact on the development of the rat young. In the following experiment, diet-induced hyperthermia will be used as a tool to alter mother-young contact time to determine if such changes in any way influences the course of pup development.

Experiment 2

In Experiment 1 it was shown that lactating rats maintained on a semipurified diet had significantly higher core temperatures than those were eating the lab chow diet. Such an addition to the chronic heat load normally experienced by dams during lactation (e.g. Leon et al, 1978) should, according to the thermal model of influences on nest-bout durations, result in a decrease in time that the SP dam spends with her litter. Females whose body temperature was already higher might be expected to experience more rapid increases in body temperature while huddling with the young and as a consequence, might reach more rapidly the critical temperature associated with nest bout termination. In a number of studies it has been shown that manipulating acute and chronic temperature factors alters the patterns of mother-young contact. Removing the dam's tail, thereby decreasing the efficiency of body heat loss, decreased the time that females spent with their young (Leon et al, 1978) as did placing the dam on a warm nest surface (Jans and Leon, 1983; Leon and Woodside, 1981; Leon et al, 1978) or presenting her with warm pups (Jans and Leon, 1983). Moreover, adrenalectomized-ovariectomized dams demonstrate a chronic depression in body temperature accompanied by an increase in nest time relative to intact females (Leon et al, 1978; Woodside and Leon, 1980).

If decreased contact time did result from increased maternal body temperature brought about by manipulating diet, this method might provide a somewhat more natural means for studying the effects of the patterns of maternal contact on pup development. For example, dams which raise their litters in a warm ambience show a reduction in contact

time with the young accompanied by a reduced rate of litter weight gain. However, under these conditions the energy balance of the dam is changed (Leon and Woodside, 1983). One criticism of the studies examining the effects of undernutrition on offspring development is that the observed outcomes result from changes in maternal behaviour rather than food availability. One might be able to address this question by assessing the effects of simply changing maternal behaviour. Thus, one possible application of the diet-induced hyperthermia might be to allow separation of the effects of nutrition from maternal care in assessing development of the rat young.

In the following experiment, we determined whether the thermal consequences of SP diet consumption would, in fact, alter maternal behaviour by comparing the mother-litter contact time of dams fed either the semipurified or the lab chow diets. In addition, any differences between the two diet groups on offspring development were also assessed. Since no diet-related difference in pup growth was found in the previous experiment, a battery of tests examining behavioural development and organ growth was administered to help determine if any more subtle differences in pup development exist.

Method

Subjects Sixteen virgin female Wistar rats, obtained from Charles River Breeding Farm, St. Constant, Quebec, were mated in our laboratory. See Experiment 1 for details. Eight dams were assigned to each of the semipurified or to the lab chow diet group two days prior to parturition. Litters were adjusted to 8 pups on Day 1 postpartum. Animals were housed in a 12 hour light/12 hour dark cycle room with lights on at 0800 hours and lights off at 2000 hours. Mean ambient temperature during the course of the experiment was 21.97 $\pm$ .03 ranging from 17.0 to 26.0 ^{o} C.

Apparatus The diets and apparatus used in Experiment 1 were also used here but with the following additions. Maternal ventral temperature and pup skin temperature were recorded using a YSI "Banjo" surface temperature probe (Yellow Springs Instruments, model no. 408). Pup core temperature was recorded with a YSI (no. 402) temperature probe. All organs were weighed on a Sartorious Analytical Balance (model no. 2842). To measure nesting behaviour, each mother reared her young in cages designed for continous recording (Croskerry, Leon, Smith and Mitchell, 1976) which were adapted for interfacing with the Apple II + microcomputer. Each cage (38 x 33 x 17 cm) was fitted with a tray (28.25 X 10.5 cm) which had a nest box (14.25 X 11.25 X 6.0 cm) constructed on one side. The nest trays were sufficiently small as to ensure contact with the pups when the dam entered the nest box. The other side of the tray could be counterbalanced to accomodate for the increasing weight of the growing litter. The tray was balanced on a fulcrum so that when the dam entered the nest box the tray would tilt and depress a microswitch which would break the current running from the

computer to each cage and which would return to its resting state when the dam left the nest area. Upon each activation of the circuit a software counter which recorded nest frequency was incremented. In addition, time of each bout onset and termination was also monitored by the computer system. Only nest bouts which were greater than 5 sec. were used for all computations in order to exclude activations of the system which did not involve contact with the young. A similar monitoring system has yielded recordings of contact time virtually identical to direct experimenter observation (Croskerry, Leon, Smith and Mitchell, 1976). Diets and water were freely available but only from outside of the nest boxes.

Procedure The procedure that was used in Experiment 1 was followed in the present experiment (see Experiment 1 for details) with the following additions. On the day of parturition (Day 0) each mother along with her litter was introduced to the nesting cages and was allowed approximately 24 hours to familiarize themselves with the new environment so that nesting behaviour data was available from Day 2 until Day 14 postpartum, when pups became mature enough to leave the nest box. Nest time and nest frequency during both the light and dark phases of the light cycle were continuously recorded for each day during this period. The computer system was restarted and all counters zeroed daily at 0830 and 2030 h for the first 14 days postpartum. Since some data were lost due to occasional system malfunction, data were condensed by calculating the mean value between days 2-4, 5-7, 8-10 and 11-14 for each subject on all measures, yielding four blocks of days but individual days data are shown in graphs.

Food and water intake for dams in both diet groups were recorded twice daily between 0830 and 1230 h and between 2030 and 2230 h. Caloric intake for each period was also determined on a daily basis. Dam and litter weight were also recorded at this time as were mother core and ventral and pup skin temperatures. As in Experiment 1, dam core temperature was recorded by placing the temperature probe 4 cm. into the dam's rectum. Mother ventral and pup skin temperatures were determined by placing the banjo probe directly over the sternum. During the first two weeks postpartum, all temperature recordings were conducted daily between 0830 and 1230 h and between 2030 and 2230 h. Pup core temperature was also recorded on day 22/23 postpartum by inserting the temperature probe 2 cm. into the rectum. Mother body temperature was also recorded on day 22/23 postpartum just prior to recording pup temperature.

Pup Development

(a) Behavioural: In order to obtain assessments of development other than weight gain, the following tests of behavioural development in the pups were carried out over a period of 23 days postpartum. The time period of individual test administration is described below.

(i) Righting Response. The righting response is demonstrated when the pups are placed in the supine position and is characterized by limb extention and bidirectional rocking of the fore and hind limbs in attempt to return to an upright position. Daily, between days 2-10 postpartum, one half of each litter was placed on a horizontal surface in the supine position and upon release, the latency to return to an upright position was recorded. Each animal was allowed a maximum of 15 seconds to perform this response.

(ii) Curling Response. The curling response is exhibited by rat pups when they are carried by their mothers and, as such, is also referred to as the "transport response" (Leon and Brewster, 1980). This response is demonstrated by the young when a compact bundle is formed by tucking the limbs and tail close to the body and ceases when the pups are capable of moving on their own. The curling response was assessed between days 3-13 inclusive. Each pup from all litters was picked up by the nape of the neck and shaken lightly. The number of pups exhibiting at least a 3-point curl (i.e. at least 3 extremities pulled into the trunk) was recorded. Criterion for this measure was the day on which at least 75% of the litter demonstrated at least a three-point curl.

(iii) Head Lifting. This response is characterized by lifting and lateral movements of the neck and head while the trunk remains flush to the surface. This response is assumed to be a general orientation response (Altman et al, 1971). The head lifting response was examined between days 6-14 postpartum. All pups were placed on a horizontal surface and the number of pups which demonstrated this response within a 60-second interval was recorded. The first day when 100% of the all pups in each litter demonstrated this response was also determined.

(iv) Eye Opening. Eye opening is said to occur when the pup has at least one eye open (Galler, 1980). The presence of at least one eye open was examined daily between days 11-14 postpartum. The number of pups in each litter demonstrating this ability was recorded.

(v) Thermoregulation. Thermoregulatory ability was also used as an index of maturation to determine whether the consequence of being reared by a warm versus cool mother is similar to being raised in a warm

versus cool environment. Rats which are raised in a warm environment usually show a delay in the development of this ability since, during the early stages of life, there is less need for them to self-regulate body temperature (Krecek, Krechkova and Martinek, 1957). One male and one female each from 7 litters in both diet conditions were tested for thermoregulatory abilities on day 22 postpartum. One male and one female from the remaining litter in each diet condition was tested on day 23. Pups were subjected to cold stress by being placed in a cold chamber (Canadian General Electric, model no. 812) maintained at 6 ^{o} C for a 40 minute period. During this time pup temperature was recorded every 10 minutes by placing the temperature probe 2 cm. into the pup's rectum. All pups were weighed prior to cold exposure.

(b) Organ Analysis

(i) Adrenal Weights. For this measure, as well as for those which follow, organ weight was determined on Day 22 or 23 postpartum for one male and one female from each litter on the same day that thermoregulatory ablilities were assessed. All animals were weighed prior to organ removal and were sacrificed by sodium pentabarbitol overdose (65% solution) administered i.p.. Adrenal weights were determined since it has been shown that the rearing environment can affect adrenal weights in gerbils (Clark and Galef, 1980). To determine adrenal weights, bilateral adrenalectomies were performed after weighing each subject.

(ii) Brown Adipose Tissue. Cold exposure and dietary factors can influence the activity and size of brown adipose tissue (Himms-Hagen, 1984). Since SP dams would be expected to spend less time with their litters than would LC dams, pups maintained by dams on the SP diet

might be expected to face greater cold stresses and hence, have larger BAT:body weight ratios than would pups maintained by dams fed the stock lab diet. Interscapular brown fat pads were weighed after removal of surrounding white fat and connective tissue for pups in both diet conditions. Similary, interscapular brown fat pad weights were also determined on Day 28 postpartum for SP- and LC-fed dams.

(iii) Gonad and Brain Weights. Since mothers consuming the semipurified diet may potentially spend less time with their young than may mothers consuming the lab chow, they may, as a result spend less time delivering nourishment to them. This reduction in available nourishment may result in decreased morphological growth which may be seen in gonad as well as brain weight. Bilateral ovariectomies were performed on one female pup from each litter. Similarly, both testes were removed from one male in each litter. Moreover, in addition to determining gonad weight, whole brain and brain weight less the cerebellum, were also determined.

Results

Nest Time

(i) Total Nest Time. Figure 8 shows mean total daily nest time for dams in the two diet conditions during the first 14 days postpartum. Although dams consuming the semipurified diet tend to spend less total time with their litters than do dams fed the lab chow, this reduction was not statistically significant ($F(1,14)=3.60$, $p>.05$). Dams in both diet conditions did demonstrate a significant reduction in total daily nest time over days (Days; $F(3,42)=67.19$, $p<.001$). The Diet X Days interaction was not significant.

(ii) Light Nest Time. Figure 9a represents the mean time that dams in the two diet conditions spent with their young during the light part of the day/night cycle for this first 14 days postpartum. SP dams spent less time with their litter than did LC dams ($F(1,14)=9.04$, $p<.01$). In addition, the significant Days effect ($F(3,42)=49.53$, $p<.001$) indicates a progressive decrease in nest time over days and that this pattern was similar for both diet groups since the the Diet X Days interaction was non-significant ($F(3,42)=.56$, $p>.05$).

(iii) Dark Nest Time. During the dark phase, there was no difference between the two diet conditions in the amount of time that dams spent with their litters ($F(1,14)=2.62$, $p>.05$). The Days effect was significant but the Diet X Days interaction was not ($F(3,42)=29.58$, $p<.001$ and $F(3,42)=.21$, $p>.05$, respectively). Mean nest time for SP and LC dams during the dark phase during the first two weeks postpartum is shown in Figure 9b.

(iv) Mean Nest Bout Duration. Mean LC and SP nest bout durations

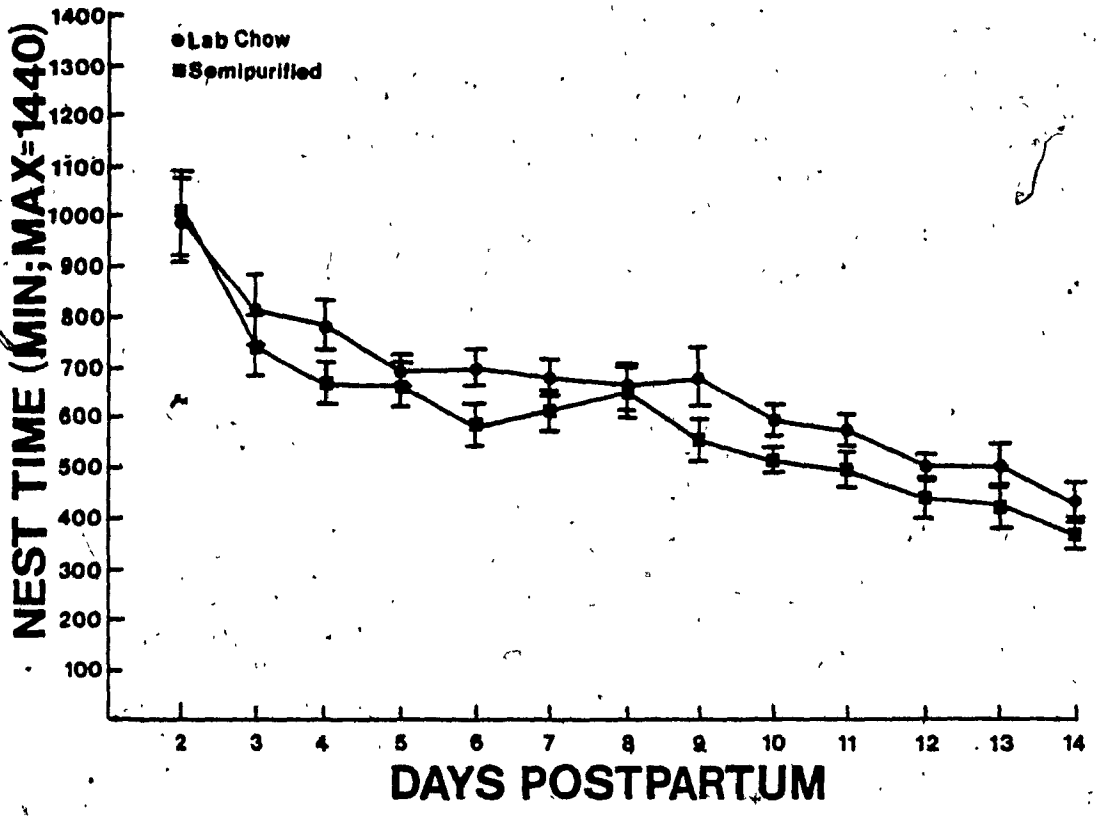

Figure 8. Mean total LC and SP nest time during first two weeks postpartum. SEM's are shown.

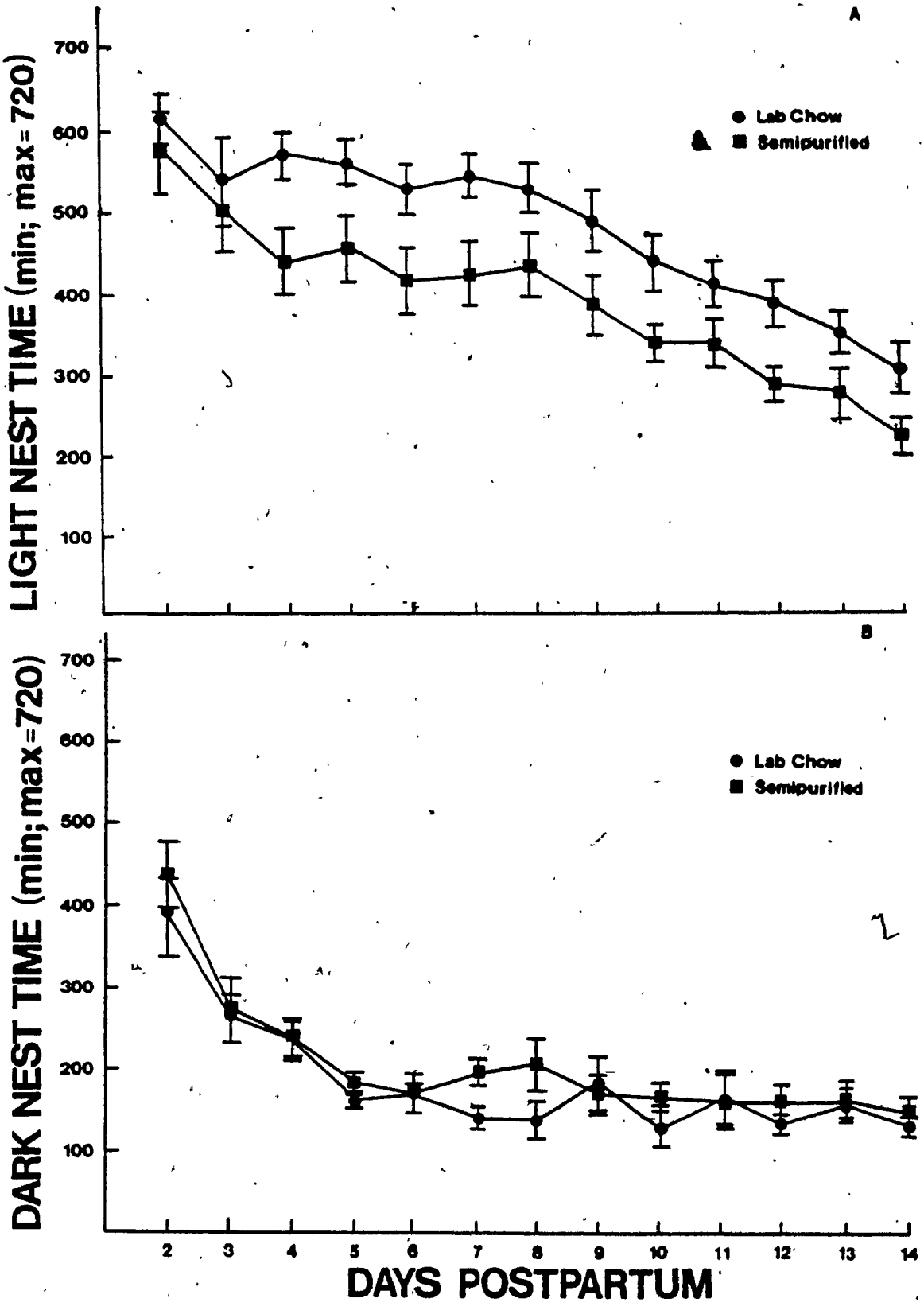

Figure 9. a) Mean daily LC and SP light nest time during first two weeks postpartum. SEM's are shown. b) Mean daily LC and SP dark nest time during first two weeks postpartum. SEM's are shown.

during the light and dark cycle on Days 4 and 10 postpartum are shown in Figure 10. The results from the analysis revealed statistically significant shorter mean nest bout durations for females in the semipurified diet group ($F(1,14)=4.48$, $p=.05$). In addition, mean nest bout duration was shorter at night than during the day ($F(1,14)=80.78$, $p<.001$) and shorter on Day 10 than on Day 4 postpartum ($F(1,14)=7.42$, $p<.05$. The Diet X Time interaction was statistically significant ($F(1,14)=8.72$, $p<.05$).

