Effects of pre- and post-natal exposure to a supermarket diet on the development of obesity.

EFFECTS OF PRE- AND POST-NATAL EXPOSURE TO A SUPERMARKET DIET ON THE DEVELOPMENT OF OBESITY

A Thesis Presented

By

SUE ANNE ASSIMON

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Department of Psychology

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Approved as to style and content by:

Richard M. Gold, Chairman of Committee

George N. Wade, Member

Melinda A. Novak, Member

Seymour M. Berger, Chairman
Department of Psychology

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ABSTRACT

Effects of Pre- and Post-Natal Exposure
to a Supermarket Diet on the
Development of Obesity

February 1984

Sue Anne Assimon, B.A., Colgate University

M.S., University of Massachusetts

Directed by: Professor Richard M. Gold

The developmental effects of pre- and post-natal exposure to a supermarket diet (SMD) on mothers and their offspring was examined. First, in SMD-fed mother rats, the excess weight typical of SMD feeding was maintained but did not increase during gestation. However, during the lactation period, the SMD-exposed mother rats lost weight such that by the end of this period they weighed no more than the pellet-fed control mothers. Overweight SMD-fed mothers gave birth to the same number of offspring as normal weight mothers, however, had a higher offspring mortality rate than normal mothers for the first half of the lactation period but not the latter half. Second, SMD availability to mothers influenced both their female and male offsprings' pre-weaning growth. At 2 days of age, pups' body weights were the same, regardless of mother's diet. However, at 10, 15, and 21 days of age, offspring whose mothers were eating SMD had lower body weights but heavier retroperitoneal and perigonadal fat depot weights at 21 days of age. Finally, exposure from weaning

to a SMD resulted in delayed onset of excessive weight gain in both female and male rats irrespective of maternal pre-weaning dietary exposure. From weaning, SMD-fed offspring consumed more calories than pellet-fed groups with no consistent effect of mother's diet exposure upon pups' subsequent caloric intake or diet component selection. As adults, female and male SMD-fed rats had elevated carcass lipid content, heavier retroperitoneal and perigonadal fat pads, and larger adipocyte diameters than pellet-fed rats. SMD exposure from weaning increased fat cell number in male rats' retroperitoneal fat pads, but not their epididymal fat pads, whereas in female rats fat cell number tended to increase in both retroperitoneal and parametrial fat pads. No significant adipose tissue effects were attributable to mother's diet. Hence, although pre- and post-natal exposure to a supermark-type diet produced early effects upon offspring, it was not found to potentiate an animal's later susceptibility to dietary obesity.

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INTRODUCTION

Adult rats given highly palatable diets, such as a supermarket-type (SMD) (1, 2, 3), cafeteria-type (4, 5), high fat (6, 7), or carbohydrate (6, 8) diets, rapidly become obese in contrast to control animals given a standard chow diet. The expression of this diet-induced obesity is greater in old adult rats than in young adult rats (2). Several physiological measures have been utilized to assess the development of dietary obesity. One measure often used is the analysis of adipocyte cellularity. Morphological changes in adipose tissue have been demonstrated in adults fed highly palatable diets, such as high fat diets (9, 22, 23), sucrose solutions (23), and cafeteria diets (24).

In comparison to adult animals' response to hypercaloric diets, weanlings placed on such diets don't show an immediate weight gain response. Weanlings fed a palatable SMD (3), a high fat diet (6, 9), or high carbohydrate diet (10, 11, 12) demonstrate delayed excessive weight gains, usually beginning at 8-10 weeks of age. Also the effects of starting such ingestive experiences as early as weaning on adult feeding behavior and body weight have been examined. For example, male rats given a high fat diet for a period at weaning exhibited differential feeding responses upon subsequent

re-exposure to the high fat diet than same age control rats newly exposed to this diet (9). The rats with previous high fat diet exposure consumed more and gained more weight. However, in another study prior weanling exposure to a SMD did not alter body weight gains produced upon adult exposure to a SMD versus diet-naive adult-started controls (3).

Early nutritional manipulations also change adipose tissue morphology. Weanling rats fed a high fat diet possessed a greater mean epididymal fat depot DNA content at 62 days than did control animals (9). Swiss mice exposed to mothers fed a high fat diet during both gestation and lactation, and then weaned on to a similar calorically dense diet, possessed heavier parametrial fat pads at 6 weeks of age due to increases in fat cell size. Hyperplasia of fat cells occurred between 18 and 32 weeks of age. Weanling female rats on a SMD tend to show an increase in parametrial fat pad cell number, along with typical cell size increases, whereas adult-started rats' parametrial fat cell number is not affected by SMD exposure (26). However, the presence of this difference in adipose tissue morphology does not translate into body weight differences.

Finally, although weanling rats typically exhibit a delayed excessive weight gain after exposure to obesity-inducing hypercaloric diets, some evidence suggests the presence of a subtle obese condition prior to its overt expression by body weight measures (11, 12). For example, from weaning to 70 days of age,

rats provided a lab chow and 32% sucrose solution diet consume calories in excess of control rats provided only a standard chow diet (11). Body weights did not differ between these groups during this period, but at 46, 57, and 70 days of age, the sucrose-fed animals had significantly higher Lee Obesity Index values, an indirect measure of percent body fat. A direct carcass composition analysis performed on these animals at 70 days of age confirmed that wet carcass percent fat was greater in the sucrose supplemented animals. Also, sucrose fed animals lost significantly less weight than controls during 24 hour fasts and rats fed the sucrose supplement had lower fasted blood glucose levels than controls. One possible explanation of these data is that a heightened insulin release led to increased lipogenesis.

In most weanling-started dietary response studies neither the pups nor their mothers had exposure to the experimental diets prior to weaning. Prior experience may not be important once an animal reaches maturity, for SMD experience as an adult does not influence later response to the same diet (2). Nevertheless, for weanlings prior experience with a diet may play a significant role in the subsequent maturational progress of dietary obesity. Significant developmental ingestive experiences clearly do not begin at weaning.

Early exposure to a taste may establish an 'appetite' for that taste. Thus, taste experiences could play a role in the development of dietary-induced obesity. Gustatory manipulation of pre-weaning

maternal ingestive experiences have been shown to favorably influence subsequent pup self-selective feeding (13, 14, 15), presumably via tastes imparted to the mother's milk. Early ingestive experiences could alter later feeding behavior, caloric selection, and body weight gain patterns.

Along with influencing pre-weaning taste experiences, administration of a high caloric, palatable diet to pregnant or lactating rats alters the pups' perinatal environment and nursing conditions. A cafeteria diet alters *in vitro* mammary gland lipogenesis (16), maternal lactational hyperphagia (17), and also influences pups' early growth (18). Hence, pre- and post-natal exposure to a hypercaloric diet along with excessive maternal weight gain may influence pups' post-weaning weight gain and feeding patterns, and underlying adiposity. The clarification and delineation of pre- and post-weaning early dietary effects may indicate the nature of the delayed onset of obesity phenomena typically seen with weanling exposure to a palatable diet.

Another approach used to alter the early ingestive environment is to vary litter size and thus pups' intake. Utilizing this technique, Oscai et al. (19) found that the amount of food consumed by rats during suckling plays an important role in determining voluntary food intake and resultant body weight in later life. The content and composition of the diet available during adulthood interacts with various early ingestive experiences in determining the level of obesity (19) and adiposity (21) finally obtained.

Promoting preweaning milk intake by reducing litter size increases epididymal fat pad cell numbers, the effect emerging at 5 weeks of age, and increases in cell size emerging at 10 weeks of age, both in comparison to normal litter size-raised pups (25). The increased fat cell number is not eliminated when small litter size pups are weaned onto a low caloric density diet (21) versus a standard chow diet as above.

In summary, early dietary manipulations can produce effects on adult feeding behavior, body weight, and adiposity. A range of studies has also examined adult animals' responses to the experimental overfeeding treatment. In contrast, few studies have examined the long-term developmental effects upon the offspring of mothers with pre-weaning exposure to palatable diets. The present study examines the influence of pregnant and nursing mother rats' dietary exposure and excessive maternal weight gain on pups' development and later susceptibility to dietary obesity. More specifically the study examines whether the exposure of mothers and pups to a SMD influences the post-weaning response of pups to SMD. The effects upon fat cell size and number development, carcass composition, and self-selecting food intake and body weight pattern are examined.

C H A P T E R I I

EXPERIMENTS

Experiment I

The influence of excessive weight gain and exposure to a palatable supermarket diet on pregnant and lactating rats' body weights were determined.

Methods. Twenty-nine adult female Charles River CD rats, weighing between 190-220 g, were housed individually in plastic maternity cages containing wood-chip bedding. Beginning at approximately 18-20 days prior to impregnation, each rat was administered one of two diets, either the experimental supermarket diet (SMD) consisting of ad libitum peanut butter, chocolate chip cookies, sugar sweetened milk (536 g sucrose:1.89 liters whole milk), Purina lab rat chow pellets, and water, or the control diet, consisting of ad libitum Purina lab rat chow pellets and water. The animals were divided into 4 groups; pregnant SMD-fed (n = 10), non-pregnant SMD-fed (n = 6), pregnant pellet-fed (n = 10), and non-pregnant pellet-fed (n = 3) females.

Body weights were measured every fifth day through the diet adjustment, gestational, and lactational periods.

Rats were randomly mated with one of eight male studs. The date of impregnation was determined by daily vaginal smears for motile sperm. The end of each mother's gestational period was monitored to determine date of birth. Survival of the pups was periodically noted.

Results. At the time of impregnation, the SMD-exposed rats weighed in excess of 50 grams over the pellet-exposed rats (Figure 1). During gestation, the SMD-fed rats maintained but did not increase the excess weight. After parturition, having lost the weight of their pups, the SMD-fed mothers had body weights similar to the SMD-fed non-mothers (Mann-Whitney U, $p > .05$). During the 3 week lactation period the SMD-exposed mother rats lost weight such that by the end of the lactation period they weighed no more than the pellet-fed mothers (Mann-Whitney U, $p < .05$). In contrast, at weaning pellet-fed mothers weighed approximately 50 grams more than pellet-fed non-mothers.

Overweight (SMD-fed) mothers gave birth to the same number of offspring as normal weight chow-fed mothers (Table 1). However, overweight mothers had a higher offspring mortality rate than normal weight mothers for the first half of the lactation period (Table 1). Also, within the SMD-fed group, the heaviest mothers tended to lose the most pups (Spearman rank correlation coefficient, $r_s = .84$, $p < .01$).

Fig. 1. Mean body weights of non-pregnant control rats and mother rats fed a standard pellet diet or a SMD during the diet adjustment, gestation, and lactation periods.

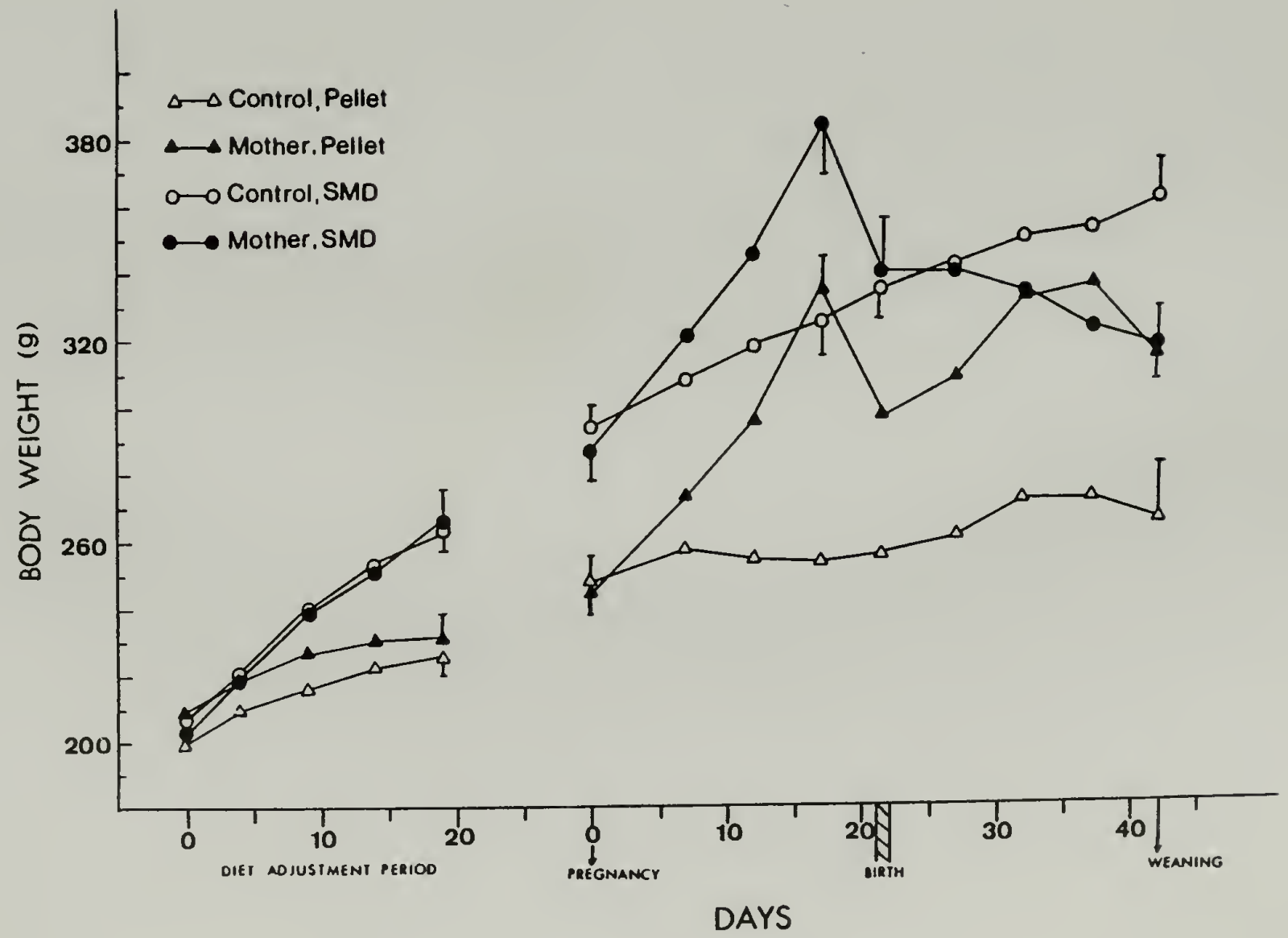
Control, Pellet
Mother, Pellet
Control, SMD
Mother, SMD
BODY WEIGHT (g)
380
320
260
200
0
10
20
0
10
20
30
40
DIET ADJUSTMENT PERIOD
PREGNANCY
BIRTH
WEANING
DAYS

Figure 1

TABLE 1

MOTHERS' BODY WEIGHTS, AND OFFSPRINGS' BIRTH AND SURVIVAL RATES
(MEAN ± SEM)

	Body Weight, Initial (g)	Body Weight, at pregnancy (g)	Body Weight, at parturition (g)	No. Offspring per litter at birth	Pup Death (in days)		
					0-2	2-10	10-21
Mothers on Pellets n=7	209.0 ± 3.0	244.0 ± 4.0	298.0 ± 8.0	12.0 ± 1.0	0	0	0
Mothers on SMD n=9	203.0 ± 3.0	288.0 ± 8.0	341.0 ±14.0	12.0 ± 1.0	2.8 ±1.3	0.9 ±0.7	0

Experiment II

The effect of SMD availability to mothers upon their offsprings' growth was examined. Lactational period body weights and adipose depot weights at weaning were measured.

Methods. Offspring of mothers from Experiment I served as experimental subjects. Their date of birth was considered day 0 of age. Litters were culled at 2 days of age to 8 ± 1 (± range) pups, approximating ½ male, ½ female. Pups' body weights were recorded at 2, 10, 15, and 21 days of age. At weaning (21 days), half of each litter, split between sexes, was sacrificed to determine parametrial or epididymal, and retroperitoneal fat pad weights.

Results. No sex differences in pups' body weight were seen at any age during lactation (Offspring's Sex: 2D - $F(1, 106) = 3.1$, $p > .05$; 10D - $F(1,106) = 1.1$, $p > .05$; 15D - $F(1, 106) = 0.9$, $p > .05$; 21D - $F(1,106) = 0.6$, $p > .05$; Sex x Mother's diet: 2D - $F(1,106) = 0.0$, $p > .05$; 10D - $F(1,106) = 0.6$, $p > .05$; 15D - $F(1,106) = 0.8$, $p > .05$; 21D - $F(1, 106) = 1.0$, $p > .05$). At 2 days of age, pups' body weights were the same, regardless of mother's diet (Figure 2). At 10, 15, and 21 days of age, offspring whose mothers were eating SMD weighed significantly less than offspring whose mothers were fed only pellets (Mother's diet: 10D - $F(1, 106) = 20.3$, $p < .05$; 15D - $F(1, 106) = 39.3$, $p < .05$; 21D - $F(1, 106) = 7.6$, $p < .05$).

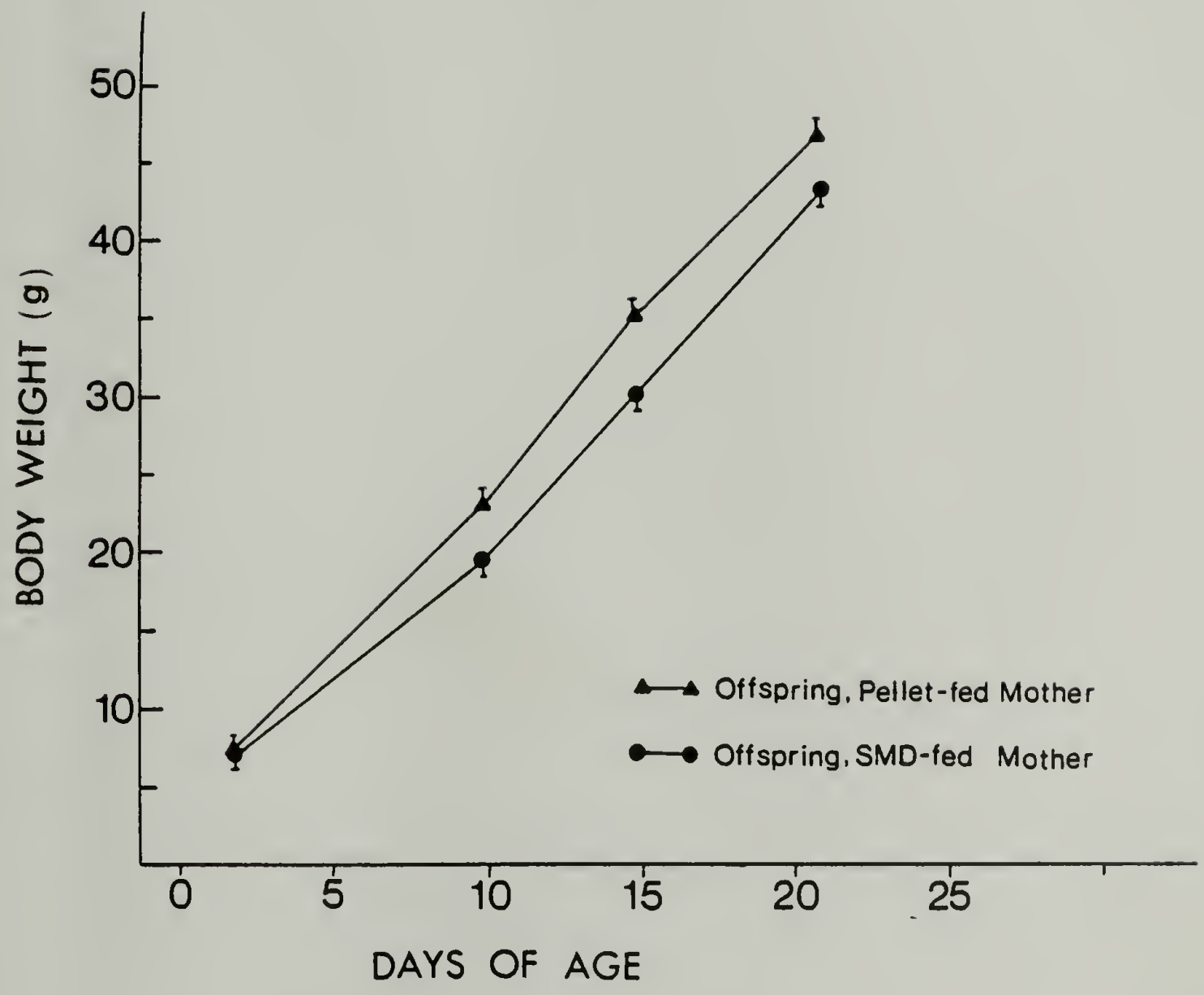

Fig. 2. Mean body weights of offspring of pellet-fed mothers and of SMD-fed mothers during the lactation period. Mean values include both female and male offspring data. No sex differences in pups' body weight resulted at any age during this period.

Despite their lower body weight at 21 days of age both male and female offspring of SMD-fed mothers possessed significantly heavier retroperitoneal fat depot weights than same sex offspring of pellet-fed mothers (Retroperitoneal: Female - $t(17) = 3.89$, $p < .05$; Male - $t(18) = 3.07$, $p < .05$)(Figure 3). Similar differences were seen for the perigonadal fat pads, although only the females reached significance (Parametrial: Females - $t(17) = 16.62$, $p < .05$; Epididymal: Males - $t(13) = 1.73$, $p > .05$).

Experiment III

This experiment examined the long-term developmental effects of pre-weaning maternal exposure to a SMD on their female and male offsprings' subsequent ingestive behavior, nutritive state, body weight, and adiposity.

Methods. The remaining female and male offspring from Experiment I served as subjects. At 21 days of age, these rats were weaned and housed singly in hanging wire cages and maintained either to their respective maternal diets (SMD or chow), or switched to the other experiment diet. The resulting experimental groups were: offspring of a pellet-fed mother weaned onto a pellet diet (P/P; female - n = 6, male - n = 6), offspring of a pellet-fed mother weaned onto a SMD (P/S; female - n = 8, male - n = 8), offspring of a SMD-fed mother weaned onto a pellet diet (S/P; female - n = 4, male -