Nest Frequency

(i) Total Nest Frequency. Figure 11 shows mean total daily nest frequency for dams in the two diet conditions during the first two weeks postpartum. Although it appears that SP dams entered the nest box more frequently than LC dams, there was no statistically significant Diet effect on total nest frequency ($F(1,14)=1.72$, $p>.05$). Further, the total mean number of daily visits to the nests remained stable over days ($F(3,42)=2.62$), $p>.05$) and the Diet X Days interaction was not statistically significant ($F(3,42)=1.82$, $p>.05$).

(ii) Light Nest Frequency. Figure 12 shows mean nest frequency during the light phase over the first 14 days postpartum for dams in both diet conditions. Dams fed the semipurified diet tend to enter the nest box more often than those fed the lab chow but this effect was not statistically significant ($F(1,14)=1.59$, $p>.05$). In addition, the mean number of visits to the nest remained stable over days ($F(3,42)=1.23$, $p>.05$). The Diet X Days interaction did not yield statistically significant results ($F(3,42)=1.07$, $>.05$).

(iii) Dark Nest Frequency. During the dark phase, SP dams visited the

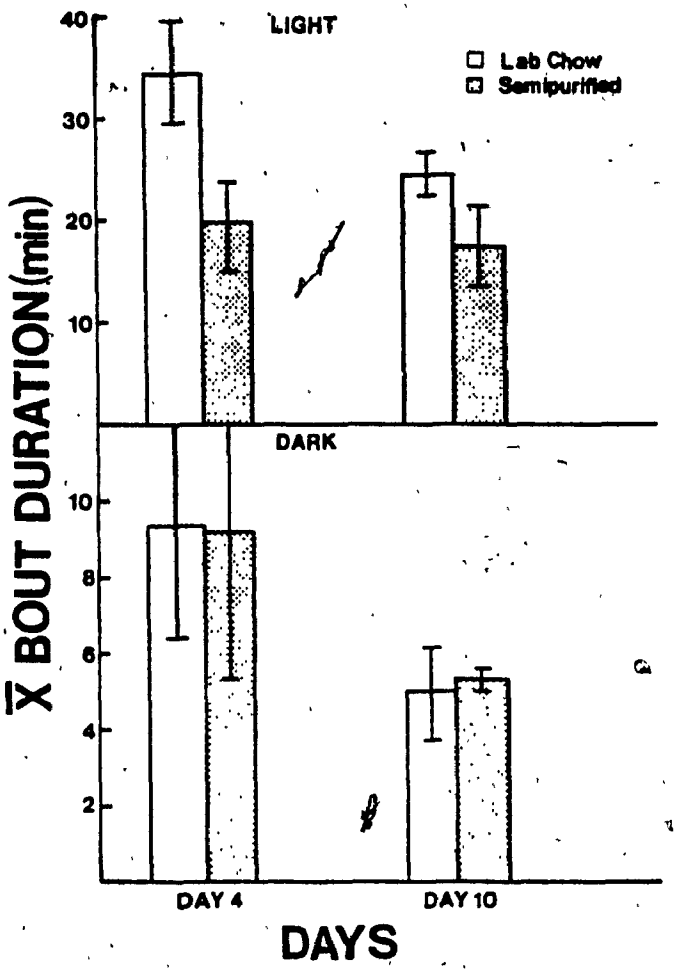

Figure 10. Mean LC and SP bout duration during the light and dark cycle on Day 4 and Day 10 postpartum. SEM's are shown.

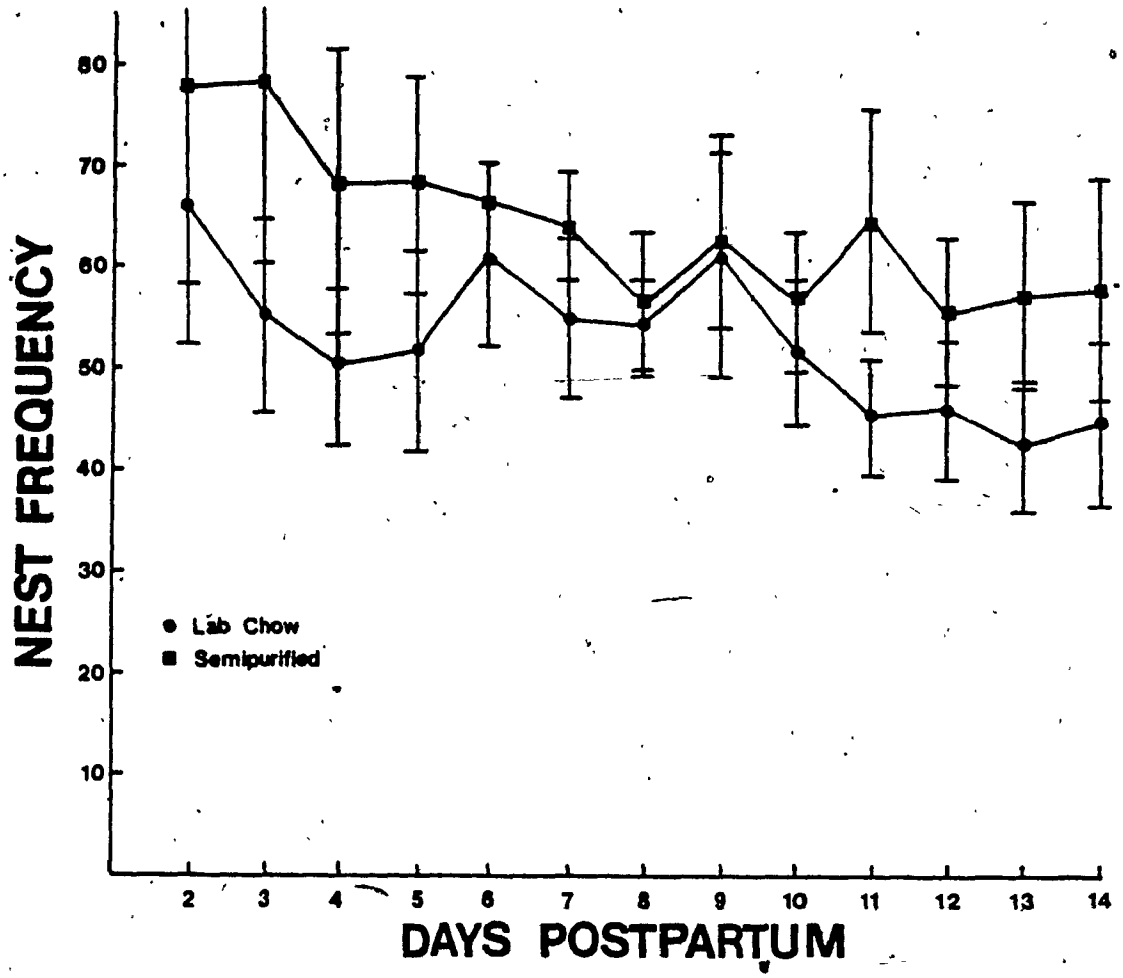

Figure 11. Mean daily LC and SP total nest frequency during first two weeks postpartum. SEM's are shown.

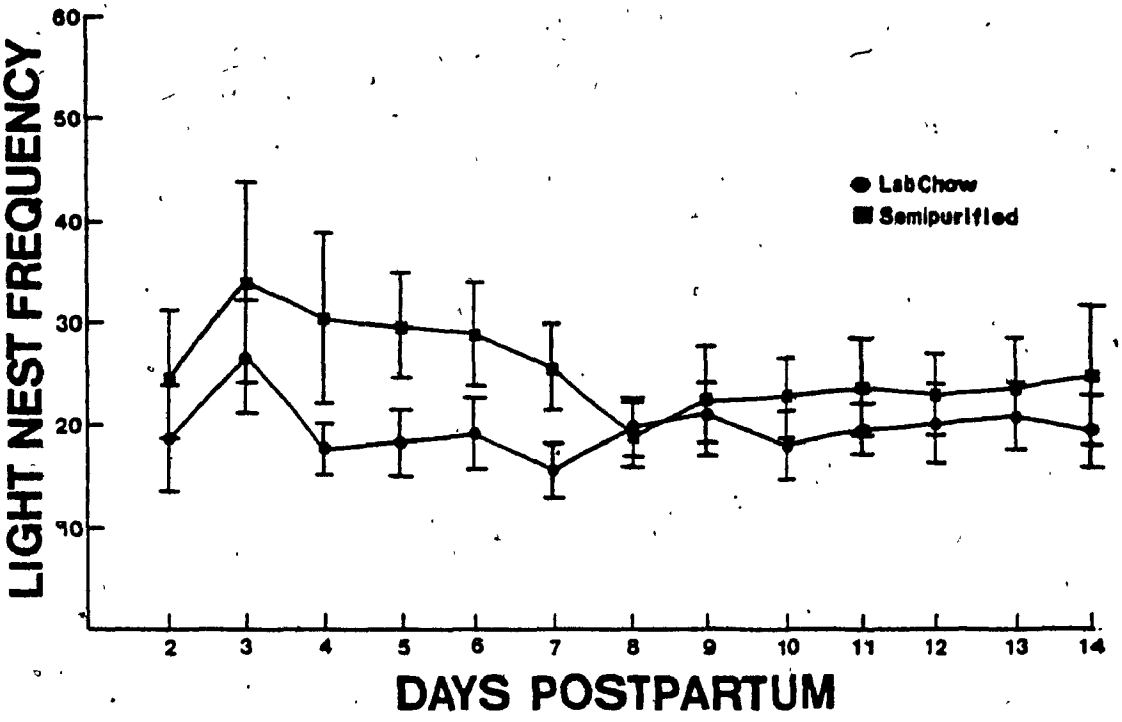

Figure 12. Mean daily LC and SP light nest frequency during first two weeks postpartum. SEM's are shown.

nest as often as did LC dams as indicated by the nonsignificant effect of Diet on frequency ($F(1,14)=2.00$, $p>.05$). The Days effect was significant ($F(3,42)$, $p<.05$) although this may be due to the aberrantly high frequencies on Days 2 and 3. Similar patterns of nest frequency were, however, maintained by dams in both diet conditions since the Diet X Days interaction was not significant ($F(3,42)=2.19$, $p>.05$). Mean nest frequency during the dark phase for SP and LC dams for the first two weeks postpartum is shown in Figure 13.

Temperature

(i) Dam Core Temperature. Figure 14 represents colonic temperature at both 0830 and 2030 h during the first 14 days postpartum for mothers maintained on either the standard lab chow or the semipurified diet. As in Experiment 1, dams maintained on the semipurified diet had higher core temperatures than did dams maintained on the lab chow. This is indicated by a significant main effect for Diet ($F(1,14)=33.26$, $p<.001$). In addition, temperatures were higher at night than during the day ($F(1,14)=37.32$, $p<.001$) and increased over days ($F(3,42)=11.21$, $p<.001$). None of the interactions were statistically significant.

(ii) Dam Ventral Temperature. The SP diet group dams had elevated ventral temperatures relative to LC dams ($F(1,14)=16.48$, $p<.01$). As with core temperature, ventral temperature was higher at night than during the day ($F(1,14)=8.28$, $p<.05$) and increased over days ($F(3,42)=7.82$, $p<.001$). None of the interactions were significant. Mean morning and evening ventral temperature for SP- and LC- fed dams during the first fourteen days postpartum is shown in Figure 15.

(iii) Pup Skin Temperature. Mean daily pup skin temperature is shown

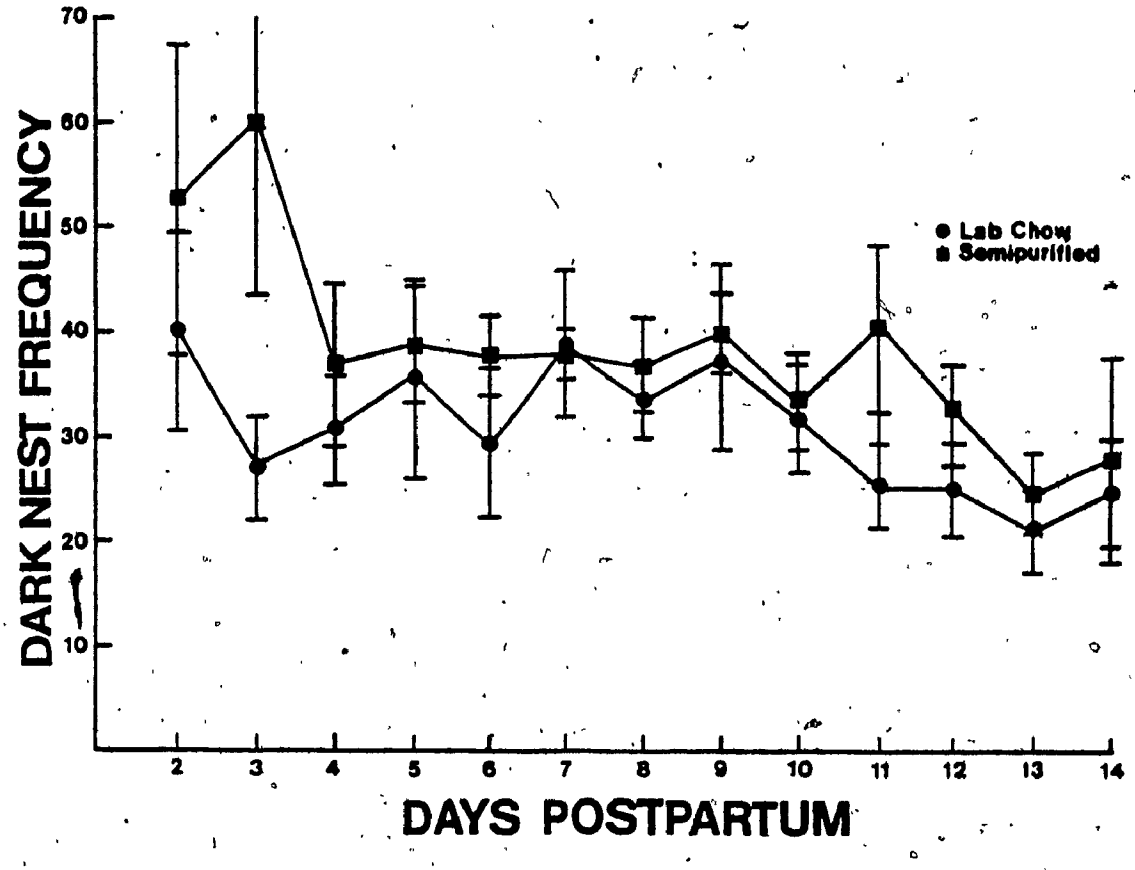

Figure 13. Mean daily LC and SP dark nest frequency during first two weeks postpartum. SEM's are shown.

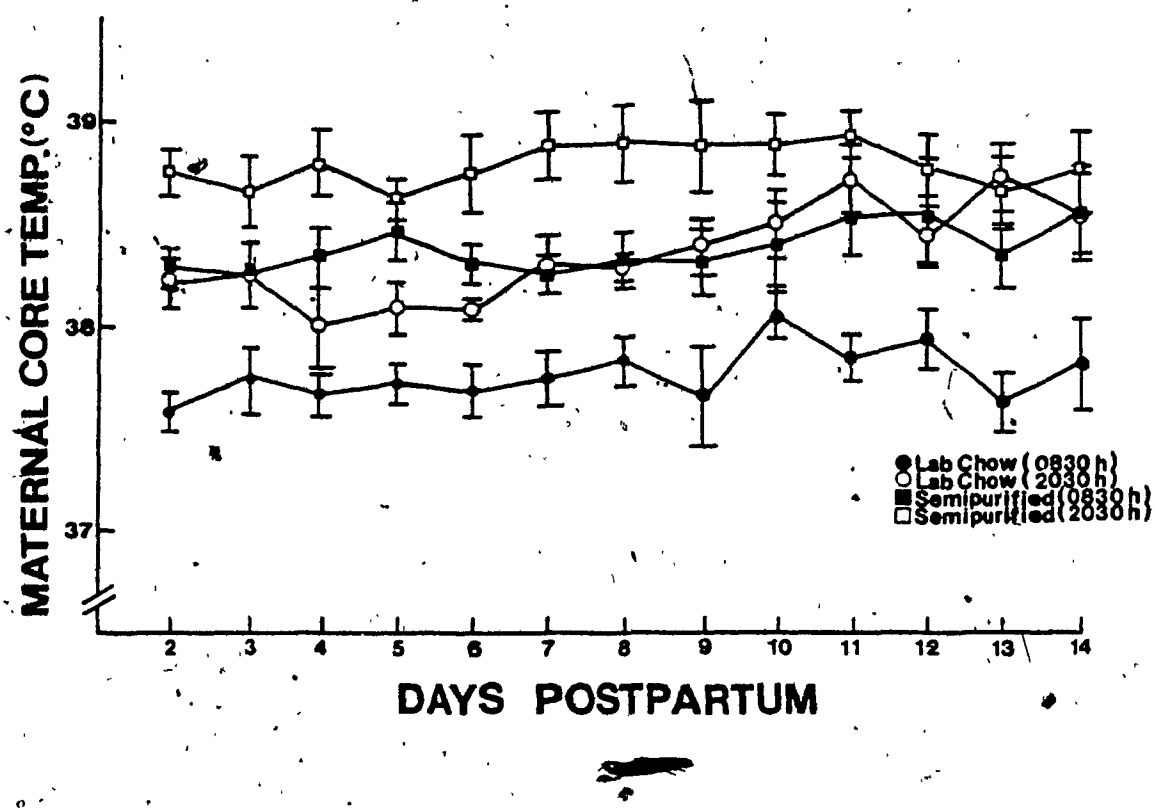

Figure 14. Mean daily LC and SP dam core temperature at 0830 and 2030 h during first two weeks postpartum. SEM's are shown.