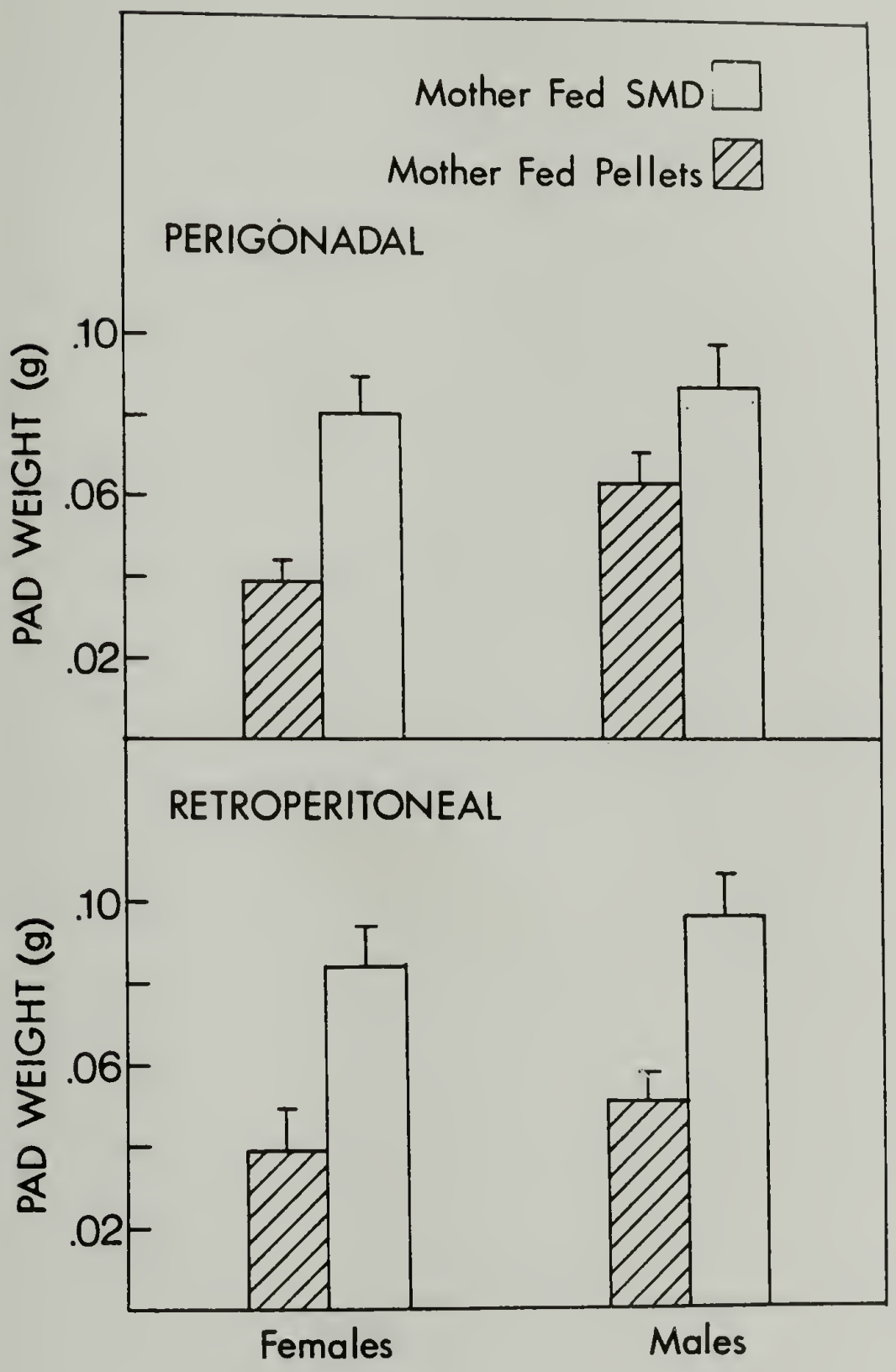

Fig. 3. Mean perigonadal and retroperitoneal fat pad weights of 21 day old female and male offspring of pellet-fed mothers and of SMD-fed mothers.

n = 6), and offspring of a SMD-fed mother weaned onto a SMD (S/S; female - n = 5, male - n = 7).

Food intake, accounting for spillage of solid foods, and body weights were measured every fifth day until 41 days of age, then weekly. The total caloric intake and caloric intake as protein, carbohydrate, and fat components were determined. Feed efficiency, defined as change in grams body weight per kilocalories eaten, was determined.

At 132-140 days of age, the rats were sacrificed for measure of white adipose tissue pad weight and cellularity (cell size and number), carcass composition, obesity by Lee Index, and organ weights.

Adipose cellularity. At sacrifice, retroperitoneal and perigonadal (parametrial or epididymal) fat pads were removed and weighed. Except for a portion of each pad taken for adipocyte analysis, the fat was returned to the carcass. A fat sample (~ 1 g) was incubated (37°C) in a Krebs Ringer Phosphate/collagenase solution for 1 hour. Suspended fat cells were then filtered through a 250 µm nylon mesh, and washed twice with collagenase-free KRP. Fat cell aliquots (50 µl) were pipetted onto a microscope slide and photomicrographed with a yellow filtered light source (29). Negatives of adipose samples were projected onto a translucent grid and adipocyte diameters digitized using a Graf pen interfaced with a computer (30). At least one hundred cells were digitized per pad. Cell volumes were computed and mean cell weights derived using

Obesity indices. Two to 3 days prior to the experiment's conclusion, rats were placed under deep anesthesia, and measured for naso-anal lengths using a "stretched" technique (34). Obesity indices are computed by the formula:

$$\text{Obesity Index} = 10^4 \times \frac{\sqrt[3]{\text{Body Weight(gm)}}}{\text{Naso-anal length(mm)}}$$

Organ weights. At sacrifice, the adrenal glands, liver, kidney and uterus or testes were blotted and weighed to the nearest 0.001 gram. The organs were then returned to the carcass for inclusion in the carcass analysis.

Data analysis. Data matrices were established and stored in the IDAP system (Interactive Data Analysis Package, University of Massachusetts, Amherst). BMDP4V program package (University of California, L.A.) was utilized to perform 2-way ANOVA and 2-way repeated measures ANOVA analyses. IDAP system commands were utilized to perform comparison tests (two sample, Student U, t-tests), and correlations.

Results

Females.

Body weight. Regardless of the mother's diet, female offspring placed on a SMD at weaning outgained offspring placed on a standard pellet diet at weaning (Diet: $F(1, 18) = 25.65$, $p < .05$) (Figure 4).

the density of triolein as representative of cellular triglycerides. Total cell number was determined as a function of mean cell weight.

Fat pad weight was corrected for non-lipid components such as stroma, water, and connective tissue. The correction factor was arrived at via a lipid extraction. A 250 mg adipose tissue sample was homogenized in 4 ml Hexane-Isopropanal (H-I, 3:2) solution. The homogenate was poured into a plastic Falcon tube, along with an additional 2 ml H-I solution used to wash the homogenizing tube, and centrifuged for 5 minutes at a 3660 g force. The supernatant was then pipetted into a preweighed citric acid tube, combined with 2 ml of 0.5 M Na_2SO_4, and vortexed. The organic layer was removed and dried to constant weight. Extraction efficiency was monitored with corn oil standards.

Carcass composition. The carcasses were prepared for analysis as described by Gray and Wade (31), modified from Leshner, Litwin, and Squibb (32). The carcass was shaved, eviscerated (stomach and intestine less fat removed), weighed, and placed in a disposable aluminum baking pan. Water content was determined by drying in an 80-90°C oven to a constant (± 1 g/day) weight. The dehydrated carcass was ground in a Waring blender from which a homogenous sample (> .5 g) was obtained for further analysis. To determine carcass lipid content, weighed homogenate samples were delipidated by repeated extractions with 10 ml petroleum ether. The remaining sample was then dried and weighed. Carcass protein was determined according to the method of Lowry et al. (33).

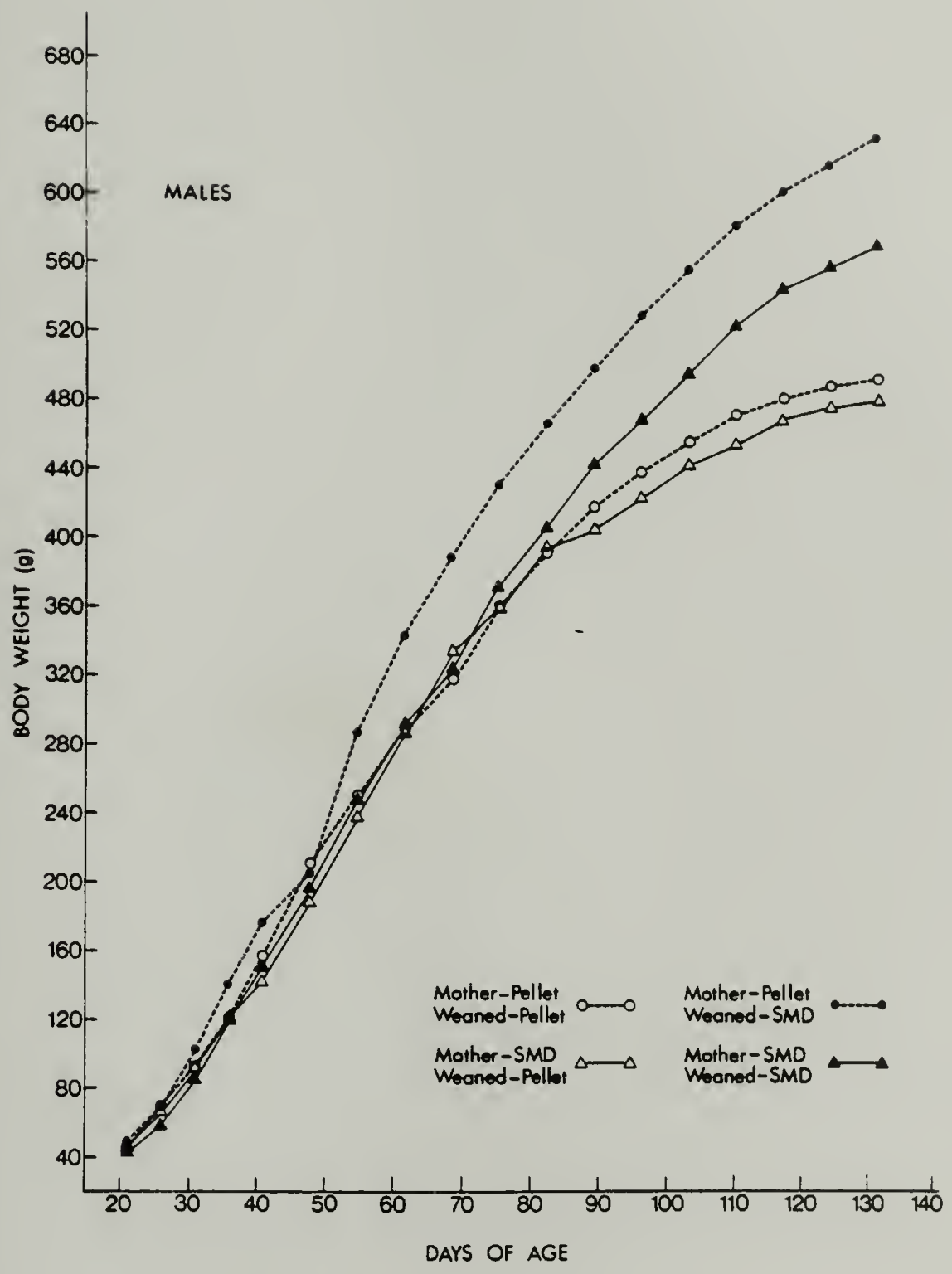

Fig. 4. Mean body weights of female offspring of pellet-fed mothers of SMD-fed mothers provided ad libitum either a standard chow pellet diet or a SMD at weaning, 21 days of age.

No significant mother's diet treatment main effect ($F(1,18) = 1.96$, $p > .05$), or mother's diet x offspring's diet interaction ($F(1,18) = 0.70$, $p > .05$) were revealed.

Exposure from weaning to SMD resulted in a delayed onset of excessive weight gain. Evidence of excessive weight appeared at about 69 days of age, at which time both SMD-fed weanling groups showed an enhanced weight gain over the P/P group (P/P, vs. P/S: Day 69 - $t(11) = 2.95$, $p < .05$; P/P vs. S/S: Day 69 - $t(8) = 2.34$, $p < .05$). As seen in Figure 4, the offspring of SMD-fed mothers consistently weighed less than the respective groups with pellet-fed mothers. However, this difference did not reach statistical significance (P/P vs. S/P: Day 76 - $t(7) = 2.18$, $p > .05$; Day 104 - $t(11) = 1.84$, $p > .05$; Day 132 - $t(7) = 1.56$, $p > .05$; P/S vs. S/S: Day 118 - $t(11) = .49$, $p > .05$, Day 132 - $t(11) = .80$, $p > .05$).

Caloric intake.