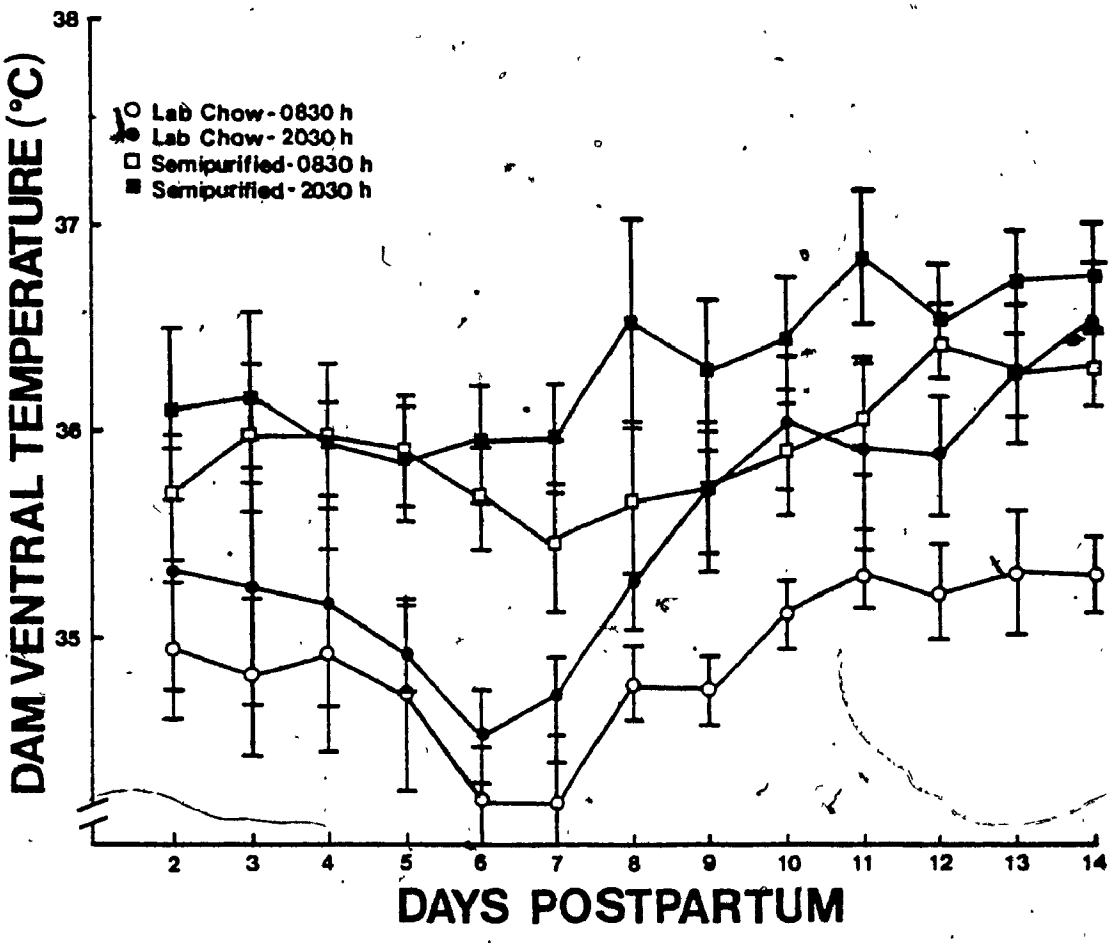

Figure 15. Mean daily LC and SP dam ventral temperature at 0830 and 2030 h during first two weeks postpartum. SEM's are shown.

in Figure 16. Pups nursed by dams maintained on the SP diet had elevated skin temperatures compared to those pups which were nursed by dams fed the stock chow ($F(1,14)=28.95$, $p<.001$). The significant Days effect ($F(3,42)=7.82$, $p<.001$) indicates that pup skin temperature increased over days but the main effect for Time of Day was not statistically significant ($F(1,14)=.67$, $p>.05$). There was a significant Time of Day X Days interaction ($F(3,42)=4.07$, $p<.05$).

Intake

Food Intake Mean daily gram food intake during both the light and dark phases in both diet conditions during the first 14 days postpartum is shown in Figure 17. Dams in the LC diet group consumed greater amounts of their diet than did SP dams ($F(1,14)=11.54$, $p<.01$). There were also significant effects of Time of Day and Days, resulting from increased food intake during the dark phase and over days ($F(1,14)=87.59$, $p<.001$ and $F(3,42)=89.79$, $p<.001$). The Diet X Time of Day interaction was statistically significant and this is due to the fact that SP dams generally consumed more food than LC dams during the light phase and less during the dark phase ($F(1,14)=10.02$, $p<.01$). The Diet X Days interaction was also statistically significant: LC dams showed a greater gram food increase over days than did SP dams ($F(3,42)=4.98$, $p<.01$). The Diet X Time of Day X Days interaction was not statistically significant ($F(3,42)=.46$, $p>.05$).

Proportional Food Intake. The proportion of grams of food consumed during each phase of the light cycle to total daily food consumed was determined. The result of the analysis (Diet X Time of Day X Days) revealed no difference between the diet groups in proportional food

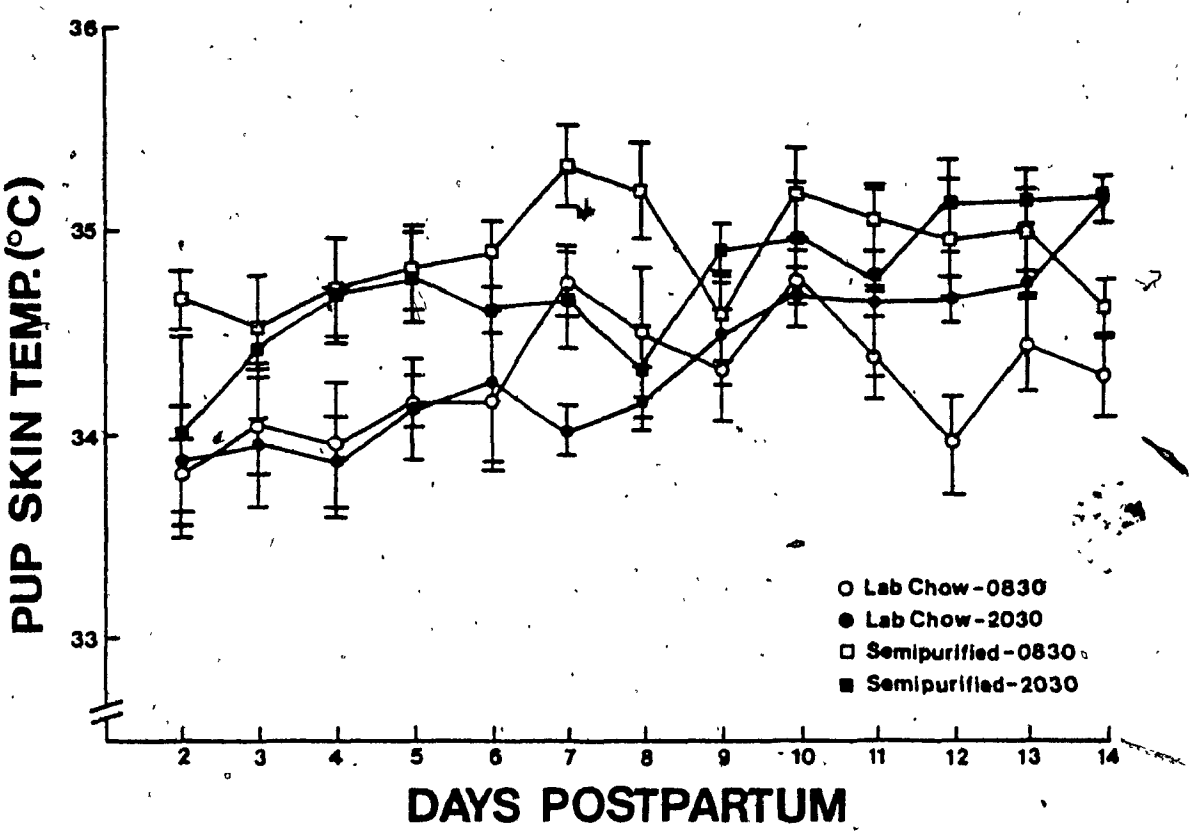

Figure 16. Mean daily LC and SP pup skin temperature at 0830 and 2030 h during first two weeks postpartum. SEM's are shown.

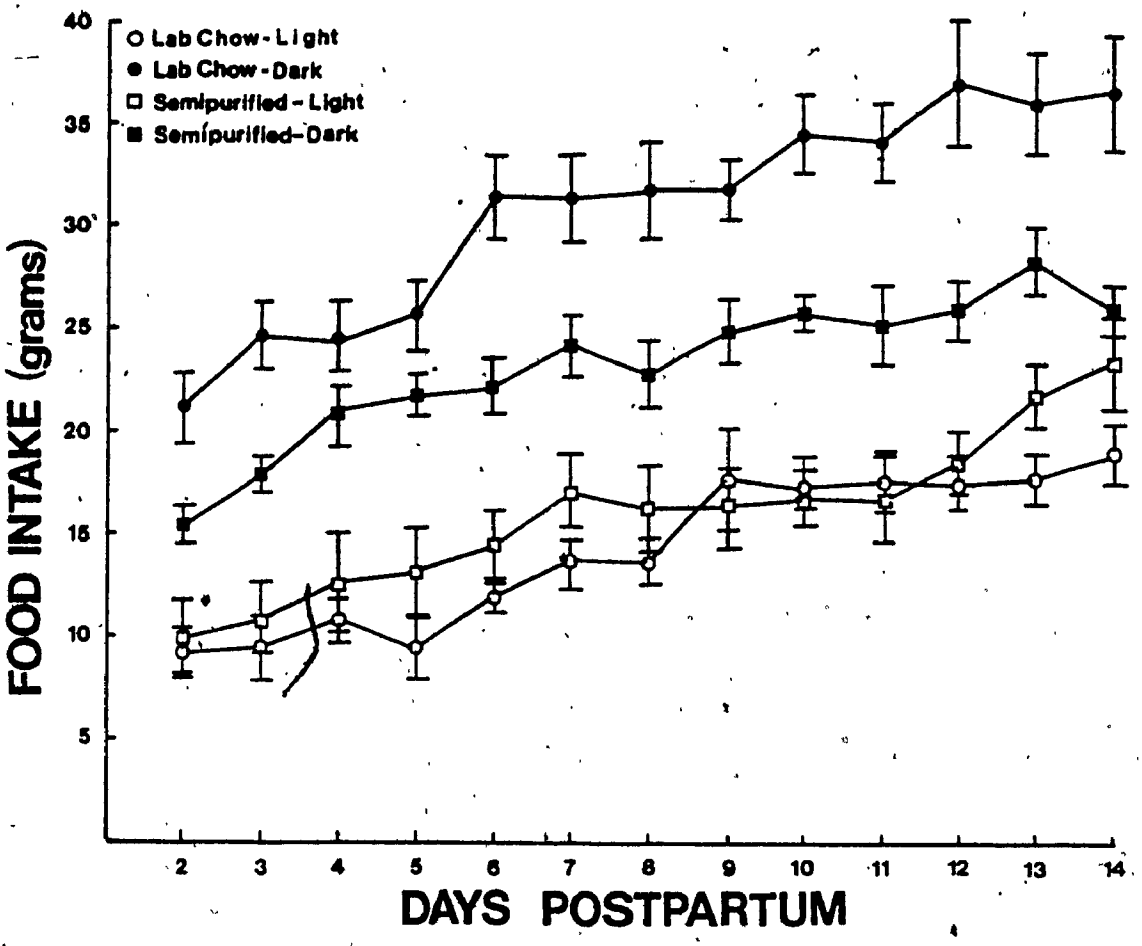

Figure 17. Mean daily LC and SP dam food intake during the light and dark phase during the first two weeks postpartum. SEM's are shown.

intake ($F(1,14)=1.00$, $p>.05$) but a greater proportion of food was eaten during the dark phase than during the light phase ($F(1,14)=73.84$, $p<.001$). The Diet X Time of Day interaction was statistically significant and this is due to the fact that compared to LC dams, SP dams consumed a greater proportion of food during the light phase and less during the dark phase ($F(1,14)=4.44$, $p=.05$). The Time X Days interaction was also statistically significant and this results from a greater increases in proportional food intake over days during the dark phase than during the light phase ($F(3,42)=3.12$, $p<.05$).

Caloric Intake Figure 18 represents mean caloric intake during the first two weeks postpartum for LC and SP females. The results from the analysis revealed a nonsignificant effect for Diet ($F(1,14)=.76$, $p>.05$) but caloric intake increased at night ($F(1,14)=78.44$, $p<.001$) and over days ($F(3,42)=111.84$, $p<.01$). SP dams consumed more calories than LC dams during the light phase and less during the dark phase ($F(1,14)=6.22$, $p<.05$). In addition, LC dams showed a greater increase in caloric intake over days than did SP dams ($F(3,42)=4.64$, $p<.01$). The Time of Day X Days and Diet X Time of Day X Day interactions were nonsignificant ($F(3,42)=1.30$, $p>.05$ and $F(3,42)=.55$, $p>.05$, respectively).

Water Intake The results from the analysis indicate a significant Diet effect ($F(1,14)=93.06$, $p<.001$) for water intake due to a greater volume of fluid ingested by LC dams compared to SP dams. Further, over days both groups demonstrated increased water intake resulting in a significant effect of Days ($F(3,42)=59.88$, $p<.001$). The Diet X Days interaction was also statistically significant ($F(3,42)=13.71$, $p<.001$),

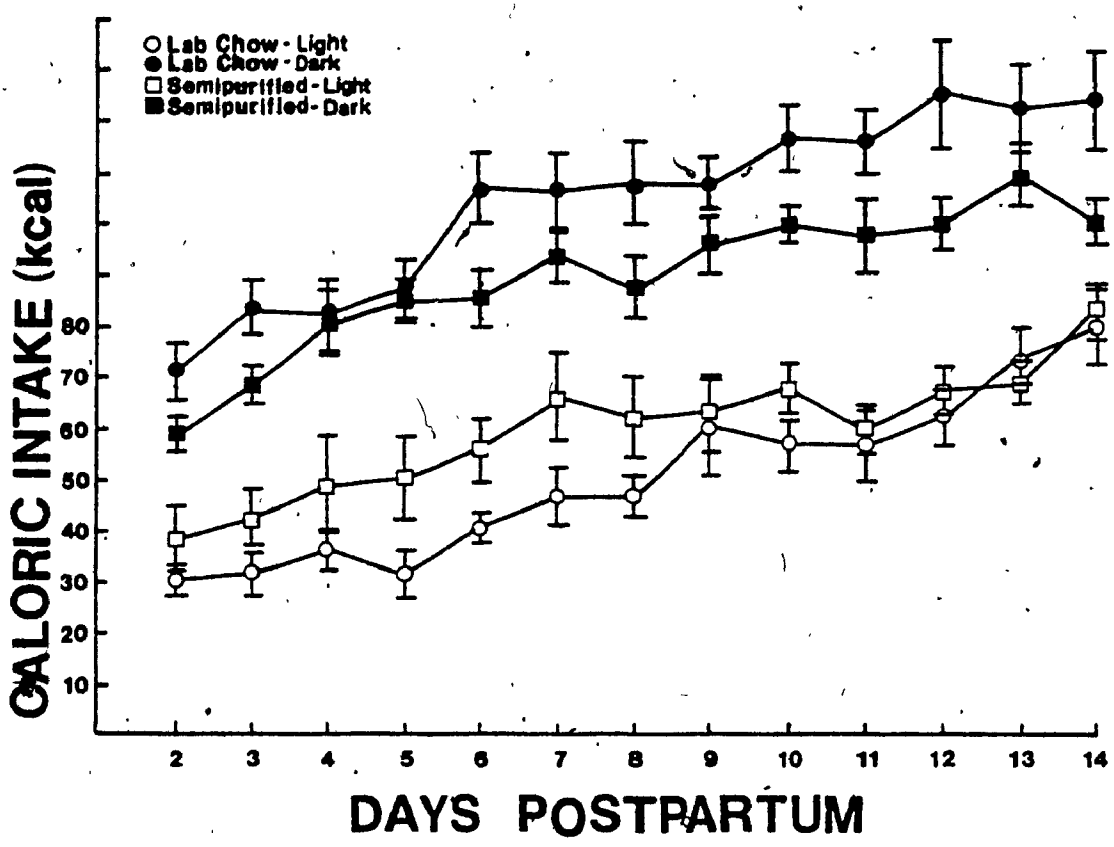

Figure 18. Mean daily LC and SP dam caloric intake during the light and dark phases during the first two weeks postpartum. SEM's are shown.

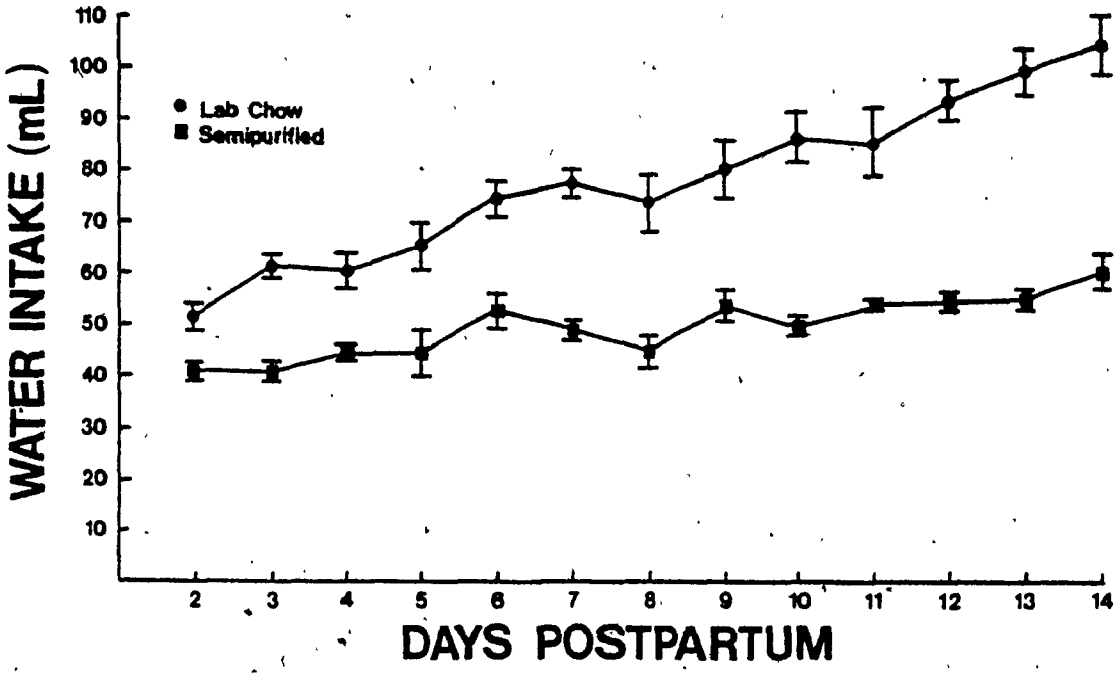

Figure 19. Mean daily LC and SP dam water intake during the first two weeks postpartum. SEM's are shown.

apparently due to LC dams demonstrating greater increases in water intake over days than did SP dams. Mean daily water intake is shown in Figure 19.

Development

(a)Behavioural

(i) Curling Response. Figure 20a shows mean number of days when at least 75% of each litter in both diet conditions demonstrated a three-point curl. A 2-tailed independent t-test revealed no difference between the two diet conditions.

(ii) Head Lifting. A two-tailed independent t-test revealed no difference between the diet conditions on the first day when 100% of all litters demonstrated the head lifting response (see Figure 20b).

(iii) Righting Response. Figure 20c shows mean latency to right from the supine postion for pups in both diet conditions between days 2 though 10 postpartum. The results of a two-way analysis of variance (Diet X Days) revealed no difference in the latency to right between the two diet conditions ($F(1,14)=.06$, $p>.05$) but over days the latency to right decreased ($F(8,112)=20.14$, $p<.001$). The Diet X Days interaction was not statistically significant ($F(8,112)=.27$, $p>.05$).

(iv) Eye Opening. The distribution of number pups having at least one eye open between Days 10 and 14, inclusive, is shown in Figure 20d for both diet conditions. These distributions were not statistically different (Kolmogornov-Smirnov test; $D_{MAX}=.068$; $p>.05$).

(v) Thermoregulation. Figure 21 shows mean colonic temperature at 10 minute intervals over a 40 minute period of cold exposure for male and female pups in both diet conditions. A three-way (Diet X Time X

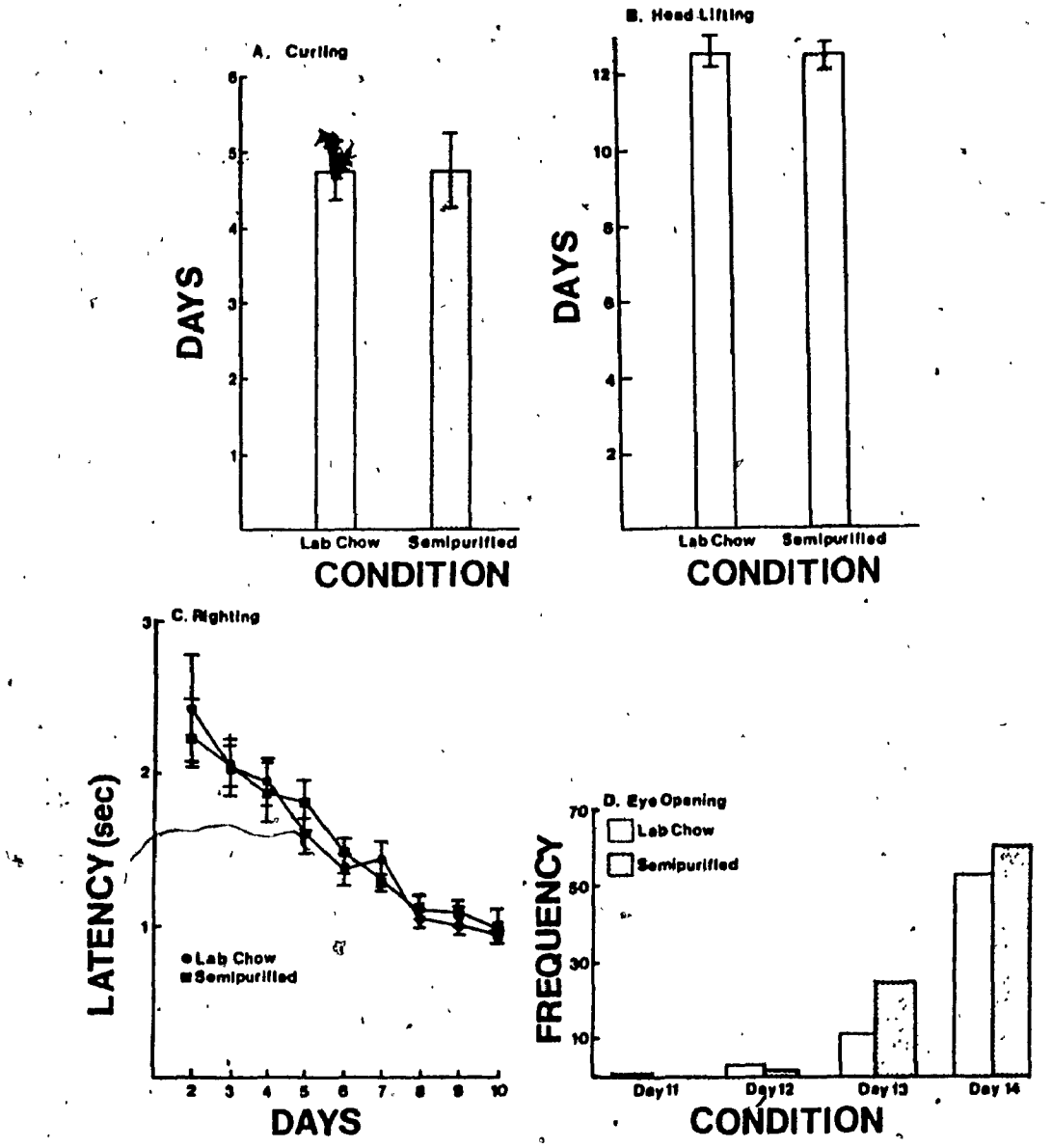

Figure 20. a) Mean number of days for LC and SP pups to demonstrate curling response. b) Mean number of days for LC and SP pups to demonstrate head lifting response. c) Mean latency for LC and SP pups to right. d) Frequency distribution for number of pups demonstrating eye opening. SEM's are shown.

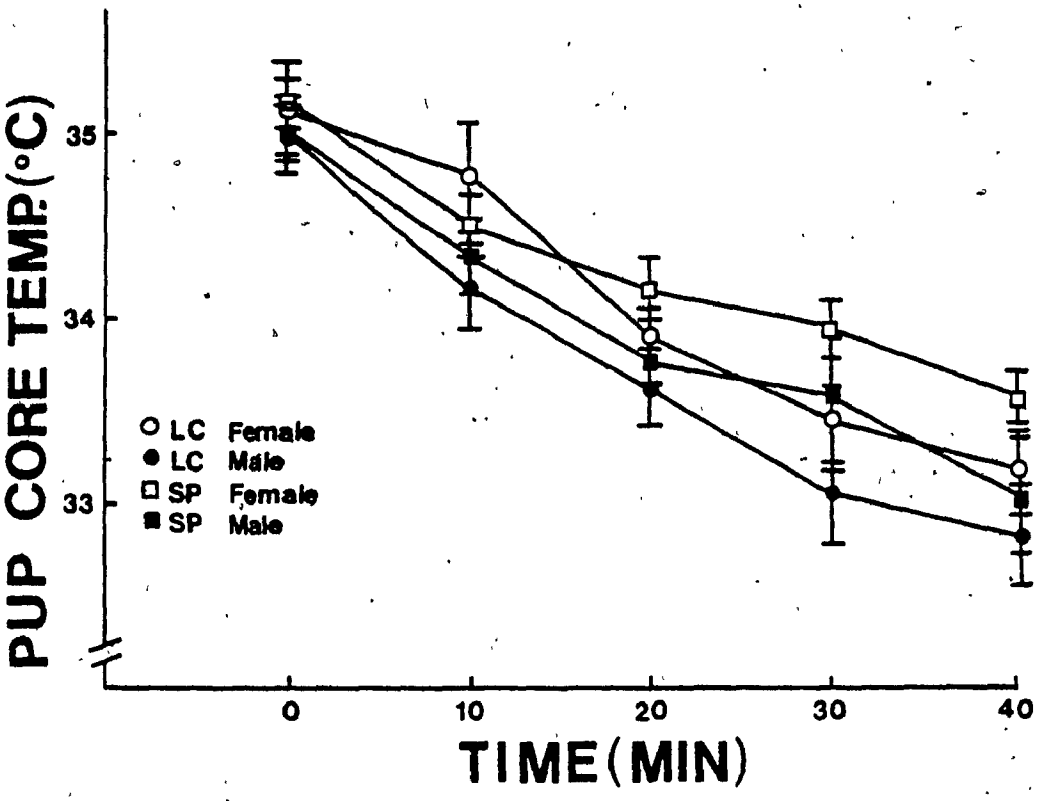

Figure 21. Mean body temperature of LC and SP pups over forty minutes during cold stress. SEM's are shown.

Sex) analysis of variance revealed a significant effect of Time ($F(4,112)=154.74$, $p<.001$) indicating that body temperature decreased during the forty minute cold exposure. The Diet X Time interaction was statistically significant ($F(4,112)=3.46$, $p=.01$): SP pups lost less body heat over time than did LC pups. All other effects on this parameter were not statistically significant.

(b) Pup Organ Weights, Body Weights and Core Temperatures

(i) Pup Brown Adipose Tissue. Figure 22a represents mean weight of BAT expressed as a percentage of body weight for both male and female pups in the LC and SP diet conditions on days 22/23 postpartum. There were no significant main effects or interactions in this analysis (Diet; $F(1,28)=.1.08$, $p>.05$; Sex; $F(1,28)=.43$, $p>.05$ and Diet X Sex ($F(1,28)=1.70$, $p>.05$)

(ii) Adrenals. The results from the analysis of variance performed on adrenal weight:body weight ratios indicated no significant effects of Diet, Sex or interaction of these factors ($F(1,28)=.44$, $p>.05$; $F(1,28)=.12$, $p>.05$ and $F(1,28)=1.70$, $p>.05$, respectively). Mean adrenal weight expressed as a percentage of body weight for LC and SP pups is shown in Figure 22b.

(iii) Gonads. Mean gonad weight, expressed as a percentage of body weight is presented in Figure 22c for both males and females in the LC and SP diet conditions. There was no difference in gonad weight between the two groups ($F(1,28)=.97$, $p>.05$) but male gonads were much heavier than female gonads ($F(1,28)=1221.08$, $p<.001$). The Diet X Sex interaction was not significant ($F(1,28)=.17$, $p>.05$).

(iv) Whole Brain Weight. There was no diet effect or sex

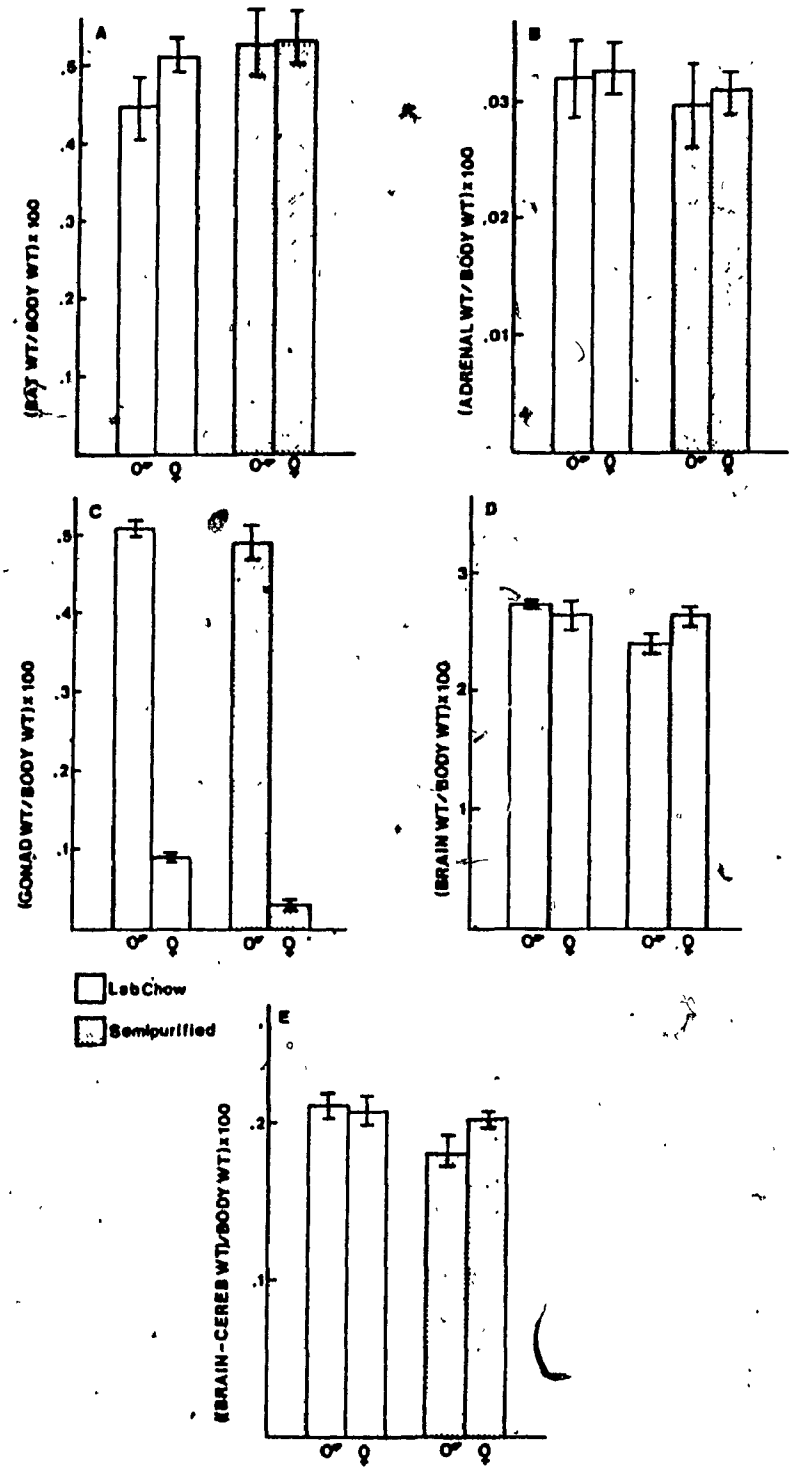

Figure 22. a) Mean LC and SP BAT weight:body weight ratios. b) Mean LC and SP Adrenal weight:body weight ratios. c) Mean LC and SP Gonad weight:body weight ratios. d) Mean LC and SP Whole brain weight:body weight ratios. e) Mean LC and SP brain less cerebellum weight:body weight ratios. SEM's are shown.

difference in pup whole brain weight as indicated by the nonsignificant main effects ($F(1,28)=3.28$, $p>.05$ and $F(1,28)=.51$, $p>.05$, respectively). The Diet X Sex interaction was nonsignificant ($F(1,28)=2.63$, $p>.05$). Mean whole brain weight expressed as a percentage of body weight for LC and SP males and females is presented in Figure 22d.

(v) Brain less Cerebellum Weight. Mean brain less cerebellum weight:body weight ratios are shown in Figure 22e. LC pups tend to have elevated brain weight:body weight ratios compared to SP pups ($F(1,28)=3.92$, $.05<p<.06$). All other effects were not significant.

(vi) Pup Body Weight. Mean body weight for pups on day 22 is presented in Figure 23. A two-tailed independent t-test revealed no difference between the diet groups ($t(14)=-1.0736$, $p>.05$).

(vii) Pup Core Temperature. Figure 24 shows mean pup core temperature measured on day 22 for the LC and SP diet conditions. A two-tailed independent t-test revealed no difference between the groups on this measure ($t(14)=.0104$, $p>.05$). Moreover, pup core temperture at this time was similar to temperatures obtained at the onset (T_o) of the thermoregulation test.

(c) Dam Body Weight, Organ Weight and Core Temperature

(i)Dam Body Weight. A two-tailed independent t-test performed on Day 1 mother body weight revealed no difference between the two diet groups. However, LC dams demonstrated a significantly greater percentage weight change than did SP dams ($t(14)=2.21$, $p<.05$). There was no difference in female body weight on day 22 postpartum. These data are shown in Figure 25a and b.

(ii) Dam Core Temperature. Mean colonic temperature for females

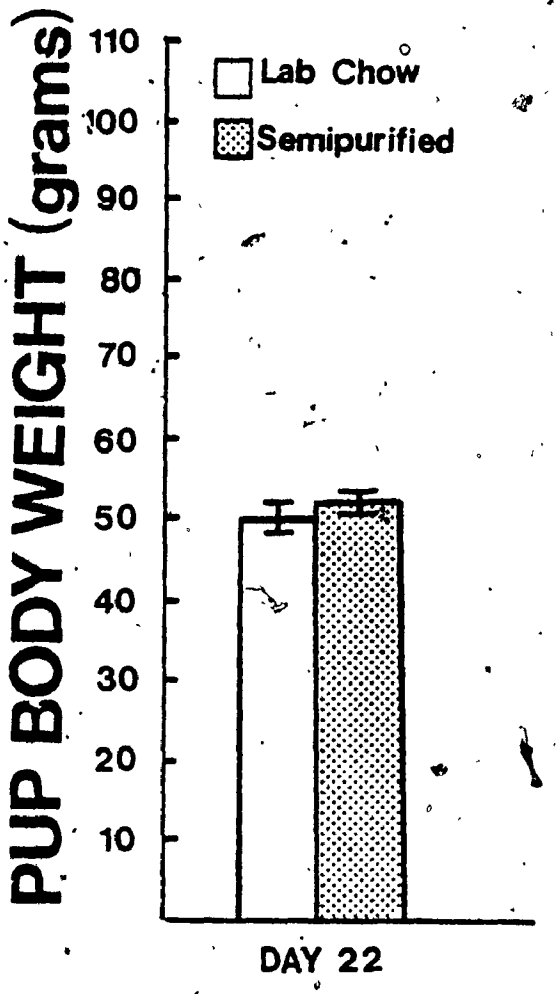

Figure 23. Mean LC and SP pup Day 22 body weight. SEM's are shown.