(a) Total caloric intake. Despite the delayed onset of excessive weight, SMD-fed offspring consumed more calories from 21 days of age until 132 days of age than did the pellet-fed groups (Diet: $F(1,18) = 253.19$, $p < .05$) (Figure 5). No significant mother treatment main effect ($F(1,18) - 0.16$, $p < .05$) or mother's diet x offspring's diet interaction ($F(1,18) = 0.01$, $p > .05$) resulted. The effect of mother's diet exposure had no consistent effect upon their pups' subsequent caloric intake.

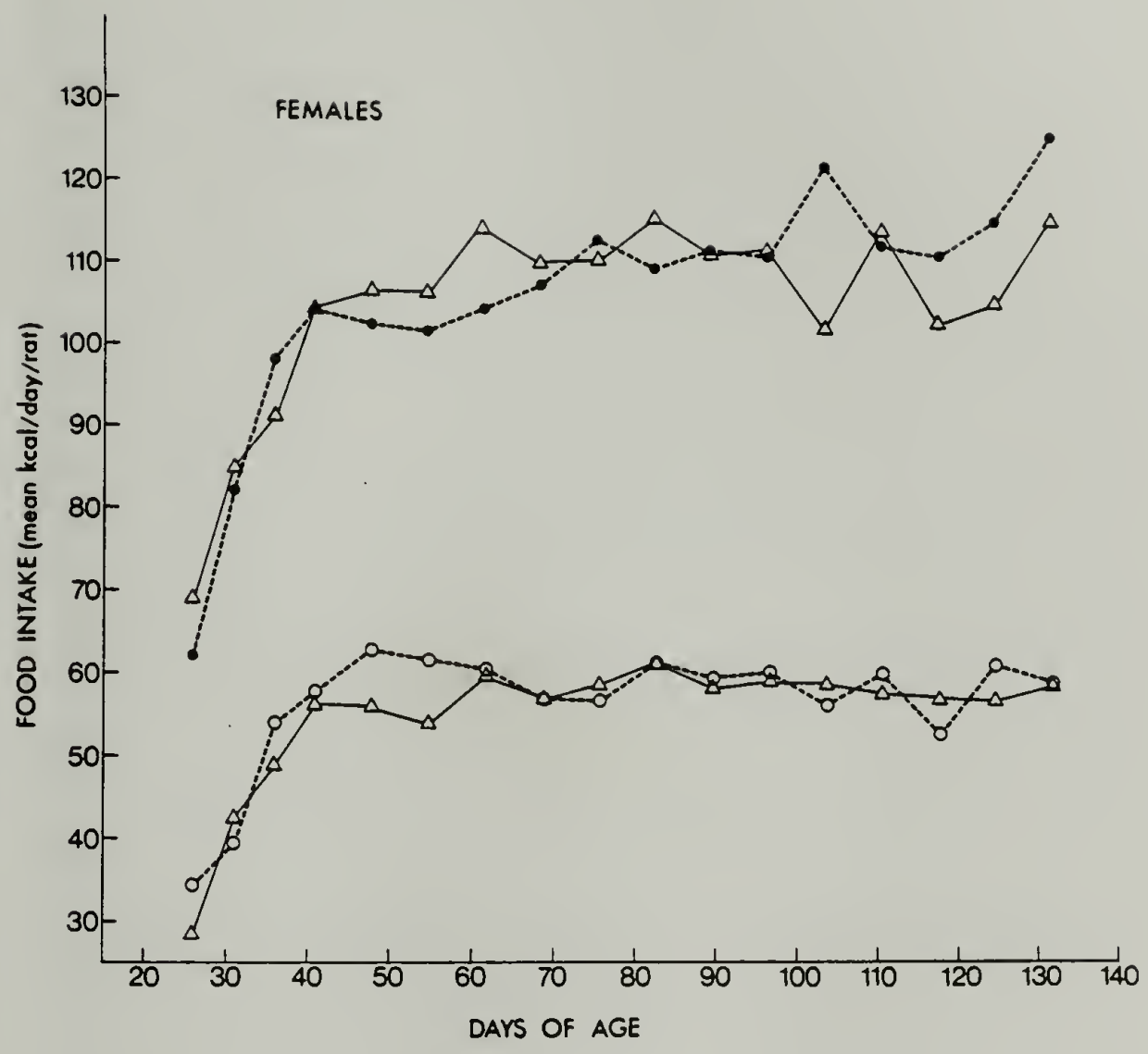

Fig. 5. Mean daily total caloric intake for female offspring of pellet-fed mothers and of SMD-fed mothers weaned either onto a standard chow pellet diet or onto a SMD. Symbols are as indicated in Fig. 4.

(b) Diet component caloric intake. A similar increase pattern in protein intake in all groups was seen from 21 to 41 days of age (Figure 6). At 48 days of age SMD-fed offspring began consuming some 30-40% fewer calories as protein than pellet-fed offspring (Protein: $F(1,18) = 58.30$, $p < .05$). Thus, the increased total caloric intake of offspring placed on a SMD over offspring provided solely a chow diet consisted of increased fat and carbohydrate consumption (Fat: $F(1,18) = 191.13$, $p < .05$; Carbohydrate: $F(1,18) = 185.73$, $p < .05$). In contrast to protein, this difference in fat and carbohydrate component intake between diet treatment groups was present at weaning. Finally, no consistent influence of mother's diet upon female offspring's diet selection was seen.

Feed efficiency. Regardless of mother's diet experience, female offspring weaned onto SMD initially exhibited lower feed efficiency than offspring weaned onto pellets (Diet: $F(1,18) = 18.62$, $p < .05$) (Figure 7). On both diets, feed efficiency decreased with age (Age: $F(3,16) = 104.62$, $p < .05$), but more so for the pellet diet. By day 62 of age there were no differences between the groups (Age x Diet: $F(2,42\ adj) = 5.92$, $p < .05$).

Naso-anal length, and obesity index. No significant differences in offspring's naso-anal length resulted. As expected, SMD-fed offspring had higher Lee Obesity Indices than pellet-fed offspring (Offspring's Diet: $F(1,18) = 32.09$, $p < .05$) (Table 2).

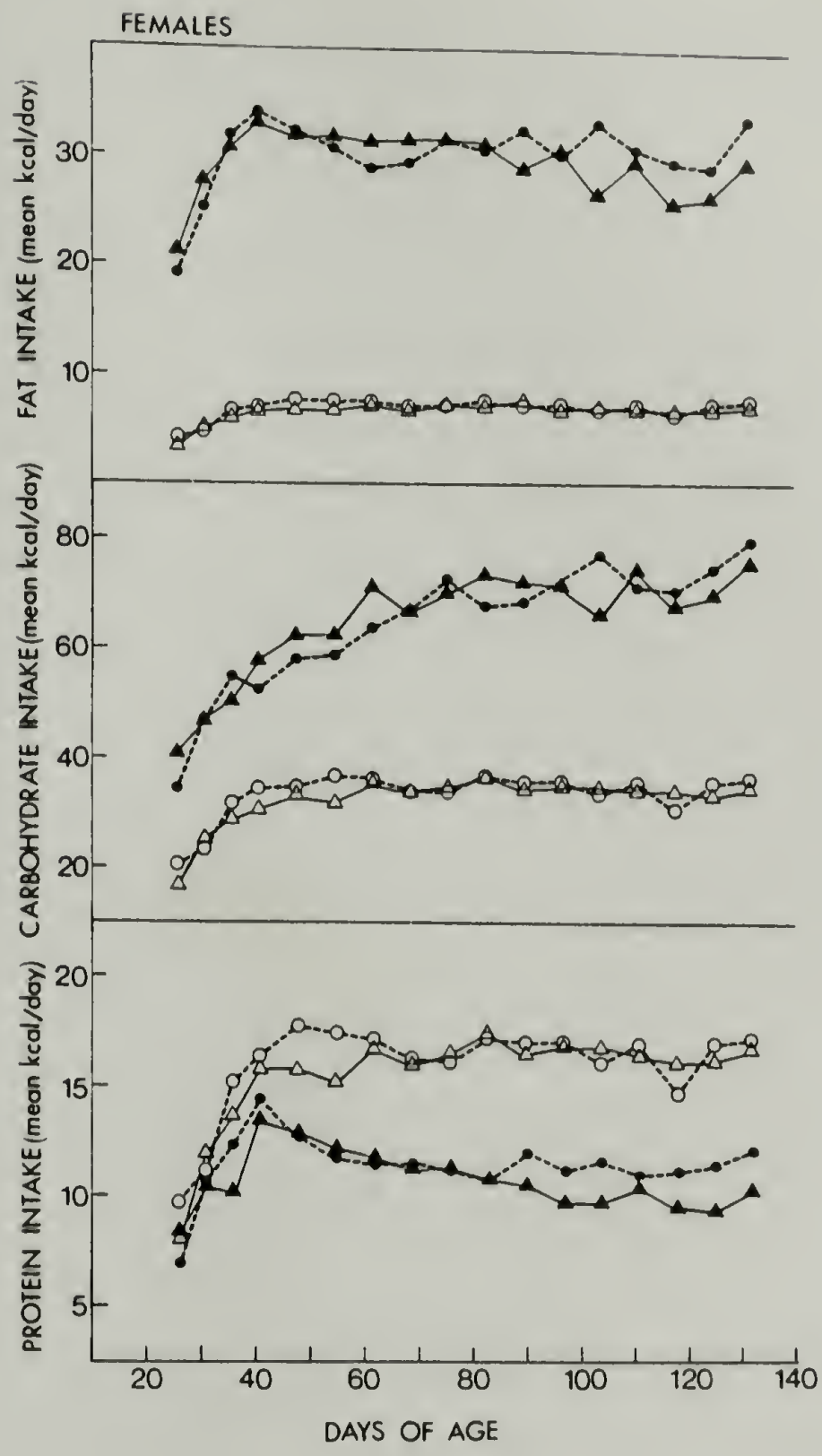

Fig. 6. Mean daily component caloric intake for female offspring of pellet-fed mothers and of SMD-fed mothers placed either on a standard pellet diet or a SMD at 21 days of age. Diet component intake is expressed as mean daily protein, carbohydrate, and fat caloric consumption. Symbols are as indicated in Figure 4.

Fig. 7. Mean feed efficiency values expressed as grams body weight change per kilocalorie eaten for female offspring of pellet-fed mothers and of SMD-fed mothers provided either a standard pellet diet or a SMD at weaning. Symbols are as indicated by Fig. 4.