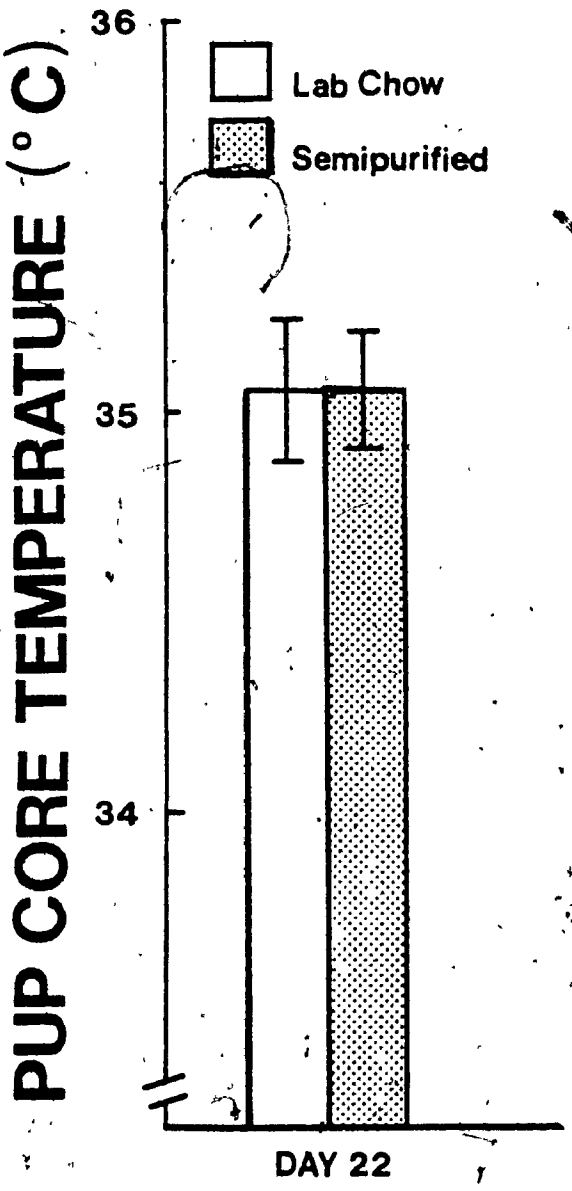

Figure 24. Mean LC and SP pup Day 22 core temperature. SEM's are shown.

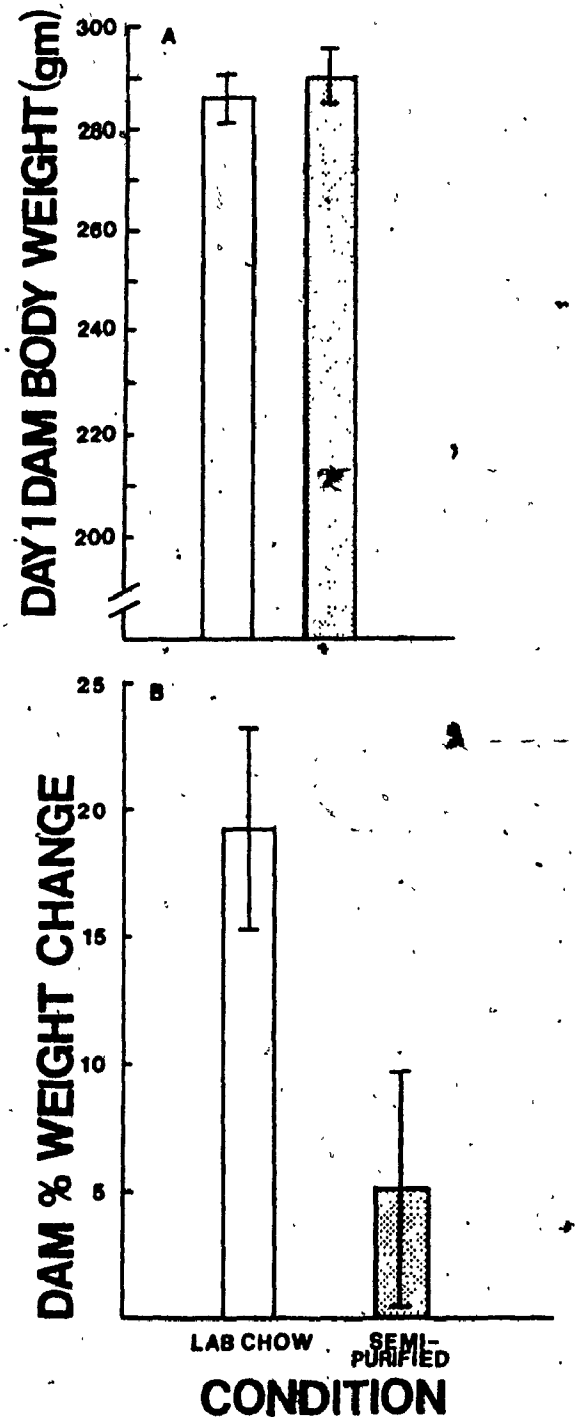

Figure 25. a) Mean LC and SP dam body weight on Day 1 b) Mean LC and SP dam percentage weight gain during first two weeks postpartum. SEM's are shown.

in the SP and LC diet groups on days 22 and 28 postpartum are presented in Figure 26. SP dams had higher core temperatures than LC dams ($F(1,14)=5.38$, $p<.05$). The Days effect was statistically significant ($F(1,14)=18.08$, $p<.01$): core temperaure was higher on Day 22 than on Day 28. The Diet X Days interaction was nonsignificant ($F(1,14)=.10$, $p>.05$).

(iii) Dam Brown Adipose Tissue. A two-tailed independent t-test revealed no difference between the two diet groups in brwon adipose tissue weight ($t(14)= -.9842$; $p>.05$). Mean BAT weights:body weight ratios for dams in both diet conditions are shown in Figure 27.

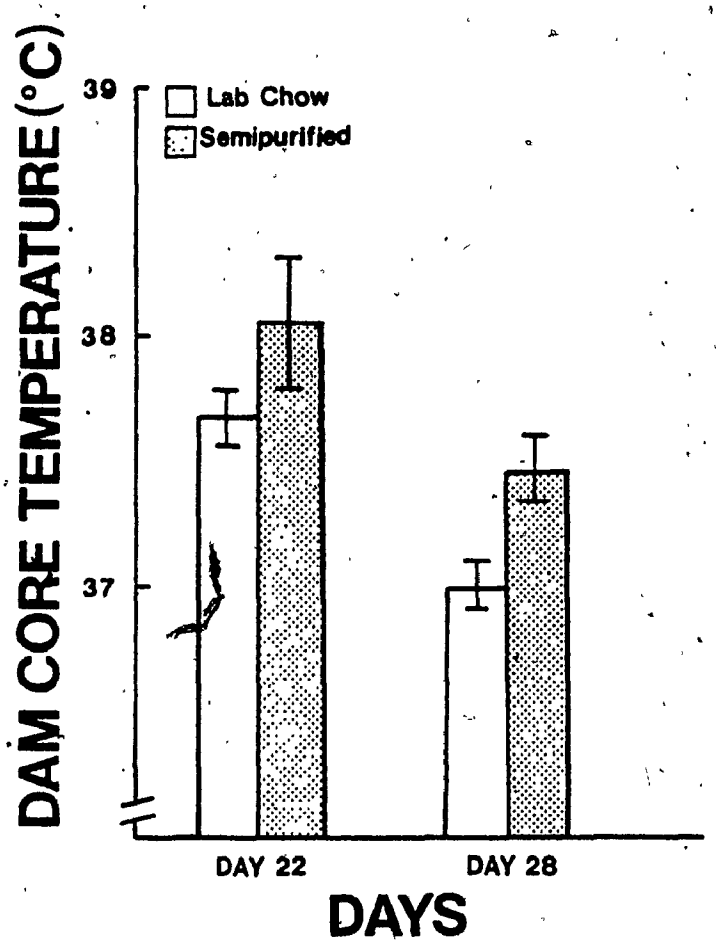

Figure 26. Mean LC and SP dam core temperature on Days 22 and 28 postpartum. SEM's are shown.

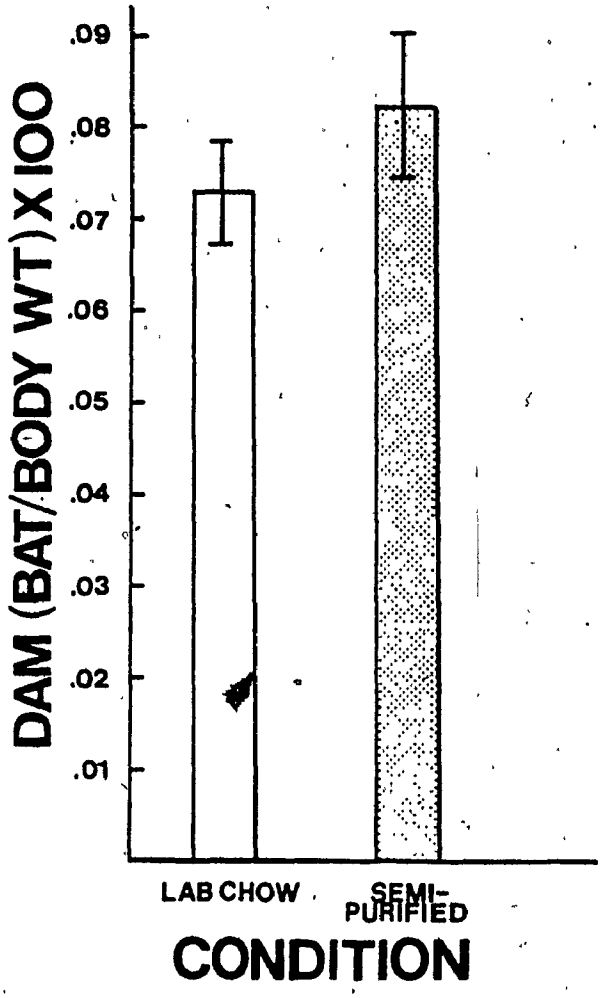

Figure 27. Mean LC and SP dam BAT weight:body weight ratios. SEM's are shown.

Discussion

As in Experiment 1, dams which consumed the semipurified diet mixture had higher core and ventral temperatures at both 0830 h and 2030 h than dams presented with the stock lab chow diet and temperatures in both diet conditions were higher at night than during the day. Moreover, both diet group females showed increases in both core and ventral temperatures over the course of lactation.

The most important finding in this experiment was that dams fed the semipurified diet spent significantly less time on the nest during the light portion of the light/dark cycle when most nursing occurs. Dams in this diet condition spent approximately 100 min. less with their young than did LC females. No difference between the diet groups was obtained in the dark portion when total nursing time is much reduced. This diet effect on light nest time implies that the increased temperature of SP dams is not the result of an elevation in the thermal set-point for maternal body temperature regulation (e.g. Woodside and Leon, 1980) for if the diet manipulation had resulted in an increase in set-point then the SP females would have been able to tolerate the elevated heat load which they experienced and thus prolong contact with the young. Rather, these results reflect an increase in the thermal load that made the dams more vulnerable to the acute temperature effects of huddling with their young. In defense of the unaltered temperature set-point for body temperature regulation, nest bout durations were shortened in order that thermal homeostasis might be maintained. These results further support the thermal model of influences on mother-young interactions. However, despite the chronic elevation in SP dam temperature, a similar reduction

in dark nest time was not obtained. That no differences due to diet during this phase were obtained may be accounted for by a floor effect on nest time since during the dark cycle, ambient temperature was quite high, usually reaching 25-26 ^{o} C and dams exposed to a warm ambience typically show a marked reduction in nest time (Jans and Leon, 1983; Leon et al, 1978).

The obtained reduction in nest time during the light phase may not be attributable only to a diet effect on maternal temperature per se since the skin temperature of the SP pups was significantly higher than that of the LC pups during the first 14 days postpartum. It has been shown that dams presented with warm pups show a reduction in nest time relative to females presented with cool pups (Leon et al., 1978; Jans and Leon, 1983). An interaction of both elevated maternal temperature and elevated pup skin temperature most probably contributed to the reduction in light nest time seen for dams in the SP diet condition.

It is not clear why SP pups were warmer than LC pups. One possibility may be that the warmer SP dams may produce and deliver warmer milk to their young than do LC females thus causing SP pup skin temperatures to become elevated. Alternatively, pups from the SP diet condition may be warmer simply because they are in contact with warmer dams when more body heat may be transferred from mother to young. The increased skin temperature of the SP pups seems not be a direct effect of the diet on the pups because by Day 22, when the young pup was actually consuming the diet, a temperature difference between SP and LC pups no longer existed. It is unclear why eating the semipurified diet would result in body temperature increases in the dams but not in the pups at this time.

There was no significant diet effect on nest frequency, implying that the difference in light nest time obtained between the LC and SP diet groups arose from differences in the duration of nest bouts rather than the number of bouts. Moreover, the nonsignificant change in mean nest frequency over time suggests that the progressive reduction in nest time over days resulted from gradual decreases in nest bout durations. The data obtained for mean nest bout duration support this argument; mean nest bout duration was shorter for SP dams than for LC dams during the light portion of the light/dark cycle and shorter at night than during the day. Moreover, nest bout durations were shorter on Day 10 than on Day 4. In accordance with similar nest time during the dark cycle between the diet conditions, mean nest bout durations, as well, were similar.

It was hypothesized that because SP dams would spend less time with their young during the light cycle there would be less time available for the pups to reveive nourishment from their mothers and that this might affect pup growth. However, the measures used in this study provide no indication of differential growth. Pups from each diet condition grew equally well despite the difference in nest time during the light phase. Even by Day 22 postpartum pup body weights between the two diet conditions were similar. There is evidence to suggest that when pups are reared in a warm nest environment the amount of energy needed to maintain body temperature may be reduced thereby allowing more energy to be allocated to growth (Hall, 1973; Jans, de Villers and Woodside, in press). Since SP pups were maintained by warmer dams, it is conceivable that they were reared in conditions analagous to the warm nest where the

amount of energy used for growth is maximized. Another possibilty is that SP pups may not have experienced a decrease in the amount of nourishment received despite reduced contact with the dam during the light period. It has been shown that dams exposed to warm pups or to a warm ambience show a shorter latency to the first milk ejection as well as shorter inter-milk ejection intervals than do control dams (Jans and Woodside, manuscript in preparation). Because SP dams and pups were warmer than their LC counterparts, a similar situation may have resulted where SP pups may have received similar amounts of milk than LC pups, but in shorter periods of time.

The analysis of pup organ weights on day 22 postpartum revealed no significant differences between the two diet conditions. Moreover, no consistently different patterns of developmental trends were found between pups raised by LC-fed females and pups raised by dams maintained on the lab chow. Of the five behavioural measures used to assess pup development the only difference that was found was in thermoregulatory abilities where upon exposure to cold stress SP pups retained more body heat over the forty minute exposure than did LC pups. This finding, at first, appears to be contradictory to the findings of Krecek, Krechkova, and Martinek (1957) where rat pups raised in a warm environment showed a delay in the ability to thermoregulate. However, by Day 22, when thermoregulatory abilities were assessed, pups were already eating the semipurified diet on their own and this may have better enabled them to maintain body temperature when exposed to cold (Heroux, 1969; Heroux et al, 1971). Alternatively, Himms-Hagen (1984) suggests that the activation of brown adipose tissue, which is involved in the maintenance of homeothermy, is dependent upon the degree of cold exposure. SP pups

may have indeed been exposed to greater cold stresses since they would not have benefitted from the thermal-insulating properties that the dam would have provided during her relatively prolonged absence from the nest area. Although increased weight of brown adipose tissue has been associated with enhanced activity, no diet effect on this measure was obtained. However, the quantity of brown adipose tissue may not be indicative of its heat producing activity or potential (Senault, Cherqui, Cadot and Portet, 1981).

As in Experiment 1, even though there were no differences in the caloric consumption of females in the two diet groups, hyperthermia was still obtained in the SP-fed females. Furthermore, over days, females in both diet conditions demonstrated a progressive increase in both gram food and caloric intake, again reflecting the dams' attempt to meet the increasing energy demands of lactation. In conjunction with a greater gram food intake, dams consuming the lab chow also consumed greater volumes of water than did females consuming the semipurified diet. Unlike in Experiment 1, however, dams consuming the lab chow ate more of their diet than did dams eating the semipurified diet. One possible factor contributing to these discrepent results may be that the pups from Experiment 2 were hardier than those from Experiment 1 and as such would have provided the dam with greater amounts of suckling stimulation which influences food intake.

One interesting finding with respect to food intake was that SP females consumed more diet than LC females during the light cycle but less during the dark cycle. That different patterns of daily food intake were obtained imply that the diet manipulation may have produced

a shift in the circadian rhythm of body temperature in SP dams that gives the appearance of an overall increase on this parameter. However, because SP dams consumed a greater proportion of their food than LC dams during the light phase when nest time was reduced suggests that such a shift did not occur. Rather, this result indicates that dams eating the semipurified diet had the opportunity to engage in other behaviours (e.g., eating, drinking, elimination) instead of tending to the young.

Similar to the results obtained in Experiment 1, dams that consumed the semipurified diet gained less weight than lab chow-fed females during the first two weeks postpartum. This difference in weight gain might reflect processes similar to diet-induced thermogenesis where the amount of weight gained is reduced since some of the excess energy consumed in eliminated in the form of heat production through increased brown adipose tissue activity (Rothwell and Stock, 1979). Dams fed the semipurified diet may have obtained, and subsequently, eliminated more energy from their diet since a purified diet is more readily assimilated than a stock lab chow (Shaw and Greep, 1949).

In summary, the results from this experiment have yielded three interesting findings. First, consumption of the semipurified diet led to a significant elevation in body temperature. It would be interesting to determine the time taken to produce this response. In this experiment, dams were already consuming this diet two to three days prior to the first day of temperature measurement and studies suggest that diet-related hyperthermia can occur slowly or rapidly after the consumption of a diet (Glick et al, 1983; Armitage et al, 1983).

Second, the increase in SP dam temperature contributed to a reduction in nest times relative to females consuming the stock lab chow

diet, but only during the light portion of the light/dark cycle. This reduction in nest time suggests that the temperature increase in SP dams was not the result of an increase in thermal set-point. If this had occurred, SP females would have been able to tolerate the increased heat load experienced, thus prolonging contacts with the litter. Rather, the increased core temperature appears to have contributed to the heat load that dams experiences while set-point remains unchanged thereby rendering her even more vulnerable than LC dams to the acute temperature aspects of huddling with the young. Thus, one way by which SP dams were able to maintain body temperature homeostasis was to remove themselves from the litter sooner than LC females.

Third, despite reduced light nest time, SP pups grew as well as LC pups on all measures. This suggests that even though these young might receive less nourishment, the energy that may have otherwise been needed for heat production may have been used for growth since they were maintained by warmer dams. However, it was found that SP pups were better able to thermoregulate than LC pups, a result which appears to contradict previous findings concerning rat pup ability to thermoregulate after being reared in a warm environment. It is not clear whether this effect is the result of reduced mother-young contact or whether it can be attributed to semipurified diet consumption by the pup.

One strategy that has been used to examine the influence of temperature on patterns of mother-young interactions has been to manipulate ambient temperature or surface temperature, thereby changing the entire thermal environment in which such interactions occur (e.g.

Jans and Leon, 1983; Leon et al, 1978). In the present experiment alterations to the external thermal environment have been minimized. As a consequence of the diet manipulation, only dam temperature increased, apparently without altering her thermal set-point, and indirectly skin temperature of the pups. Although the present experiment adopts only one of several possible approaches in examining the effects of patterns of maternal contact on pup development, if future studies can maximize nest time differences by directly changing the temperature of only the dam, then perhaps a clearer indication of the influence of nutritional and maternal factors on pup growth may be obtained.