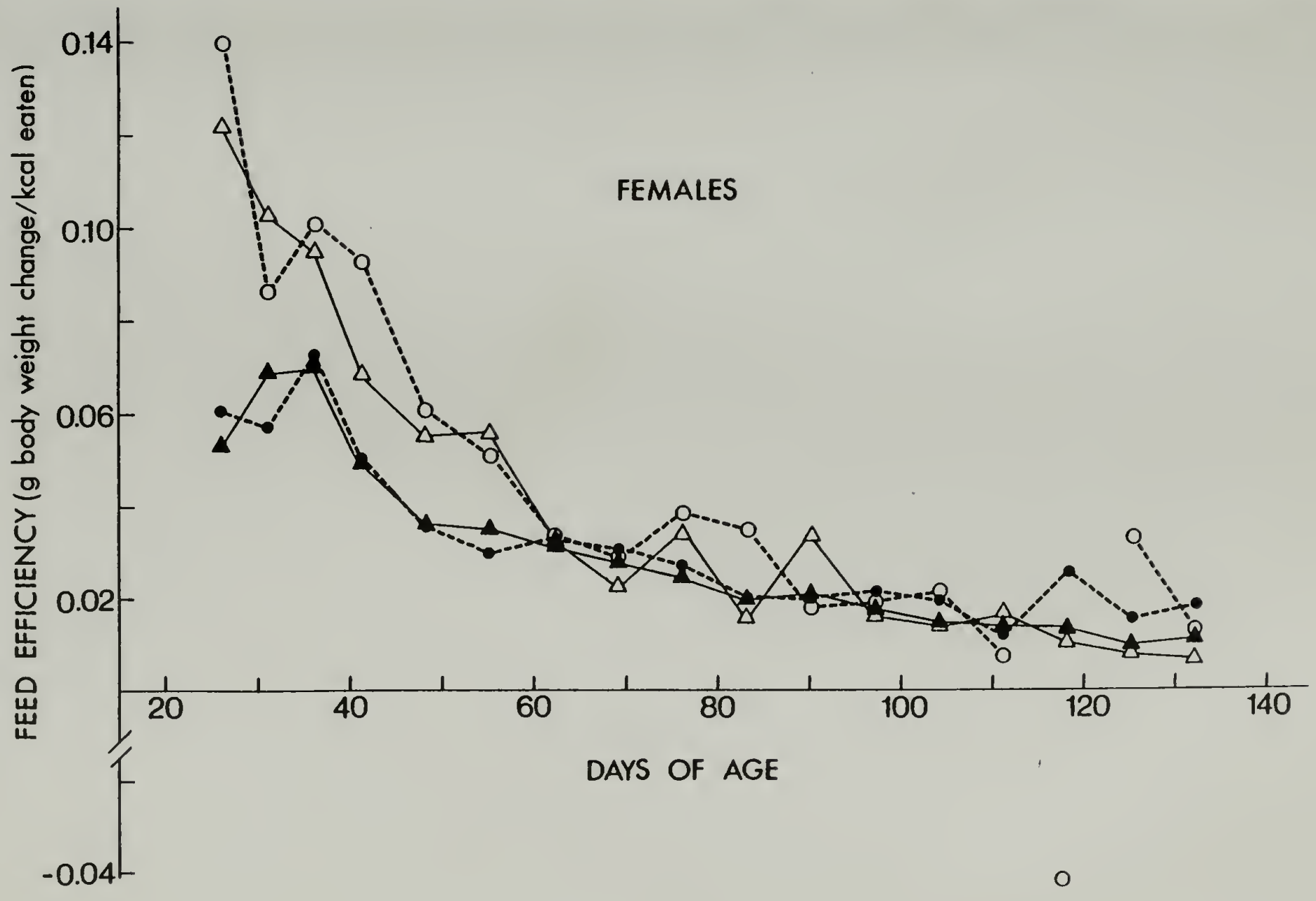
FEMALES
FEED EFFICIENCY (g body weight change/kcal eaten)
0.14
0.10
0.06
0.02
-0.04
20
40
60
80
100
120
140
DAYS OF AGE

Figure 7

TABLE 2

MEAN NASO-ANAL LENGTHS (mm ± SEM) AND MEAN OBESITY INDICES (± SEM) OF FEMALE OFFSPRING AT 132 DAYS OF AGE

	NASO-ANAL LENGTH	OBESITY INDEX
FEMALES:		
Pellet Weaning Diet:		
Mother on pellet	225.1 ± 1.2	292.4 ± 4.5*
Mother on SMD	226.2 ± 2.0	324.0 ± 5.9*
SMD Weaning Diet:		
Mother on pellet	219.4 ± 2.7	286.9 ± 4.9†
Mother on SMD	225.6 ± 1.4	319.2 ± 4.0x

† differs from other mother treatment, same weaning diet treatment, $p < .05$.

* differs from same mother treatment, other weaning diet treatment, $p < .05$.

x differs from other mother treatment, other weaning diet treatment, $p < .05$.

Carcass composition. At 132 days of age, carcass lipid content was elevated, with a corresponding depressed carcass water content, for SMD-fed female rats in contrast to pellet-fed females (Diet: Lipid - $F(1,18) = 90.86$, $p < .05$; Water - $F(1,18) = 115.23$, $p < .05$). SMD-fed groups consistently had lower percent of wet carcass protein than chow-fed groups (Figure 8). However, no significant differences were found between groups in absolute grams lean body mass (calculated as shaved carcass weight minus carcass water and lipid content) (Figure 9). Finally, no effect due to mother's diet was seen in any of the carcass composition measures.

Adipose tissue. At 132 days of age SMD-fed rats had heavier retroperitoneal and parametrial fat pads than pellet-fed rats, irrespective of mother's diet (Offspring's diet: retroperitoneal - $F(1,17) = 55.18$, $p < .05$; parametrial - $F(1,17) = 63.95$, $p < .05$) (Figure 10). This difference can be ascribed primarily to enlarged fat cell diamaters in SMD-fed females (Offspring's diet: retroperitoneal - $F(1,17) = 63.15$, $p < .05$; parametrial - $F(1,17) = 55.18$; $p < .05$). SMD exposure increased fat cell number, but statistical significance was reached only for the retroperitoneal pad (Offspring's diet: $F(1,20) = 7.90$, $p < .05$; P/P versus P/S: $t(10) = 2.26$, $p < .05$; P/S versus S/P: $t(10) = 2.16$, $p < .056$).

Organ weights. Offspring of SMD-fed mothers had lighter livers than offspring of pellet-fed mothers (mother's diet: $F(1,18) = 11.97$, $p < .05$), whereas offspring fed a SMD had heavier livers than offspring fed a pellet diet (Offspring's diet:

Fig. 8. Carcass composition, including water, lipid, and protein components expressed as percent wet carcass, of ~ 132 day old female and male rats from Experiment III. P/P represents pellet-fed mothers' offspring weaned onto a pellet diet. S/P represents SMD-fed mothers' offspring weaned onto a pellet diet. P/S represents pellet-fed mothers' offspring weaned onto a SMD. S/S represents SMD-fed mothers' offspring weaned onto a SMD.

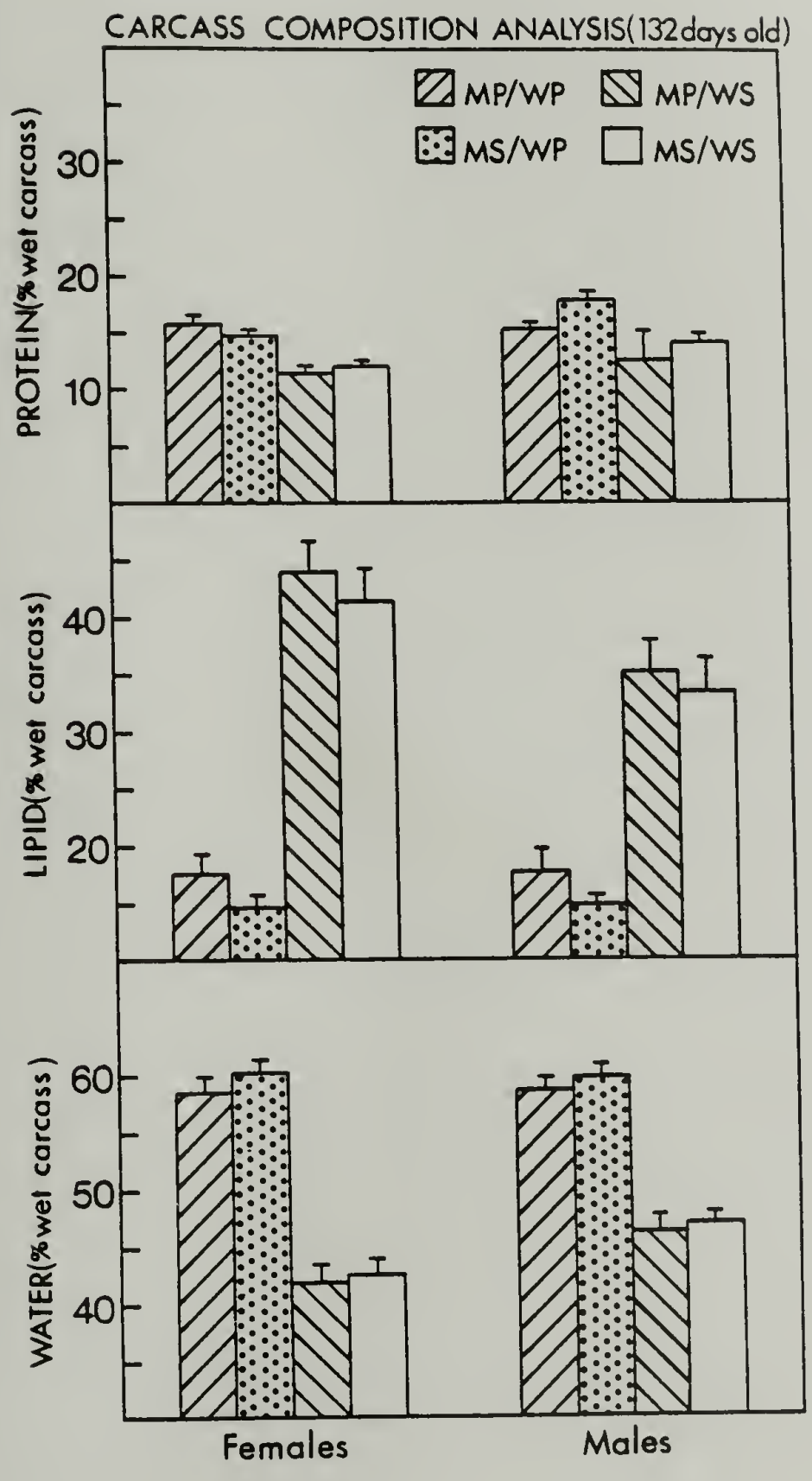
CARCASS COMPOSITION ANALYSIS(132days old)
MP/WP
MP/WS
MS/WP
MS/WS
PROTEIN(% wet carcass)
30
20
10
LIPID(% wet carcass)
40
30
20
WATER(% wet carcass)
60
50
40
Females
Males

Figure 8

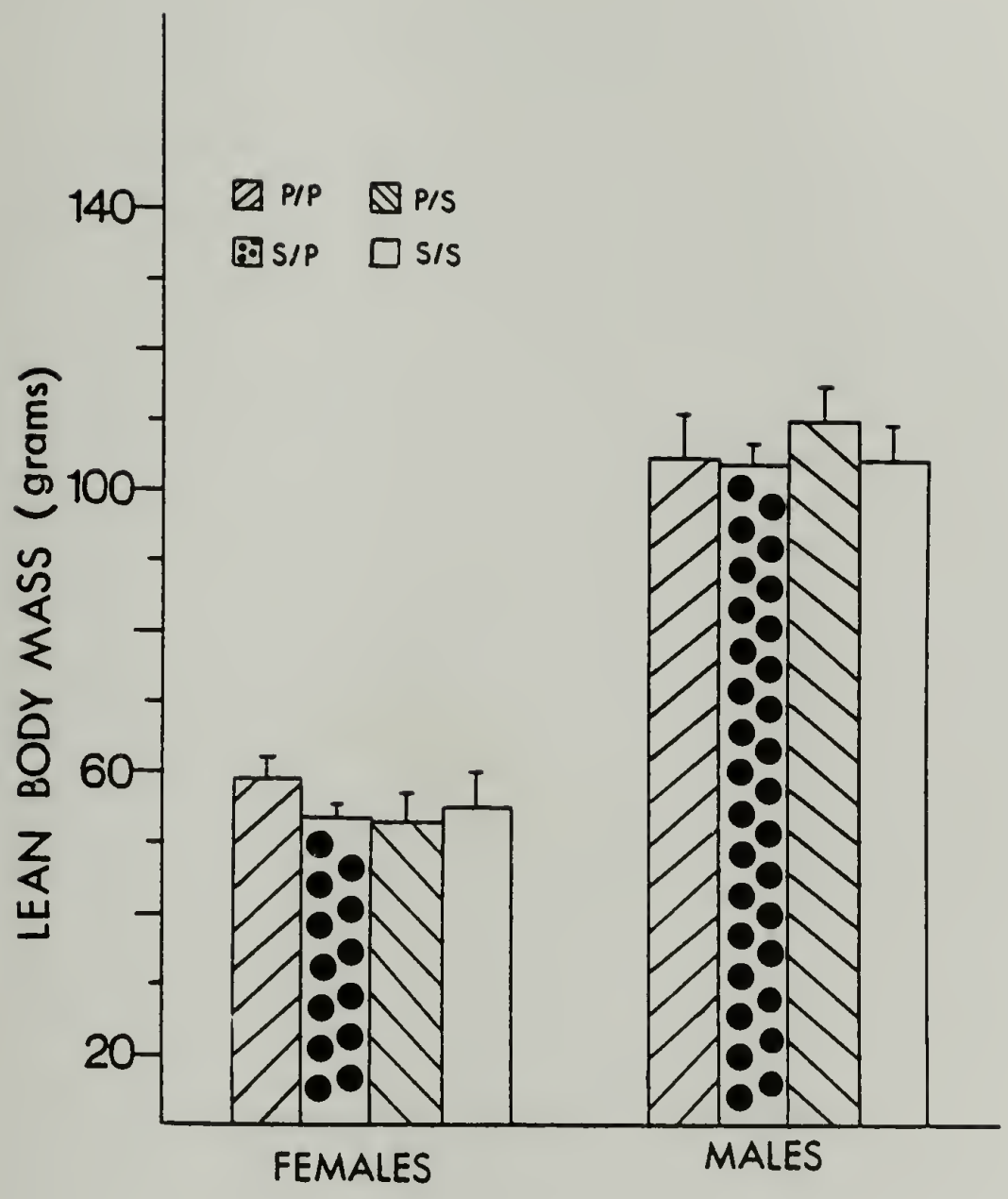

Fig. 9. Lean body mass defined as shaved carcass weight content minus carcass fat and water content for ~132 day old female and male rats from Experiment III. P/P, P/S, S/P, S/S are as represented in Fig. 8.

Fig. 10. Mean adipose pad weight, adipocyte diameter, adipocyte number for retroperitoneal and parametrial adipose tissue sites in female rats from Experiment III. P/P, P/S, S/P, S/S are as represented in Fig. 9.

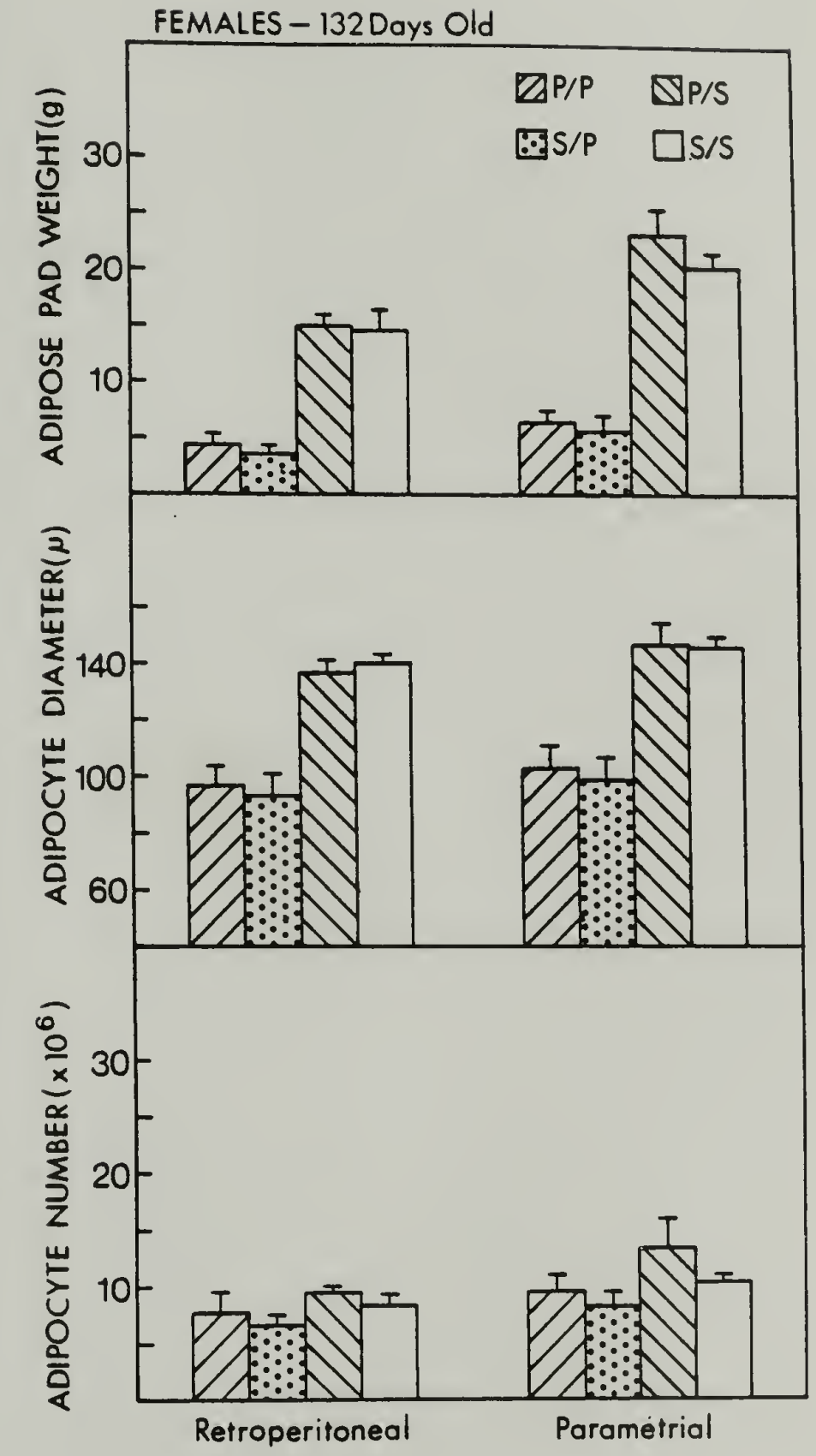
FEMALES – 132 Days Old
P/P
P/S
S/P
S/S
ADIPOSE PAD WEIGHT(g)
30
20
10
ADIPOCYTE DIAMETER(μ)
140
100
60
ADIPOCYTE NUMBER(x10^6)
30
20
10
Retroperitoneal
Paramétrial

Figure 10

$F(1,18) = 9.15$, $p < .05$). The P/S females thus possessed the heaviest livers of all, the excess weight possibly representing stored fuels (Table 3).

Kidney weights were influenced by mother's diet ($F(1,18) = 10.20$, $p < .05$). The P/S group had significantly heavier kidneys than the S/P and S/S groups.

Males.

Body weight. As with the females, male offspring placed on a SMD at weaning gained more weight than male offspring maintained on lab chow (Diet: $F(1,23) = 10.39$, $p < .05$) (Figure 11). Once again, no significant main effect for mother treatment ($F(1,23) = 3.59$, $p > .05$), or mother's diet x offspring's diet interaction ($F(1,23) = 1.68$, $p > .05$) was revealed.

A delayed onset of excessive weight gain resulted for male weanlings exposed to the palatable SMD. However, the age of body weight response to the diet differed depending upon the maternal diet. At 62 days of age, P/S males showed an enhanced weight gain relative to all other groups (P/S, P/P: $t(12) = 2.44$, $p < .05$, P/S, S/P: $t(9) = 3.40$, $p < .05$; P/S, S/S: $t(13) = 2.36$, $p < .05$). In contrast, rats whose mothers had SMD (S/S group) did not outweigh the pellet-fed offspring until 125 days of age (S/S vs. P/P: $t(11) = 2.24$, $p < .05$).

As with the females, males with SMD-fed mothers weighed less than males with pellet-fed mothers irrespective of diet after

TABLE 3

MEAN ORGAN WEIGHTS (g±SEM) FOR FEMALE OFFSPRING AT 132 DAYS OF AGE

	LIVER		KIDNEY		ADRENAL		UTERUS	
Pellet Weaning Diet:								
Mother on pellet	11.43* ±0.28	4.00[x] ±.18	2.04 ±0.07	0.70* ±0.02	0.053 ±0.004	0.019* ±0.002	0.958 ±0.156	0.35 ±0.06
Mother on SMD	10.14[x] ±0.75	3.95* ±0.17	1.77[x] ±0.06	0.70* ±0.06	0.056 ±0.002	0.023* ±0.002	0.710 ±0.071	0.280 ±0.034
SMD Weaning Diet:								
Mother on pellet	13.83[†] ±0.54	3.46[†] ±0.11	2.24[†] ±0.09	0.56[x] ±0.01	0.051 ±0.002	0.013[x] ±0.001	0.90 ±0.13	0.22 ±0.03
Mother on SMD	11.02 ±0.47	3.00 ±0.20	1.92 ±0.05	0.51[x] ±0.02	0.048* ±0.003	0.013[x] ±0.001	0.75 ±0.15	0.193[x] ±0.04

†, *, x = as defined in Table 2.

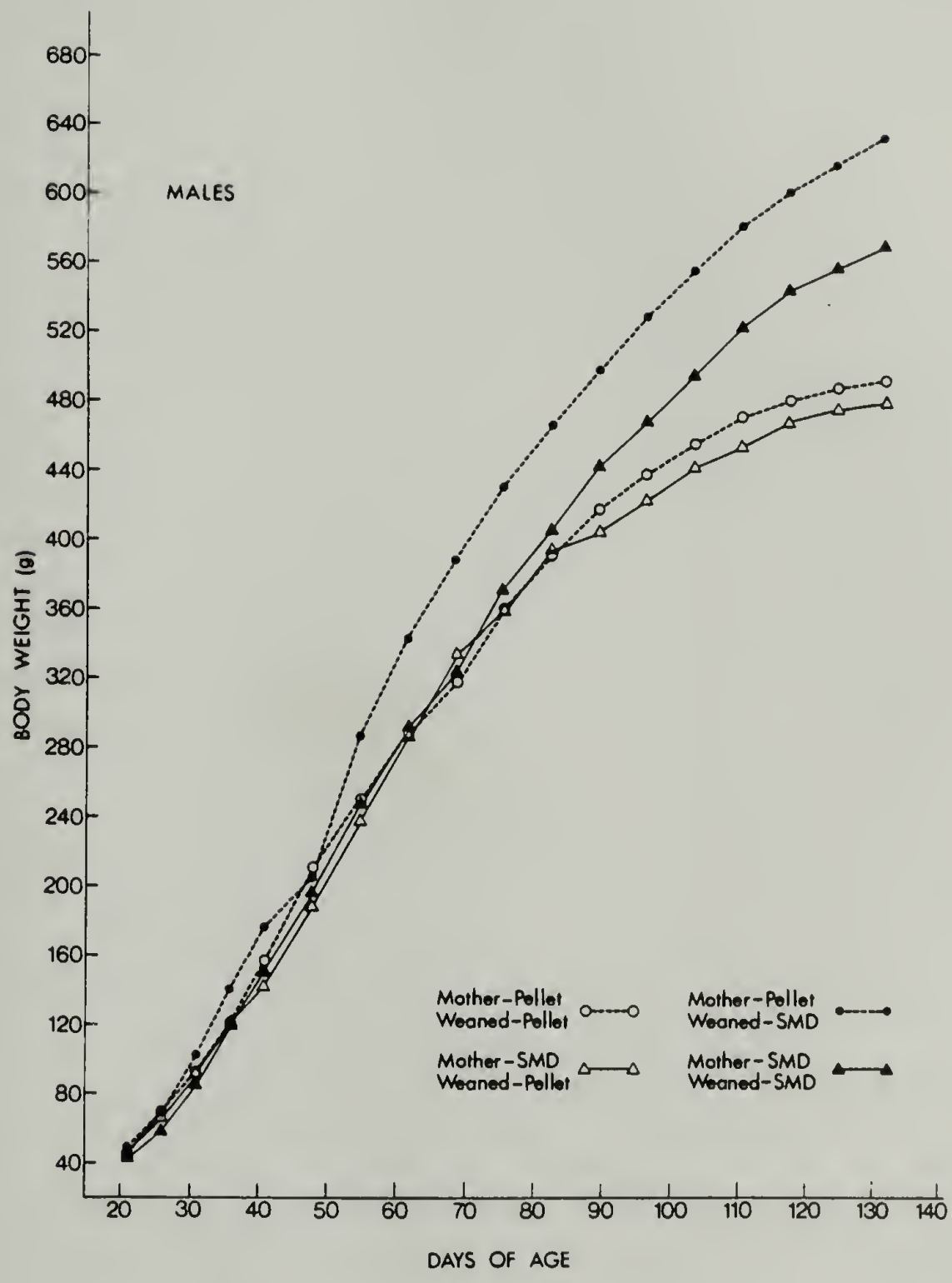

Fig. 11. Mean body weights of male offspring of pellet-fed mothers and of SMD-fed mothers provided ad libitum either a standard chow pellet diet or a SMD at weaning, 21 days of age.

weaning. These trends were never significant for the pellet fed offspring, and only briefly reached significance for the SMD fed offspring (Day 55 - $t(13) = 2.18$, $p < .05$; Day 69 - $t(13) = 2.91$, $p < .05$; Day 83 - $t(13) = 2.28$, $p < .05$).