Experiment 3

The temperature elevations obtained in Experiments 1 and 2 in lactating females consuming the semipurified diet appear to parallel those found in other studies of diet-induced thermogenesis. In the rat, for example, consumption of a cafeteria diet (Bukowiecki et al, 1982; Rothwell and Stock, 1979), a low protein diet (Swick and Gribskov, 1983), or a semipurified diet (Heroux, 1969; Heroux et al, 1971) has resulted in either a hyperthermic response or a greater ability to maintain body temperature when faced with cold challenges. Moreover, these thermal responses have also been accompanied by an increase in the metabolic activity, weight, cell size and cell number of brown adipose tissue, the reputed effector tissue of diet-induced thermogenesis.

Interestingly, studies examining diet-induced thermogenesis have shown that the hyperthermia can occur at different time intervals after the initial diet consumption. For example, the increased oxygen consumption of brown adipose tissue which is seen during diet-induced thermogenesis can occur in the rat shortly after the presentation of a low protein/high carbohydrate meal (Glick et al, 1981). On the other hand, increased energy expenditure has occurred up to four days after the presentation of a cafeteria selection of palatable food items during which time animals became hyperphagic (Armitage et al, 1981).

In the first two experiments reported here, dams fed the semipurified diet had higher body temperatures than those fed the lab chow diet and this effect was obtained in the absence of increased food intake. Body temperature increases in the semipurified-fed females were apparent on the first day of measurement (Day 1 postpartum) but these

animals had been consuming their diet two to three days prior to parturition. Consequently, it was not possible to determine the time of onset of body temperature increases nor of any transient hyperphagia. In Experiment 3, therefore, the time course of the thermal response and patterns of caloric intake were monitored in non-lactating females when the diet was switched from the stock lab chow to the semipurified diet and when original dietary status was reinstated.

Method

Subjects Eleven virgin female Wistar rats, ranging in weight from 223.1 to 241.1 grams at the onset of the experiment, were obtained from Charles River Breeding Farm, St. Constant, Quebec. Animals were housed on a 12 h light/12 hour dark cycle with lights on at 0800 h and lights off at 2000 h. Mean ambient temperature during the experiment was 19.94 $\pm$ 0.19 ^{o} C.

Apparatus and Procedure Subjects were individually housed in polycarbonate cages as described earlier. The diets presented were those used in Experiments 1 and 2. All subjects were allowed ad libitum access to the stock lab chow for a ten day period in order to establish baseline conditions. For the next ten day period subjects were presented with the semipurified diet after which the lab chow diet was reintroduced. Female body weight and water intake were recorded once daily while core temperature and food intake were recorded twice daily as previously described. To facilitate comparisons among the different diet phases data were condensed by finding the mean value over each successive five day interval for each subject on each measure. This yielded ten 5-day blocks; two five day blocks each for the initial lab chow and semipurified diet phases and six 5-day blocks for the lab chow reintroduction period.

Results

Core Temperature Figures 28a and 28b show mean morning and evening core temperature respectively for each of 10 five-day blocks across the various diet phases. The results from a two-way analysis of variance for repeated measures (Blocks X Time) showed a significant effect for Blocks and Time of day as well as a significant interaction of these factors ($F(9,90)=12.73$, $p<.001$; $F(1,10)=121.22$, $p<.001$ and $F(9,90)=1.95$, $p=.05$, respectively). Tukey post-hoc analyses revealed that morning core temperature was significantly greater during the phase of the experiment in which the females were fed on the semipurified diet than in the first lab chow phase and remained statistically significantly higher until 11-15 days after the reintroduction of lab chow.

Data for the evening temperatures are similar but there were no statistically significant differences among the blocks.

To further explore the rapidity of core temperature increase, the last five days of the lab chow baseline period and the first five days of the semipurified diet phase were examined for morning temperatures only. The results of the analysis indicated a significant effects of Days ($F(9,90)=10.94$, $p<.001$) and Tukey post-hoc analyses indicated a significant increase in temperature on the first day of semipurified diet consumption. Mean morning core temperature during the last five days of the lab chow baseline phase and the first five days of semipurified diet consumption is shown in Figure 29.

Caloric Intake Figure 30 shows mean caloric intake over the ten 5-day blocks of the experiment. A one-way analysis of variance for repeated measures revealed a significant effect of Blocks ($F(9,90)=11.44$,

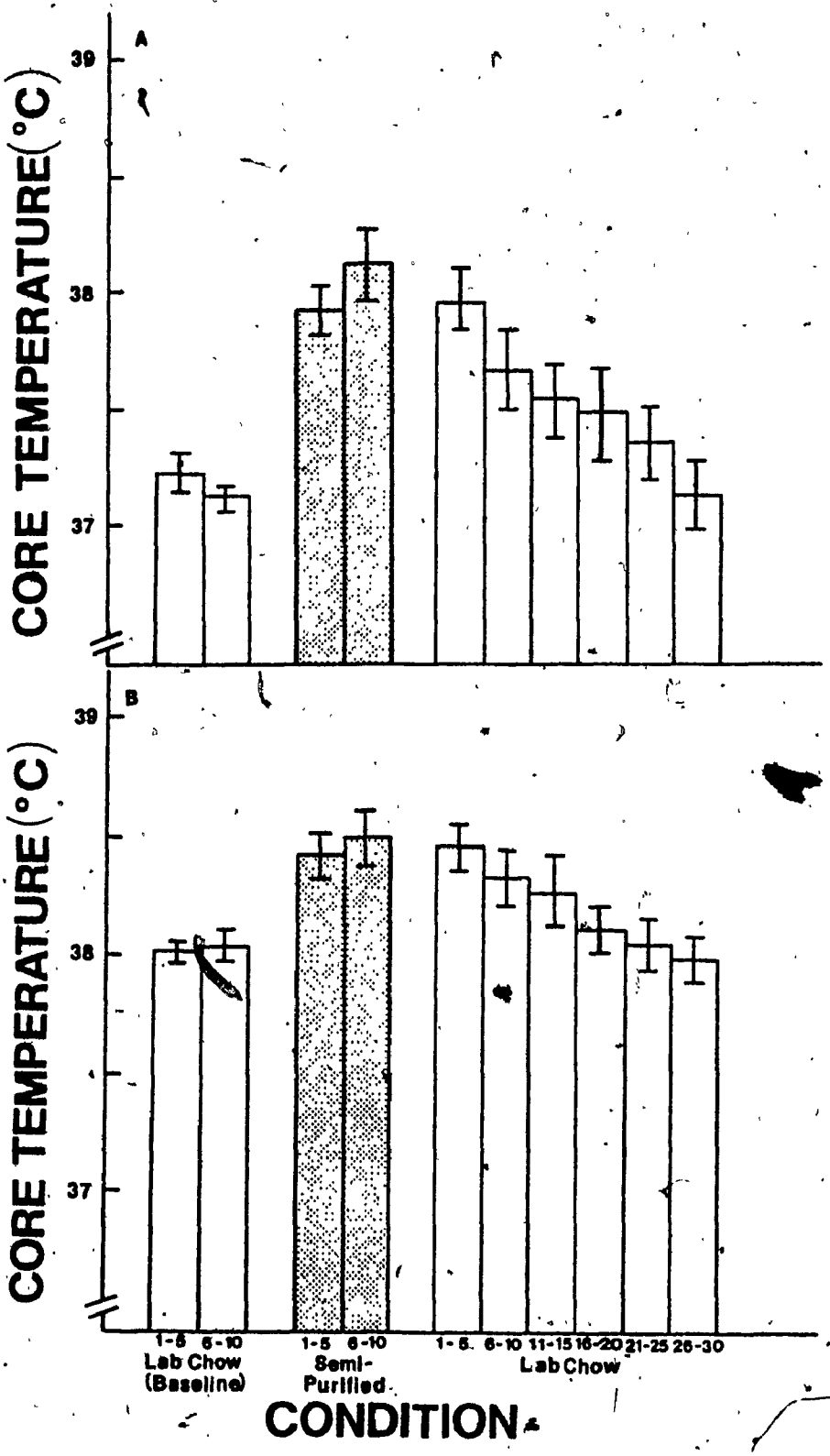

Figure 28. a) Female mean core temperature at 0830 h across diet phases. b) Female mean core temperature at 2030 h across diet phases. SEM's are shown.

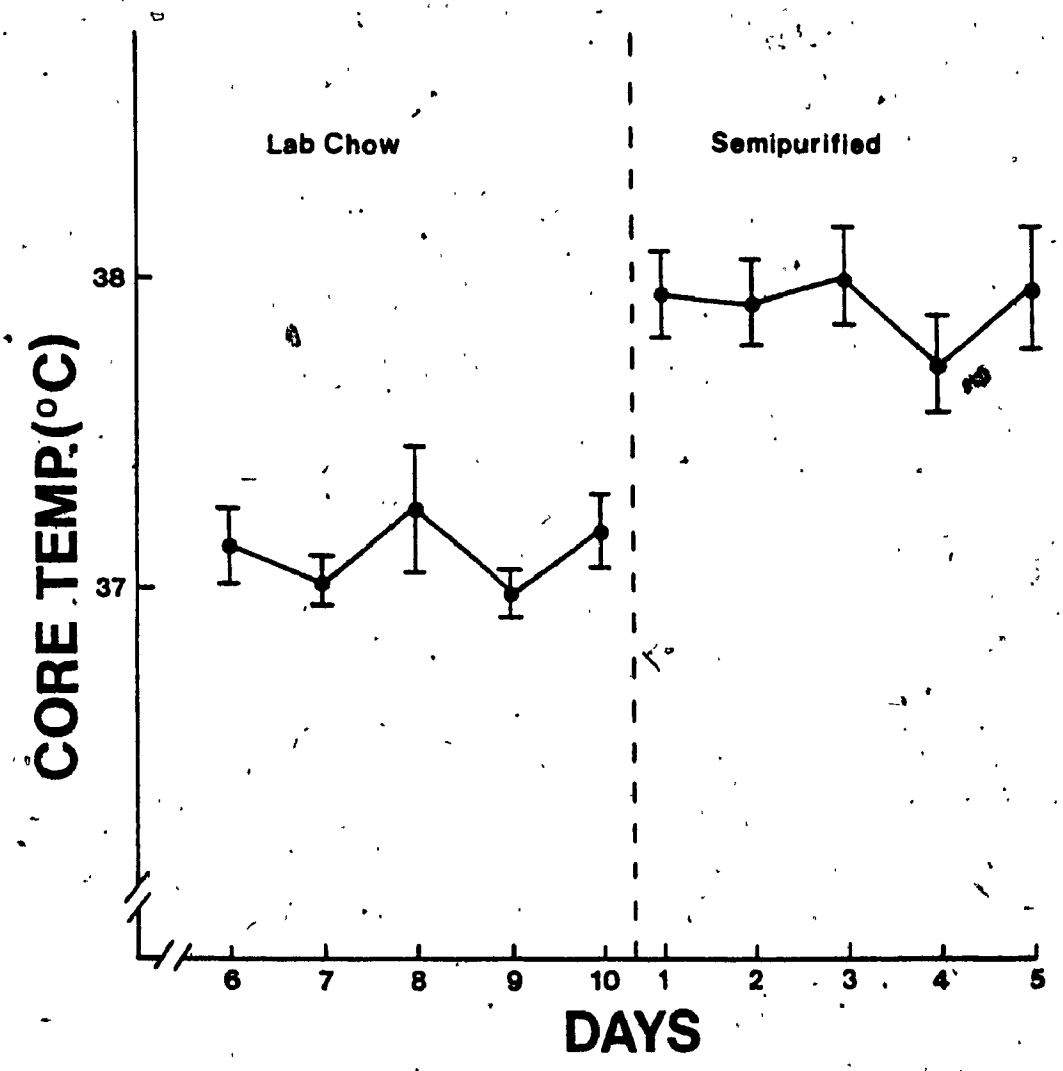

Figure 29. Mean morning core temperature during the last five days of Lab Chow (Baseline) phase and first five days of semipurified diet phase. SEM's are shown.

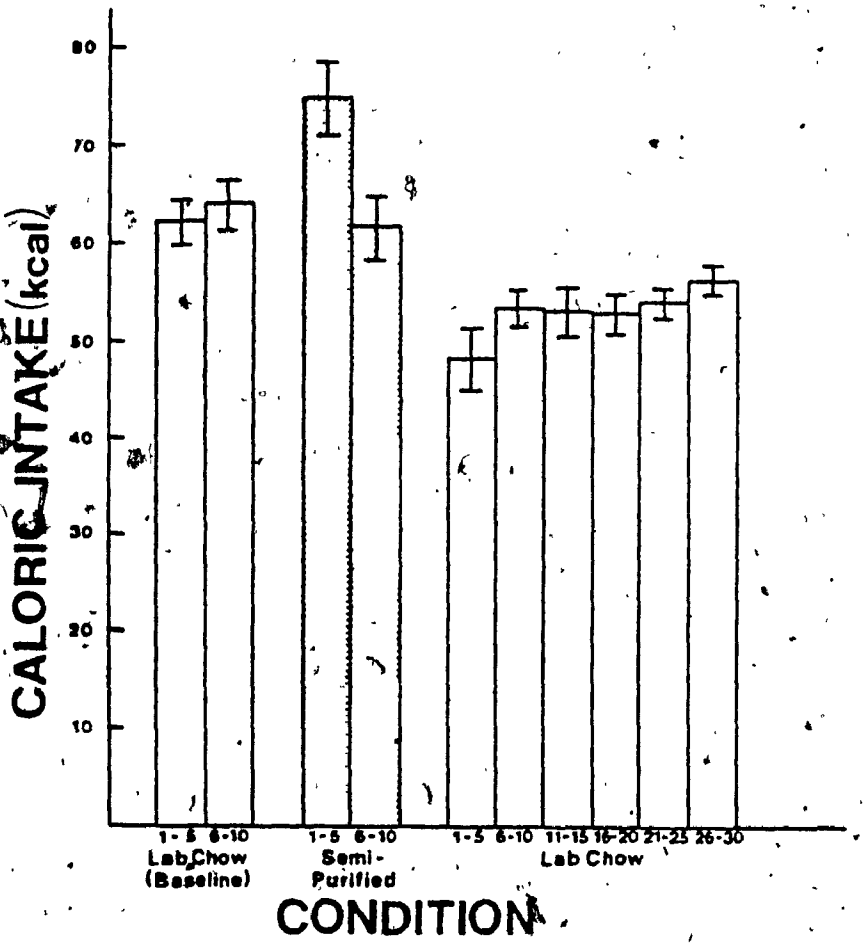

Figure 30. Mean female caloric intake across diet phases. SEM's are shown.

$p<.001$). Tukey post-hoc analyses indicated a significant increase in caloric intake between days 6-10 of baseline lab-chow presentation and days 1-5 of the semipurified diet phase and a significant decrease between days 6-10 of the semipurified diet phase and days 1-5 of lab chow reintoduction. No significant difference was found between days 6-10 of the lab chow (baseline) phase and days 6-10 of lab chow reintroduction. Mean daily caloric intake during the last five days of the lab chow baseline phase and the first five day of the semipurified phase is shown in Figure 31.

To further explore the time of onset and duration of the hyperphagia, caloric intake was examined for the last five days of the lab chow baseline period and the first five days of semipurified diet consumption. The results of the analysis indicated a significant effects of days ($F(9,90)=8.72$, $p<.001$). Tukey post-hoc analyses indicated that there was a significant increase in caloric intake on the first day of the semipurified diet phase over the entire lab chow (baseline) phase after which time caloric intake returned to baseline levels.

Water Intake Water intake for each of ten 5-day blocks is presented in Figure 32. A one-way analysis of variance for repeated measures revealed an effect for Blocks which was just significant ($F(9,90)=1.98$, $p=.05$). Tukey post-hoc analyses failed to reveal where such differences in water consumption occurred.

Percent Weight Change Figure 33 shows percent weight change over each 5-day block during each diet phase of the experiment. A one-way analysis of variance for repeated measures showed a significant effect

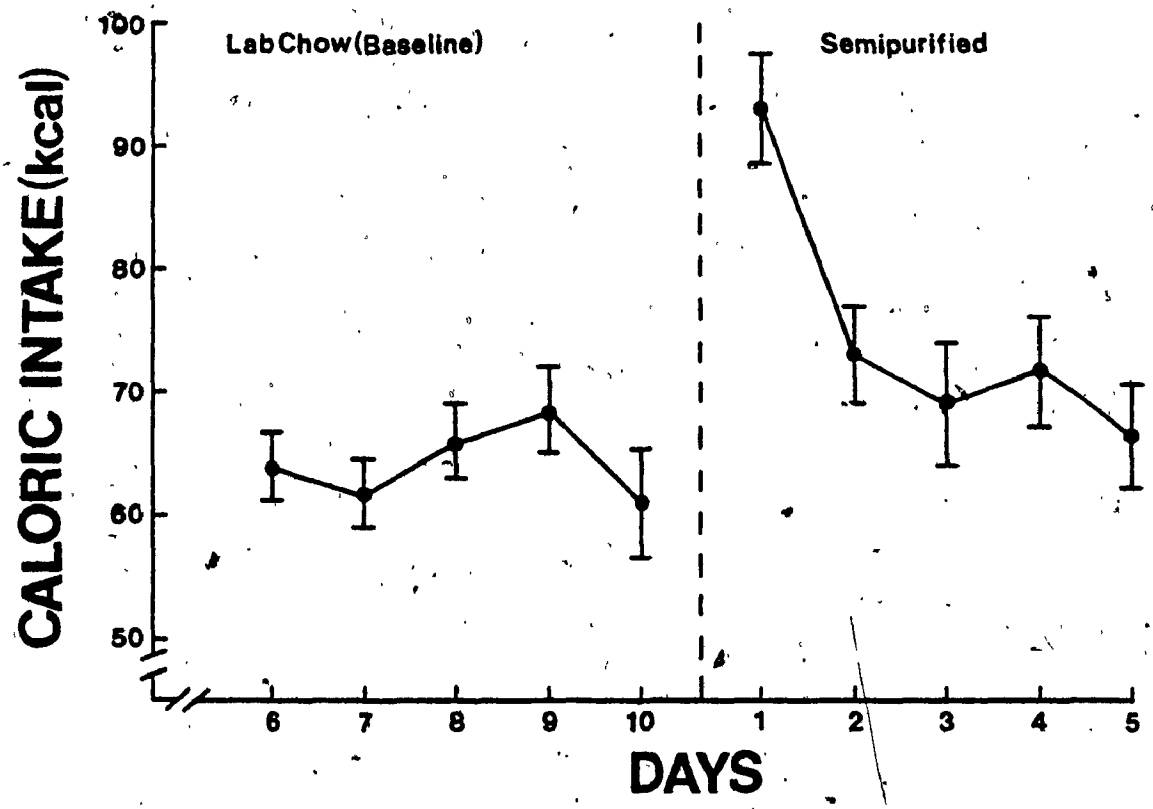

Figure 31. Mean daily caloric intake during the last five days of lab chow baseline and first five days of semipurified diet phases. SEM's are shown.