Caloric intake.

(a) Total caloric intake. Male offspring weaned onto SMD consumed significantly more calories per day than offspring weaned onto pellets (Diet: $F(1,23) = 151.79$, $p < .05$ (Figure 12). Comparisons of mean daily caloric intake revealed no significant differences attributable to mother's diet.

(b) Diet component caloric intake. From weaning SMD-fed male rats consumed more calories as fat and carbohydrate than did pellet-fed rats (Fat: $F(1,23) = 298.30$, $p < .05$; Carbohydrates: $F(1,23) = 101.96$, $p < .05$) (Figure 13). Like females, males exposed to SMD ate fewer calories as protein than rats exposed to a pellet chow diet (Protein: $F(1,23) = 78.72$, $p < .05$), this difference in protein intake not emerging until approximately 48 days of age. No clear differences in diet component intake appeared as a result of mother's diet.

Feed efficiency. SMD-fed offspring exhibited lower feed efficiency than pellet-fed offspring from weaning until approximately 90-96 days of age (Diet: $F(1,23) = 168.39$, $p < .05$) (Figure 14). At this point until the experiment's conclusion, all male rat groups showed comparable feed efficiencies.

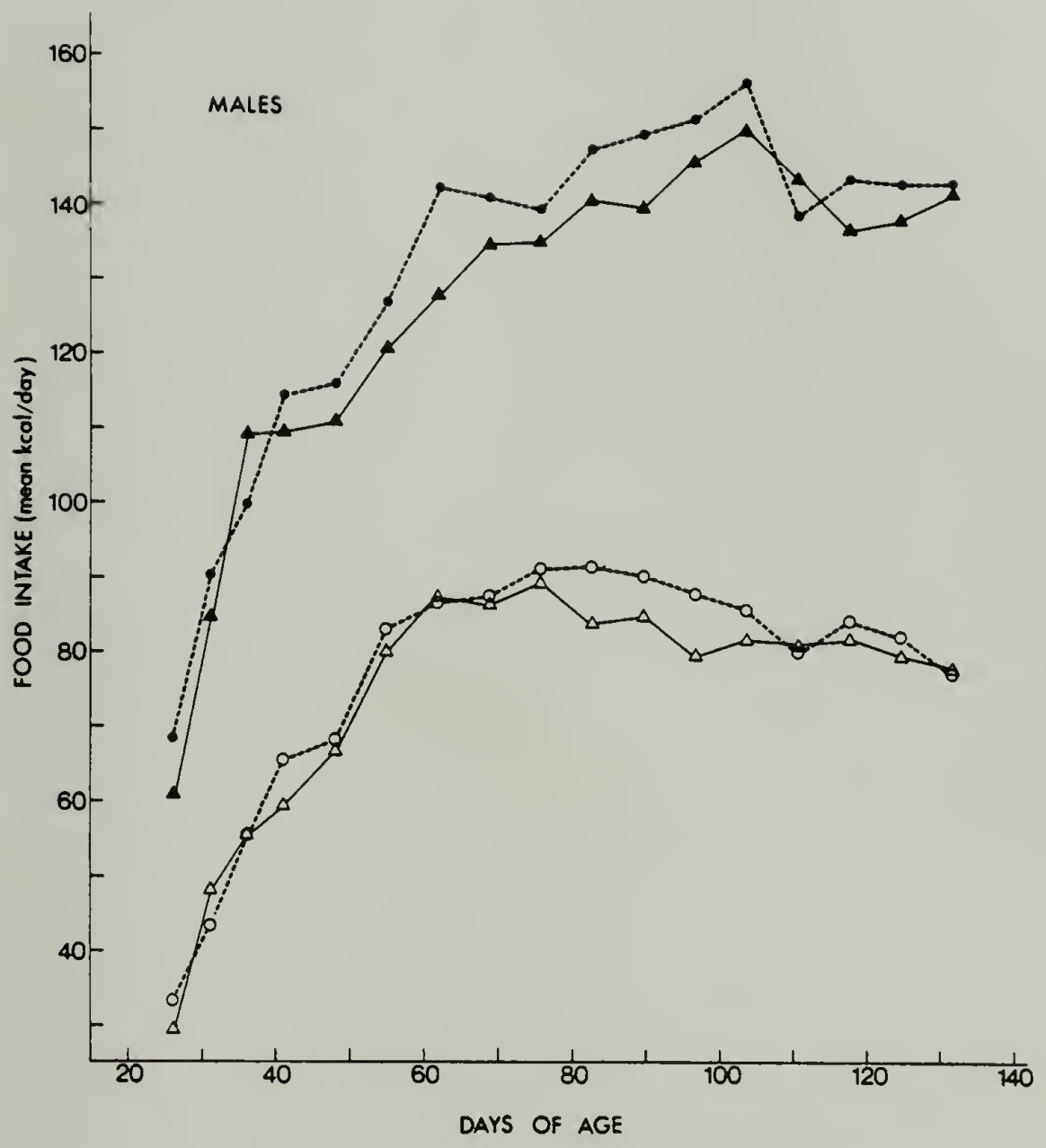

Fig. 12. Mean daily total caloric intake for male offspring of pellet-fed and of SMD-fed mothers weaned either onto a standard chow pellet diet or onto a SMD. Symbols are as indicated in Fig. 11.

Fig. 13. Mean daily component caloric intake for male offspring of pellet-fed mothers and of SMD-fed mothers placed either on a standard pellet diet or a SMD at 21 days of age. Diet component intake is expressed as mean daily protein, carbohydrate, and fat consumption. Symbols are as indicated in Fig. 11.

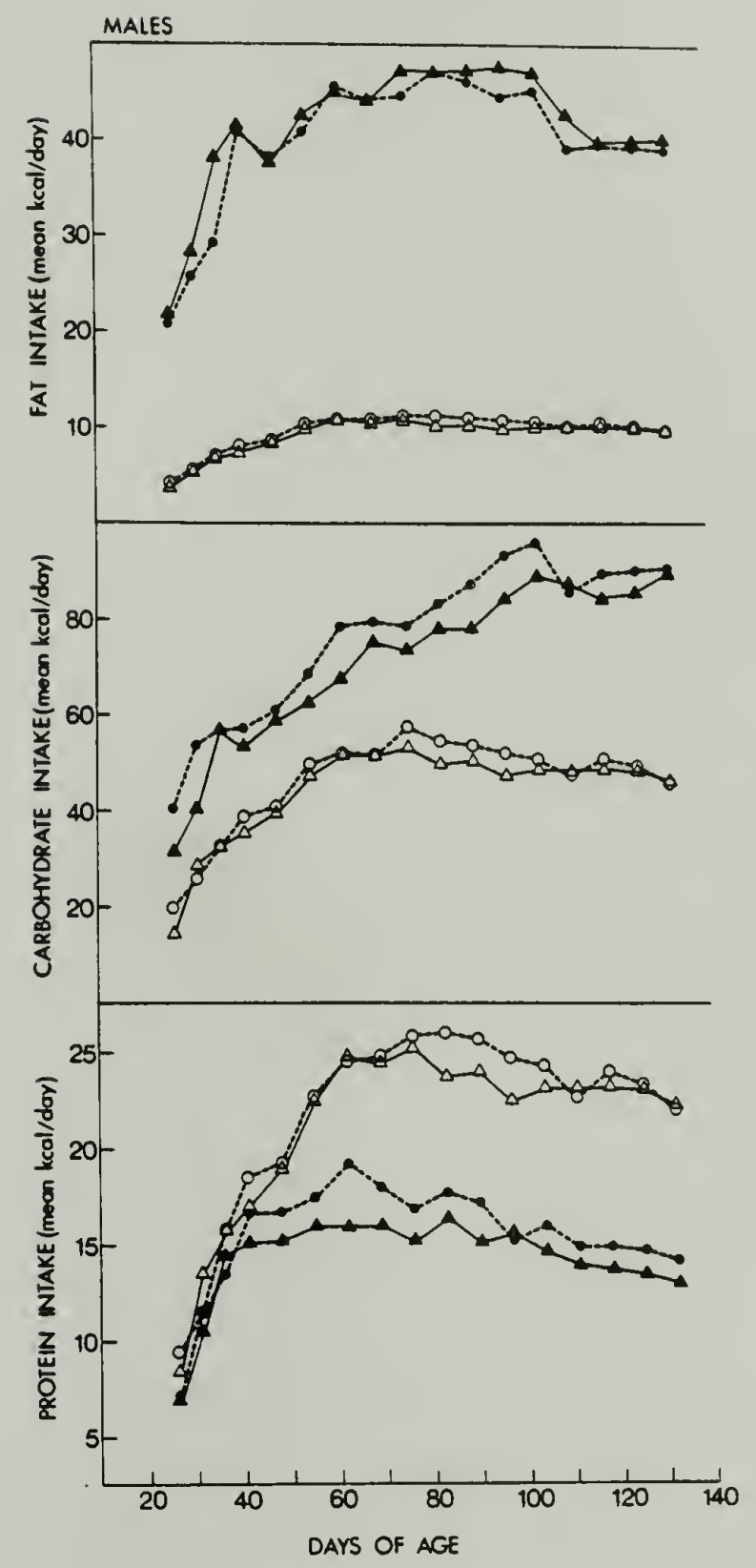
MALES
FAT INTAKE (mean kcal/day)
10
20
30
40
CARBOHYDRATE INTAKE (mean kcal/day)
20
40
60
80
PROTEIN INTAKE (mean kcal/day)
5
10
15
20
25
20
40
60
80
100
120
140
DAYS OF AGE

Figure 13

Fig. 14. Mean feed efficiency values expressed as grams body weight change per kilocalorie eaten for male offspring of pellet-fed mothers and of SMD-fed mothers provided either a standard pellet diet or a SMD at weaning. Symbols are as indicated in Figure 11.