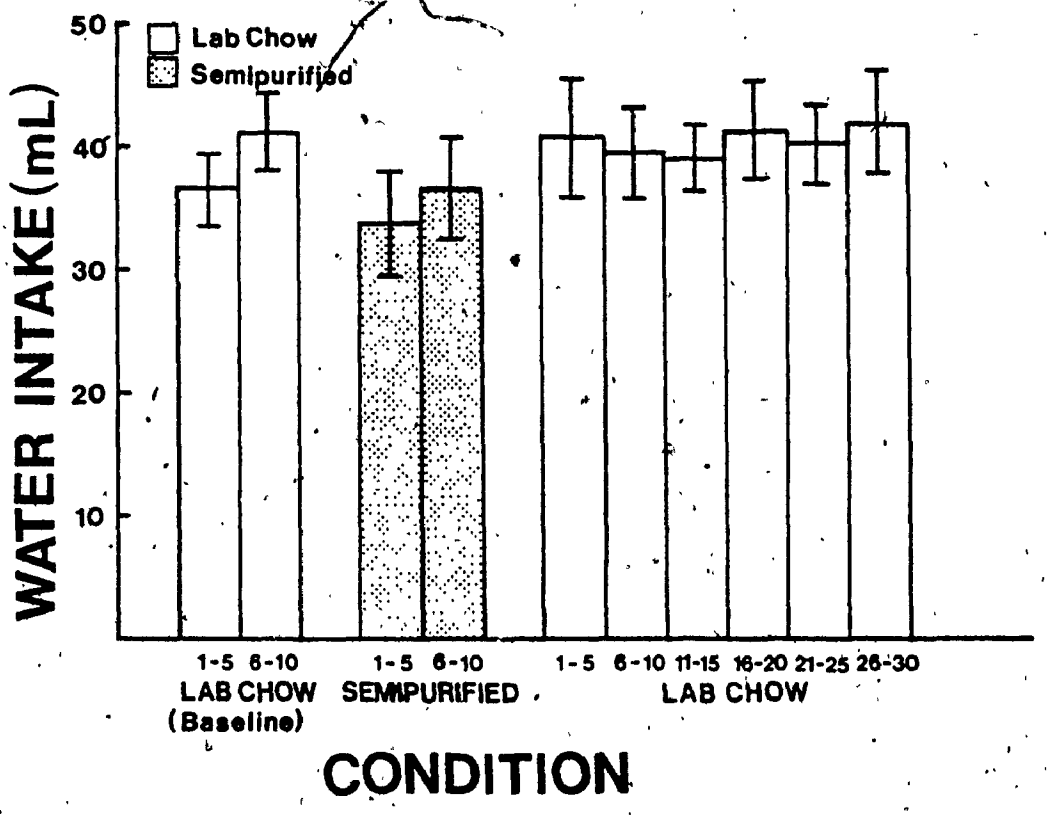

Figure 32. Mean female water intake across diet phases. SEM's are shown.

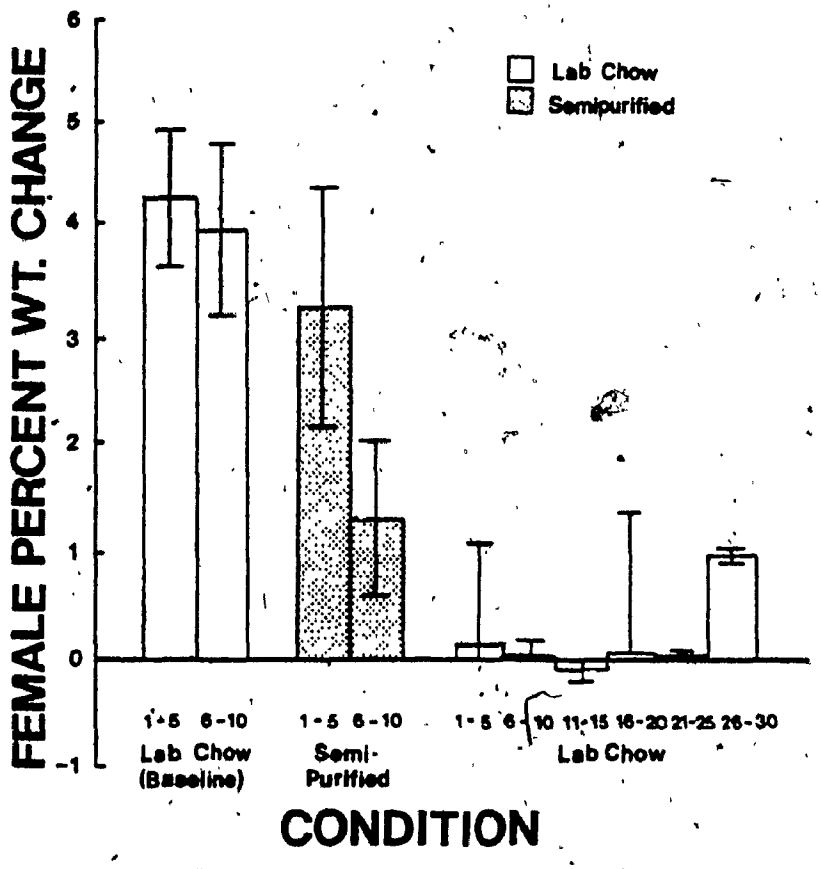

Figure 33. Mean female percentage body weight gain during each 5-day block across diet phases. SEM's are shown.

of Blocks ($F(9,90)=5.56$, $p<.001$). Tukey post-hoc analyses revealed a significant reduction in percent weight gain between days 6-10 of baseline lab chow presentation and days 1-5 of lab chow reintroduction but by days 26-30 there was no statistically significant difference between these two diet phases. No statistically significant differences were revealed between the semipurified diet phase and either of the two lab chow phases.

Discussion

These results show that the semipurified diet produces hyperthermia rapidly in the laboratory rat and that the magnitude of the effect varies as a function of the circadian temperature cycle with the greater effect at lights on (0830 h) than at lights off (2030 h). These data also suggest that the thermal influences of the semipurified diet are relatively long-lasting since body temperature did not immediately return to to LC-baseine levels when the animals were reintroduced to the stock lab chow. Instead, only during days 11-15 of the second lab chow phase did body temperature return to levels demonstrated during the initial presentation of this diet.

On the the first day of the semipurified diet phase hyperthermia was accompanied by caloric hyperphagia, a result that is reminiscent of the dietary over-consumption that accompanies diet-induced thermogenesis (e.g. Rothwell and Stock, 1979). However, hyperphagia was not a long-lasting phenomenon: on the second day of the presentation of this diet caloric intake decreased and returned to levels demonstrated during the initial lab chow phase, although the hyperthermia continued. When the original diet was reintroduced there was a further reduction in caloric intake, which returned to baseline levels after 5-10 days of presentation.

There were no statistically significant differences in the percentage weight gained between days 6-10 of the lab chow (baseline) presentation and the entire semipurified diet phase, indicating that the hyperthermia demonstrated during semipurified diet consumption was not accompanied by any change in rate of body weight gain. Rather, statistically significant decreases in percentage weight change were

found between days 6-10 of the baseline lab chow phase and throughout the period of lab chow reintroduction and this persisted until the last five day period (days 20-25) of the second lab chow phase when weight change was similar to that of the baseline period. Under conditions of diet-induced thermogenesis the amount of weight gained usually associated with this phenomenon is far less than what would be expected despite a great increase in energy intake (e.g. Rothwell and Stock, 1979). That similar results were not obtained during the semipurified phase may be due to the fact that the females were still growing and as such, the percentage weight change among the different diet phases might simply reflect a decreased rate of body weight change as the animal matures.

At least one study has presented some data that are somewhat similar to those of this experiment with respect to time course of the thermic effect and patterns of caloric consumption. When Armitage et al (1983) presented rats with a cafeteria selection of palatable food items after having maintained the animals on only a stock chow both energy intake and energy expenditure increased but only after the fourth day of presentation of the supplemented diet. In the present experiment however, such increases occurred much more rapidly than those described by Armitage et al since body temperature and caloric intake were elevated on the first day of the semupurified diet phase compared to original lab chow levels. Similar to the results obtained in this experiment, Glick et al (1981) have found a more immediate diet effect on energy expenditure. These investigators have shown that immediately following the presentation of a low protein-high carbohydrate meal there

was a two-fold increase in brown adipose tissue oxygen consumption *in vitro* and that this increase was also accompanied by an increased weight of interscapular brown adipose tissue pads, the reputed effector tissue of diet-induced thermogenesis.

During the second 5-day block (days 6-10) of the semipurified diet presentation, caloric intake decreased relative to levels demonstrated during the first block (days 1-5) of this phase and at this time caloric consumption was statistically indistinguishable from the LC baseline levels. Rolls, Rowe and Turner (1980) and Hervey and Tobin (1983) also indicated that during periods of dietary supplementation (i.e. cafeteria feeding) the excess energy intake initially demonstrated diminished over time.

Switching animals from the semipurified diet to the stock lab chow resulted in a temporary depression in caloric intake. Further, when the females were reintroduced to the lab chow caloric consumption returned to baseline levels after 6-10 days of presentation of the stock chow. Both Rothwell and Stock (1979) and Armitage et al (1983) have found similar patterns of responses when a cafeteria diet was removed and animals were presented only with a stock chow. In addition, others (Armitage et al, 1983; Rolls et al, 1980; Rothwell and Stock, 1979) have reported similar responses in caloric intake for cafeteria-to-stock chow fed animals. However, the return to baseline levels in the present experiment was more rapid than the two weeks reported by Armitage et al (1983). It is interesting that despite the use of different diet preparations among the various studies cited, results similar to those presented in this experiment were still obtained.

In summary, the data presented in this experiment are consistent

with the results presented thus far in that consuming the semipurified diet is capable of producing a hyperthermic response in the laboratory rat. Moreover, patterns of caloric consumption and the hyperthermic response vary as a function of dietary regime. The thermal response which is accompanied by a temporary caloric hyperphagia, is evident on the first day of presentation of the semipurified diet and is also relatively long-lasting. In this experiment hyperphagia was defined only in terms of caloric consumption. Since it has been previously shown that animals demonstrating diet-induced thermogenesis tend to ingest and eliminate greater amounts of food-derived energy resulting from hyperphagia, it would be of interest, therefore, to determine whether there are any differences in utilization between the two diets presently used which may be contributing to the thermal response. It may be possible that the semipurified diet is utilized more efficiently than the lab chow resulting in a greater net caloric intake even though similar gross quantities of these diets had been consumed.

Summary and General Discussion

These studies have demonstrated that when hyperthermia is produced in lactating rats by feeding them a semipurified diet, they spend less time on the nest during the light phase of the cycle than do dams that consumed a stock lab chow diet. The failure to find a similar reduction in nest time in the dark phase is most probably due to the fact that all animals spent little time in the nest, probably a result of high ambient temperatures in the laboratory at this time of day. Decreased contact time, however, did not appear to influence the development of the rat pup; on most measures of behavioural and morphological development there were no differences between the two diet conditions, perhaps because the change in contact time was very small due to the fact that there were no nest time differences at night. The one exception was that SP pups were better able to thermoregulate than LC pups.

The alteration in mother-young contact suggests that the increase in SP dam temperature did not result from an elevation in set-point for body temperature regulation (see Jans and Leon, 1983; Leon, Croskerry and Smith, 1978; Woodside and Leon, 1980). Rather the temperature elevation apparently made these females further vulnerable to the acute temperature effects of huddling with their young. The difference in nest time between diet conditions and the reduction over days both appear to result from decreased bout duration rather than a decrease in the number of such bouts. SP mean nest bout duration was shorter than for the LC group and in both diet groups became shorter over days. The reduction in light nest time probably resulted from both an effect of the diet manipulation on the body temperature of the dam and on the skin

temperature of the pups both of which might have caused SP dam temperature to rise more radiply (Woodside et al, 1980). Further, dams nursing warm pups show a marked reduction in nest time compared to dams nursing cool pups (Jans and Leon, 1983; Leon et al, 1978).

Under the present laboratory conditions, it was shown that body temperature increases were produced on the first day of the presentation of the semipurified diet and that this effect was relatively long-lasting; when the semipurified diet was replaced with the stock chow, female core temperature remained elevated until the third 5-day block of the lab chow reintroduction phase. Furthermore, presenting the animals with the semipurified diet after the original lab chow period immediately produced hyperphagia but this effect was short-lived since on the second day of the semipurified diet phase caloric intake returned to baseline levels.

Since nourishment is only available to the pups when they are with the dam it appears somewhat surprising that the reduction in contact time experienced by the SP litters did not affect their growth. However, factors such as energy conservation, rate of milk delivery and composition of the dam's milk might account for any lack of developmental differences between the diet groups. For example, Jans et al (in press) have suggested that although pups reared in a warm environment may experience reduced contact with the mother and hence receive less nourishment, energy that is received may be channelled to growth since less would need be expended to maintain body temperature. Alternatively, pups in both diet conditions may, in fact, have received similar amounts of nutrients from the dam. Investigations in this laboratory (Jans and Woodside, manuscript in preparation) have indicated

that when the dam is in a warm environment or is presented with warm pups, the latency to the first milk ejection and subsequent inter-milk ejection intervals are signifcantly shorter compared to control dams. Since both SP dams and pups are warmer than their LC counterparts, it may be that the same situation results and thus SP pups may be receiving more milk in shorter periods of time. If greater nest time differences had been obtained, more pronounced differences in offspring development would have resulted. A third possibility is that the SP diet resulted in a change in the milk composition of the dams in that group.

An interesting finding that was obtained was that SP pups were better able to thermoregulate than were LC pups when exposed to cold stress. This finding is in contrast to previous findings (e.g., Krecek et al, 1957) where pups reared in a warm environment demonstrate delayed abilities to thermoregulate. However, pups in the semipurified diet condition had less contact with the dam and may have indeed been exposed to greater cold stresses than LC pups. Alternatively, pups were already eating on their own when thermoregulatory abilities were assessed and as Heroux and others have shown rats consuming a semipurified diet are better able to withstand cold stress than those eating lab chow (Heroux, 1969; Heroux, Johnson and Flattery, 1971).

In light of the nest time data obtained and the indices used to assess pup growth, perhaps differences in offspring development should not have been expected. The difference in mother-young contact time between the two diet conditions was relatively small and this can be attributed to the fact that at night, both LC and SP dams spent similar amounts of time on the nest as a result of high ambient temperatures in

the lab at this time. Compared to other studies which have continuously monitored mother-young contact (Leon et al, 1978; Leon et al, 1982; Woodside and Leon, 1980), the amount of time that LC females spent with their young appears to be much less than that reported for control females. If the LC dams in this study had behaved similarly where dark and hence total nest time would have been greater thus increasing the difference between the two groups, further differences between LC and SP pups might have been obtained. Furthermore, the developmental measures used here were quite gross and have been used primarily in studies of severe undernutrition. Perhaps if other, more subtle indices were investigated differences between the pups in the two diet conditions would have been obtained. One possible route of investigation would be to examine the development of the opiate system. Panksepp, Herman, Conner, Bishop and Scott (1978) have indicated that morphine administration can alleviate separation distress in a variety of young animals and that social contact activates the development of the opiate system. Development of this system appears to be plastic since young animals group housed immediately post-weaning are more sensitive to the same dose of heroin than are rats housed in isolation but no differences were obtained for mature rats similarly housed (Schenk, Ellison, Hunt and Amit, 1985). Moreover, the decrease in sensitivity has been attributed to a reduction in the number of opiate-receptor binding sites (Schenk, Britt, Charelson, Attalay, 1982). In light of this evidence, one might expect SP pups to have less developed endogenous opiate system because they clearly have less contact with their mothers and thus a different social environment from the LC pups.

Another measure for consideration would be to examine the sexual

development of SP and LC pups. It has been shown that animals which are experimentally "handled" prior to weaning have lower serum levels of ACTH and glucocorticoids than do "non-handled" animals (Levine, 1968; Thoman and Levine, 1969) and elevated levels of these secretions might inhibit the reproductive system (Bediz and Whitsett, 1979). Factors that might be contributed to the "handling" effect are altered patterns of tactile and thermal stimulation that the young animal receives (Russell, 1971) and this situation may be similar to being reared in the SP condition since alerations in mother-young contact may also produce differences in these stimuli. It would be interesting, then, to determine if the altered patterns of contact obtained between the two present diet conditions might also influence, for example, day of vaginal opening and testicular descension in the pups. The more traditional measures of the handling phenomenon such as emotional reactivity and corticosterone levels might lend themselves well to the present situation since "handled" animals, which are less emotional than "non-handled" animals, are similar to the SP pups, since they too experience different early environments.

The data presented here do not of course completely elucidate the role that the pattern of mother-litter contact plays in influencing rat pup development. They do, however, suggest one route through which to evaluate this factor since differences in contact time, which resulted in some long-lasting change in the SP pups, were accomplished by manipulating only dam temperature while nutritional differences were minimized.

It has been shown that food restriction and protein restriction

also change maternal body tempertaure (Leon et al, 1983; Swick and Cribskov, 1983) but they do so at a severe cost to the dam's nutritional status. Further, changing the ambient temperature of the dam and litter also changes the dam's energy balance and thereby her ability to pass energy to her young. Unlike these manipulations, the diet manipulation used here does not involve undernourishment of the dam; SP dams may be overfed relative to LC dams because the semipurified diet may be more readily assimilated thus yielding greater nutrient availability per gram of diet consumed than the lab chow diet. The best strategy might be to look at a variety of manipulations that change the pattern of mother-litter contact and evaluate the effects of this factor by comparing the obtained effects on pup development across experiments.

The mechanism by which the semipurified diet produced the increase in body temperature is unclear but two possible factors contributing to this effect are are diet-induced thermogenesis or shifts in the circadian rhythm of body temperature resulting from the diet manipulation. SP females generally gained less weight and were hotter than their LC counterparts. Thus, they show a diet-induced thermogenesis , a phenomenon that has been described by Rothwell and Stock, 1979; 1983 in rats that become hyperphagic on a cafeteria diet. According to Rothwell and Stock, such animals do not gain as much weight as would be expected because of an increase in brown adipose tissue activity where some but not all of the excess calories consumed are eliminated in the form of heat production. Heightened brown adipose tissue activity has been associated with increased size of this tissue (e.g., Rothwell and Stock, 1979) but in Experiment 2 hypertrophy of brown adipose tissue in the SP group was not found. However, this may

mean that the size of the tissue may not be an indication of its heat producing capacity or potential. On the other hand, it may be that brown adipose tissue is not involved in producing the temperature increases. Unlike the phenomenon described by Rothwell and Stock, however, the SP animals in these studies do not demonstrate a long-lasting hyperphagia, and, as is shown in Experiment 3, the hyperthermia effect lasts considerably longer than the transient hyperphagia that is observed. An alternative is that although the SP animals are not eating more than the lab chow-fed rats they are in fact receiving more nutrients because the semipurified diet is more readily assimilated. Such an explanation implies that these animals are not controlling their nutrient intake in the same way that the rats given the lab chow diet are, for the animals in the latter group are not eating at capacity. The question then becomes one of identifying the controlled variables with respect to food intake in both conditions.

A second possibility is that introducing a novel diet changes the rat's pattern of food intake in such a way that one gets a shift in the circadian rhythm of body temperature that gives the appearance of a global increase in core temperature. The finding that female food intake in Experiment 2 was greater in the SP group than in the LC group during the light phase and less in the dark phase might argue in favour of such a shift. However, in light of other data obtained, this might not necessarily be the case. Preliminary data obtained for non-lactating females suggests that proportional intake during each light phase does not vary across dietary regime. With this in mind, perhaps the increased food intake demonstrated during the light cycle by the

lactating females in Experiment 2 simply reflects the dam's ability to engage in behaviours others than tending to her young since she does spend less time with the pups during this portion of the light/dark cycle. Further, when the lab chow diet was reintroduced after the semipurified diet phase in Experiment 3, the females continued to remain hyperthermic and remained so for a relatively long period of time and this, as well, might argue against a shift in temperature cyclicity. Finally, if solely a shift in circadian temperature cycle was contributing to the elevation in SP female temperature, one might expect to have a point of intersection where temperature was similar between the diet conditions. However, SP dam core temperatures appear to be consistently higher those of the LC females. What might be a possible explanation is that temperature cycles are not being phase shifted, rather the points of origin are altered where, for example, the patterns of temperature cycles for the SP females are similar to LC females but they only start at an initially higher level. The only way to definitively obtain a solution to this problem would be to continuously monitor dam core temperature for both diet groups over 24-hours.

Presenting lactating and non-lactating female rats with a semipurified diet can elevate body temperature compared to females which are consuming a stock lab chow although the mechanism producing the temperature elevation is not yet clear. The increased SP dam temperature did, however, produce a reduction in nest time during the light portion of the light/dark cycle. Despite this reduction, pup growth was not affected although SP pups were better able to thermoregulate than LC pups. These results suggest that rat young are, in part, buffered from the consequences of decreased contact with the

dam and hence a reduction in the time available for nourishment. Moreover, these data also indicate that by changing mother-young contact time some long-lasting changes are produced in the offspring.

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Appendix A: Analysis of Variance Summary Tables (Experiment 1) and Detailed Breakdown of Dietary Constituents

Breakdown of Dietary Constituents

Protein	Lab Chow (g/100g)	Semipurified (g/100g)
Alanine		3.1
Arganine	1.42	4.2
Aspartic Acid		6.5
Cystine	0.35	0.4
Glutamic Acid		23.6
Glycine	1.12	2.1
Hystadine	0.58	3.0
Isoleucine	1.22	6.6
Leucine	1.85	10.1
Lysine	1.36	8.2
Methionine	0.43	3.3
Phenylalanine	1.07	5.8
Proline		12.3
Serine		6.3
Threonine	0.89	4.5
Tryptophan	0.27	1.5
Tyrosine		6.3
Valine	1.17	7.4

Minerals	(%)	(g/kg mixture)
Calcium	1.01	
Calcium Phosphate		500.00
Chlorine	0.43	
Chromium Potassium Sulfate		0.55
Cupric Carbonate		0.30
Ferric Citrate		6.00
Magnesium	0.21	24.00
Manganous Carbonate		3.50
Phosphorous	0.74	
Potassium	1.08	
Potassium Citrate Monohydrate		220.00
Potassium Iodate		0.01
Potassium Sulfate		52.00
Sodium	0.36	
Sodium Chloride		74.00
Sodium Selenite		0.01
Sucrose (finely powdered)		118.00
Zinc Carbonate		1.60

	(ppm)
Cobalt	0.37
Copper	15.1
Fluorine	65.0
Iodine	1.17
Iron	197.0

Manganese	54.4	
Zinc	30.3	
Vitamins (1.0%)	Lab Chow (ppm)	Semipurified (/kg vitamin mixture)
Biotin	0.30	
Carotene	5.6	
Cholecalciferol		2.50 mg
Choline	19.0 (x100)	
Cyanocobalamin		1.00 mg
d-Biotin		20.00 mg
d-Calcium Pentophentate		1.6 g
d-l Tocopherylacetate		20.00 g
Folic Acid	1.7	200.00 mg
Menaquinone		5.00 mg
Niacine	60.0	
Nicotinic Acid		3.00 g
Pantothenic Acid	12.5	
Pyrodoxine Hydrochloride	4.5	700.00 mg
Retinyl Pelmatate		1.60 g
Riboflavin	4.5	600.00 mg
Sucrose (finely powdered)		972.90 g
Thyamine	10.9	
Thyamine Hydrochloride		600.00 mg

In addition to the following:

B-12	9.0 mcg/lb
Vitamin A	12.0 IU/g
Vitamin D	3.3 IU/g
Alpha-tocopherol	17.0 IU/lb

Ash	6.0	3.5 - 4.0 %
Moisture	10.0	5.0 - 8.0 %

Dam Core Temperature

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	9.77	1	9.77	29.81 ***
Error	4.58	14	.32	
Within Subjects/Blocks				
Time of Day	5.00	1	5.00	23.20 ***
Diet x Time of Day	.01	1	.01	.02
Error	3.02	14	.22	
Days	.14	3	.04	.73
Diet x Days	.32	3	.10	1.55
Error	2.84	42	.06	
Time of Day x Days	.06	3	.02	.44
Diet x Time of Day x Days	.02	3	.01	.15
Error	2.16	42	.05	
Total	27.96	127		

(Residual) 8.02 98

*** p < .001

Dam Food Intake

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	170.10	1	170.10	2.22
Error	1070.63	14	76.48	
Within Subjects/Blocks				
Days	1749.40	3	583.13	37.98 ***
Diet x Days	4.96	3	1.66	.10
Error	644.72	42	15.35	
Total	3639.96	63		

***p < .001

Dam Caloric Intake

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	432.02	1	432.02	.42
Error	14601.06	14	1042.93	
Within Subjects/Blocks				
Days	23119.24	3	7706.41	39.92 ***
Diet x Days	61.73	3	20.58	.11
Error	8107.92	42	193.04	
Total	46321.97	63		

*** $p < .001$

Dam Water Intake

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	4355.83	1	4355.83	38.02 ***
Error	1596.02	14	114.00	
Within Subject/Blocks				
Days	1771.94	3	590.64	13.56 ***
Diet x Days	129.91	3	43.30	.99
Error	1829.46	42	43.56	
Total	9683.18	63		

*** $p < .001$

Individual Pup Growth

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	.01	1	.01	.01
Error	12.44	14	.88	
Within Subjects/Blocks				
Days	5.96	3	1.98	12.45 ***
Diet x Days	.12	3	.04	.26
Error	6.70	42	.16	
Total	25.23	63		

*** $p < .001$

Appendix B: Analysis of Variance Summary Tables (Experiment 2)

Total Nest Time

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	65664.08	1	65664.08	3.60
Error	255261.16	14	18232.94	
Within Subjects/Blocks				
Days	1158421.26	3	386140.42	67.19 ***
Diet x Days	4016.18	3	1338.72	.23
Error	241361.05	42	5746.69	
Total	1724723.73	63		

*** $p < .001$

Light Nest Time

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	127895.64	1	127895.96	9.04 **
Error	197951.84	14	14139.42	
Within Subjects/Blocks				
Days	431379.79	3	143793.26	49.53 ***
Diet x Days	4910.92	3	1636.98	.56
Error	121929.02	42	2903.07	
Total	884067.22	63		

** $p < .01$
*** $p < .001$

Dark Nest Time

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	10025.02	1	10025.02	.12
Error	53667.22	14	3833.37	
Within Subjects/Blocks				
Days	242172.54	3	80724.18	29.18 ***
Diet x Days	1774.04	3	591.34	.21
Error	116197.66	42	2766..61	
Total	423836.48	63		

***$p < .001$

Bout Duration (Day 4 and Day 10)

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	456.36	1	456.36	4.48 +
Error	1424.06	14	101.72	
Within Subjects/Blocks				
Time of Day	4496.04	1	4496.04	80.78 ***
Diet x Time of Day	485.10	1	485.10	8.72 *
Error	779.26	14	55.66	
Days	458.92	1	458.92	7.42 *
Diet x Days	71.74	1	71.74	1.16
Error	865.46	14	61.82	
Time of Day x Days	11.02	1	11.02	.34
Diet x Time of Day x Days	48.13	1	48.13	1.49
Error	452.11	14	32.29	
Total	9548.20	63		
(Residual)	2096.83	42		

+ $p = .05$
* $p < .05$
*** $p < .001$

Total Nest Frequency

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	2434.92	1	2434.92	1.72
Error	19721.10	14	1408.65	
Between Subjects/Blocks				
Days	1751.32	3	583.77	2.62
Diet x Day	1213.42	3	404.47	1.82
Error	9335.26	42	222.26	
Total	34456.03	63		

Light Nest Frequency

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	538.88	1	538.88	1.58
Error	4775.14	14	341.08	
Within Subjects/Blocks				
Days	174.89	3	58.30	1.23
Diet x Days	151.88	3	50.62	1.07
Error	1986.51	14	47.30	
Total	7627.32	63		

Dark Nest Frequency

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	1062.10	1	1062.10	2.00
Error	7411.90	14	529.42	
Within Subjects/Blocks				
Day	1130.05	3	376.68	3.27
Diet x Days	755.82	3	251.94	2.18
Error	4833.28	42	115.08	
Total	15193.16	63		

Dam Core Temperature

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	8.96	1	8.96	33.26 ***
Error	3.76	14		
Within Subjects/Blocks				
Time of Day	7.67	1	7.67	47.32 ***
Diet x Time of Day	.28	1	.28	1.76
Error	2.27	14	.16	
Days	1.04	3	.34	11.20 ***
Diet x Days	.14	3	.04	.22
Error	1.30	42	.03	
Time of Day x Days	.08	3	.02	.51
Diet x Time of Day x Days	.32	3	.10	2.11
Error	2.10	42	.05	
Total	27.94	127		
(Residual)	5.68	98		

*** $p < .001$

Dam Ventral Temperature

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	27.02	1	27.02	16.48 ***
Error	23.10	14	1.65	
Within Subjects/Blocks				
Time of Day	6.56	1	6.56	8.28 *
Diet x Time of Day	.51	1	.51	.64
Error	11.07	14	.79	
Days	15.34	3	5.11	7.82 ***
Diet x Days	.75	3	.25	.38
Error	27.48	42	.65	
Time of Day x Days	1.33	3	.44	2.58
Diet x Time of Day x Days	.06	3	.02	.12
Error	7.21	42	.17	
Total	120.64	127		
(Residual)	45.77	98		

* $p < .05$
*** $p < .001$

Pup Skin Temperature

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	8.42	1	8.42	28.95 ***
Error	4.07	14	.29	
Within Subjects/Blocks				
Time of Day	.16	1	.16	.67
Diet x Time of Day	.49	1	.49	2.15
Error	3.24	14	.23	
Days	5.62	3	1.88	11.52 ***
Diet x Days	.30	3	.10	.62
Error	6.84	42	.16	
Time of Day x Days	1.84	3	.61	4.07 *
Diet x Time of Day x Days	.08	3	.02	.19
Error	6.31	42	.15	
Total	37.40	127		
(Residual)	16.40	98		

* $p < .05$
*** $p < .001$

Dam Food Intake

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	328.38	1	328.38	1.54 **
Error	398.23	14	28.44	
Within Subjects/Blocks				
Time of Day	4363.64	1	4363.64	87.59 ***
Diet x Time of Day	499.28	1	499.28	10.02 **
Error	697.44	14	49.82	
Days	1557.97	3	519.32	89.80 ***
Diet x Days	86.36	3	28.78	4.98 **
Error	242.90	42	5.78	
Time of Day x Days	30.20	3	10.06	1.18
Diet x Time of Day x Days	11.67	3	3.89	.46
Error	356.78	42	8.49	
Total	8572.88	127		
(Residual)	1297.12	98		

* $p < .05$
** $p < .01$
*** $p < .001$

Dam Proportional Food Intake

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	.000	1	.000	1.00
Error	.000	14	.000	
Within Subjects/Blocks				
Time of Day	2.85	1	2.85	73.84 *z*
pppp Diet x Time of Day	.17	1	.17	4.44 +
Error	.54	14	.04	
Days	.000	3	.000	.99
Diet x Days	.000	3	.000	.99
Error	.000	42	.000	
Time of Day x Days	.05	3	.02	3.12 *
Diet x Time of Day x Days	.01	3	.004	.79
Error	.24	42	.005	
Total	3.88	127		
(Residual)	.78	98		

\+ $p = .05$
* $p < .05$
*** $p < .001$

Dam Caloric Intake

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	264.24	1	264.24	.76
Error	4866.18	14	347.58	
Within Subjects/Blocks				
Time of Day	56933.30	1	56933.30	78.44 ***
Diet x Time of Day	4516.18	1	4516.18	6.22 *
Error	10160.68	14	725.76	
Days	19779.26	3	6593.08	111.84 ***
Diet x Days	821.74	3	273.91	4.64 **
Error	2475.76	42	58.94	
Time of Day x Days	404.14	3	134.71	1.30
Diet x Time of Day x Days	172.80	3	57.60	.55
Error	4365.55	42	103.94	
Total	104759.84	127		
(Residual)	17001.99	98		

* $p < .05$
** $p < .01$
*** $p < .001$

Dam Water Intake

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	12165.81	1	12165.81	93.06 ***
Error	1830.12	14	130.72	
Within Subjects/Blocks				
Days	5579.45	3	1859.82	59.88 ***
Diet x Days	1277.70	3	425.90	13.71 ***
Error	1304.49	42	31.06	
Total	22157.59	63		

$p < .001$

Pup Brown Adipose Tissue Weight

Source	SS	df	ms	F
Diet	.011	1	.011	1.08
Sex	.004	1	.004	.43
Diet x Sex	.017	1	.017	1.70
Error	.286	28	.010	
Total	.319	31		

Pup Adrenal Weight

Source	SS	df	ms	F
Diet	.000	1	.000	.42
Sex	.000	1	.000	.12
Diet x Sex	.000	1	.000	.05
Error	.002	28	.000	
Total	.002	31		

Pup Gonad Weight

Source	SS	df	ms	F
Diet	.001	1	.001	.97
Sex	1.720	1	1.720	1221.08 ***
Diet X Sex	.000	1	.000	.17
Error	.04	28	.001	
Total	1.76	31		

Pup Whole Brain Weight

Source	SS	df	ms	F
Diet	.25	1	.25	3.28
Sex	.04	1	.04	.51
Diet x Sex	.20	1	.20	2.63
Error	2.16	28	.08	
Total	2.65	31		

*** $p < .001$

Pup Brain Weight Less Cerebellum

Source	SS	df	ms	F
Diet	.24	1	.24	3.92
Sex	.06	1	.06	.94
Diet x Sex	.14	1	.14	.13
Error	1.73	28	.06	
Total	2.18	31		

Thermoregulation

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	1.70	1	1.70	1.08
Sex	5.08	1	5.08	3.24
Diet x Sex	.01	1	.01	.01
Error	43.86	28	1.56	
Within Subjects/Blocks				
Time	74.38	4	18.60	154.74 ***
Diet x Time	1.66	4	.42	3.46 +
Sex x Time	.42	4	.10	.87
Diet x Sex x Time	.40	4	.10	.84
Error	13.46	112	.12	
Total	140.98	159		

+ $p = .01$ *** $p < .001$

Righting Latency

Source	SS	df	ms	F
Between Subjects/Blocks				
Diet	.01	1	.01	.06
Error	2.12	14	.15	
Within Subjects/Blocks				
Days	29.64	8	3.70	20.14 ***
Diet x Days	.40	8	.27	
Error	20.60	112	.18	
Total	52.78	143		

$p < .001$

Appendix C: Analysis of Variance Summary Tables (Experiment 3)

Female Core Temperature Across Diet Phases

Source	SS	df	ms	F
Subjects/Blocks	24.92	10		
Blocks	15.05	9	1.67	12.73 ***
Error	11.82	90	.13	
Time	24.52	1	24.52	121.22 ***
Error	2.02	10	.20	
Days x Blocks	1.45	9	.16	1.95 +
Error	7.44	90	.08	
Total	87.24	219		
(Residual)	21.29	190		

+ $p = .05$
*** $p < .001$

Female Core Temperature During Last Five Days of Lab Chow Baseline and First Five Days of Semipurified Diet Phases

Source	SS	df	ms	F
Subjects/Blocks	7.64	10		
Days	18.88	9	2.10	10.94 ***
Error	17.26	90	.19	
Total	43.78	109		

*** $p < .001$

Female Caloric Intake Across Diet Phases

Source	SS	df	ms	F
Subjects/Blocks	1060.11	10		
Blocks	5883.52	9	653.72	11.44 ***
Error	5144.70	90	57.16	
Total	12088.93	109		

*** $p < .001$

Female Caloric Intake During Last Five Days of Lab Chow Baseline and First Five Days of Semipurified Phases

Source	SS	df	ms	F
Subjects/ Blocks	7830.33	10		
Days	8183.02	9	909.22	8.72 ***
Error	9386.94	90	104.30	
Total	25400.94	109		

*** $p < .001$

Female Water Intake Across Diet Phases

Source	SS	df	ms	F
Subjects/Blocks	10658.01	10		
Blocks	690.15	9	76.68	1.98 +
Error	3492.76	90	38.80	
Total	14840.91	109		

+ $p = .05$

Female Percent Weight Change Across Diet Phases

Source	SS	df	ms	F
Blocks/Subjects	54.48	10		
Blocks	315.49	9	35.05	5.56 ***
Error	566.83	90	6.29	
Total	936.80	109		

*** $p < .001$