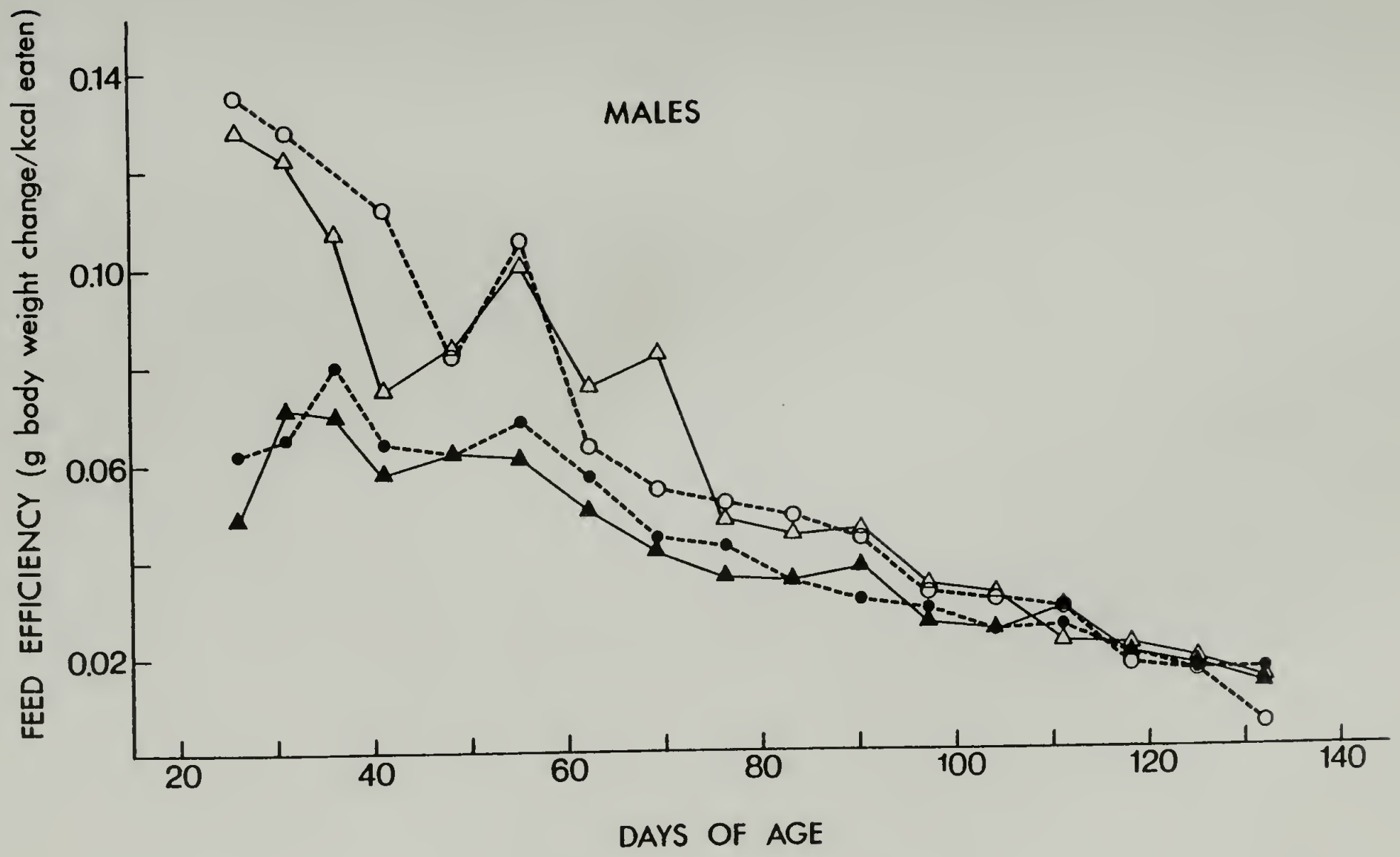
FEED EFFICIENCY (g body weight change/kcal eaten)
0.14
0.10
0.06
0.02
MALES
20
40
60
80
100
120
140
DAYS OF AGE

Figure 14

Naso-anal lengths and obesity index. No significant differences in offspring's naso-anal lengths resulted. As expected, SMD-fed offspring had higher Lee Obesity Index values than pellet-fed offspring (Offspring's diet: $F(1,23) = 23.06$, $p < .05$) (Table 4).

Carcass composition. As with females, male rats provided a SMD were composed of a higher percent lipid and lower percent water of wet carcass weight than rats provided a pellet diet, with no effect due to mother's treatment (Figure 8). For percent wet carcass protein, significant effects of mother's diet (higher in offspring of SMD-fed mothers) and offspring's diet (lower in SMD-fed rats) were present (Mother's diet: $F(1,23) = 12.58$, $p < .05$; Offspring's diet: $F(1,23) = 32.95$). However, no differences in absolute lean body mass existed (Figure 9).

Adipose tissue. Males maintained on SMD had larger adipose pads with larger adipocyte diameters than males maintained on pellets (Offspring's Diet: Retroperitoneal - pad weight, $F(1,23) = 45.40$, $p < .05$; Cell size, $F(1,20) = 31.43$, $p < .05$; Parametrial - pad weight, $F(1,23) = 41.40$, $p < .05$; cell size, $F(1,28) = 51.67$, $p < .05$). SMD exposure from weaning increased fat cell number in the male rats' retroperitoneal fat pads, but not in their epididymal fat pads (Retroperitoneal: $F(1,20) = 7.90$, $p < .05$). No significant adipose tissue effects were attributable to mothers' diet (Figure 15).

TABLE 4

MEAN NASO-ANAL LENGTHS (mm ± SEM) AND MEAN OBESITY INDICES (± SEM) OF MALE OFFSPRING AT 132 DAYS OF AGE

	NASO-ANAL LENGTH	OBESITY INDEX
MALES:		
Pellet Weaning Diet:		
Mother on pellet	257.6 ± 3.4	307.9 ± 3.3*
Mother on SMD	260.6 ± 1.9	330.5 ± 5.9*
SMD Weaning Diet:		
Mother on pellet	257.8 ± 2.1	303.8 ± 2.5†
Mother on SMD	256.4 ± 3.5	323.3 ± 3.3x

† differs from other mother treatment, same weaning diet treatment, $p < .05$.

* differs from same mother treatment, other weaning diet treatment, $p < .05$.

x differs from other mother treatment, other weaning diet treatment, $p < .05$.

Fig. 15. Mean adipose pad weight, adipocyte diameter, adipocyte number for retroperitoneal and parametrial adipose tissue sites in male rats from Experiment III. P/P, P/S, S/P, S/S are as represented in Fig. 9.

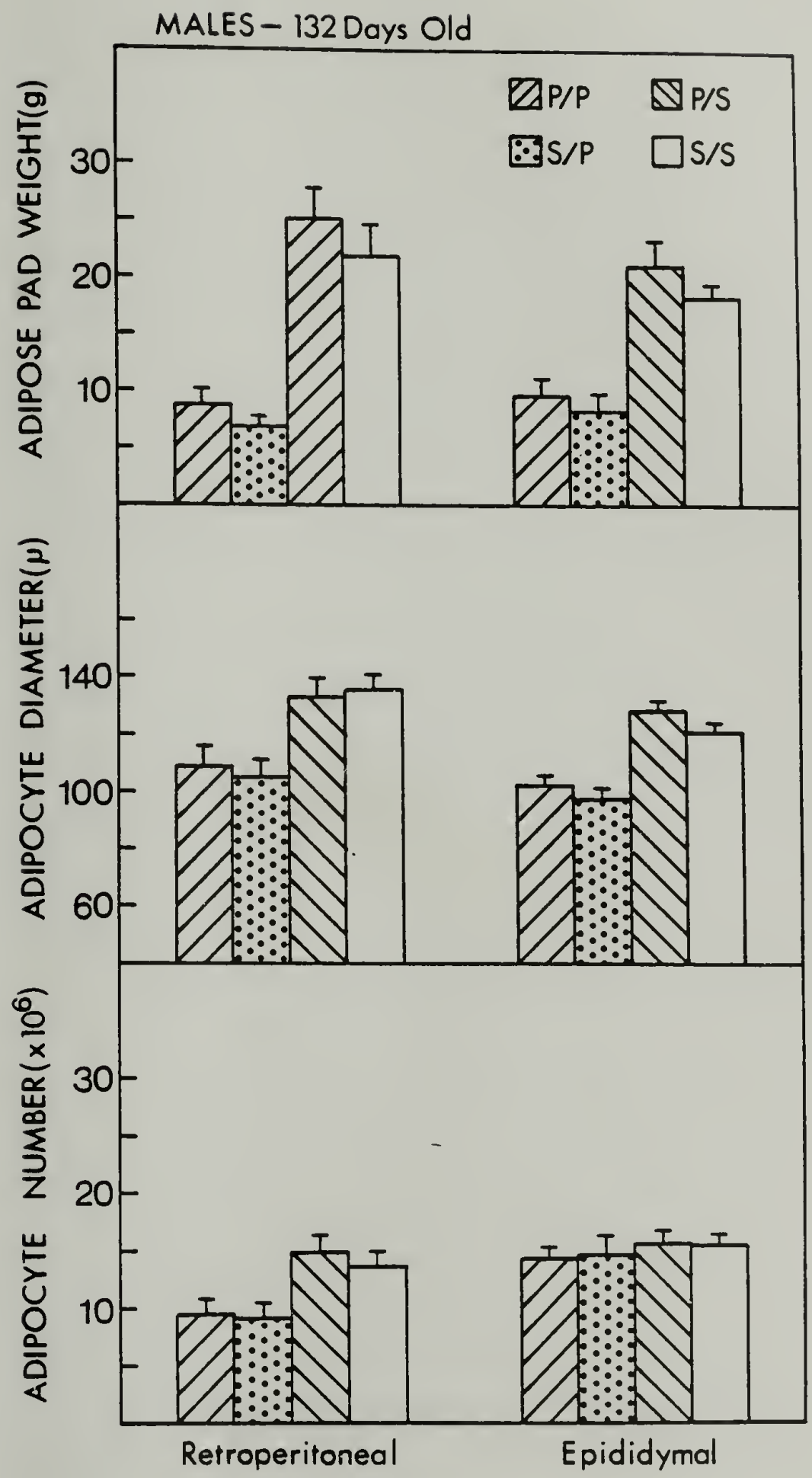
MALES – 132 Days Old
P/P
P/S
S/P
S/S
ADIPOSE PAD WEIGHT(g)
30
20
10
ADIPOCYTE DIAMETER(μ)
140
100
60
ADIPOCYTE NUMBER(x10⁶)
30
20
10
Retroperitoneal
Epididymal

Figure 15

Organ weights. No significant differences resulted for liver, kidney, and adrenal weights. Testes weights were influenced by mother's diet (Mother: $F(1,23) = 10.25$, $p < .05$), offspring of SMD-fed mothers having lighter testes, irrespective of post-weaning diet (Table 5).

TABLE 5

MEAN ORGAN WEIGHTS (g±SEM) FOR MALE OFFSPRING AT 132 DAYS OF AGE

	LIVER		KIDNEY		ADRENAL		TESTES	
Pellet Weaning Diet:								
Mother on pellet	19.21 ±1.74	4.49 ±0.68	3.47 ±0.22	0.82* ±0.13	0.044 ±0.006	0.009 ±0.002	3.90† ±0.21	0.93 ±0.17
Mother on SMD	18.29 ±0.49	3.78* ±0.17	3.32 ±0.11	0.69* ±0.03	0.051 ±0.004	0.010 ±0.001	3.33 ±0.09	0.69 ±0.03
SMD Weaning Diet:								
Mother on pellet	21.87 ±1.58	3.37 ±0.21	3.50 ±0.18	0.54x ±0.02	0.054 ±0.003	0.008 ±0.001	3.61 ±0.15	0.56* ±0.02
Mother on SMD	19.11 ±0.84	3.32 ±0.13	3.01 ±0.14	0.52 ±0.02	0.047 ±0.002	0.008 ±0.001	3.22x ±0.12	0.56x ±0.03

†, *, x = as defined in Table 4.

C H A P T E R I I I

DISCUSSION

The early dietary manipulation performed here did produce effects on offspring, but these effects were not initially apparent. Excessive SMD-induced weight gain prior to and during pregnancy produced no striking perinatal effects on the offspring. There were no differences in number of offspring born, or pups' 2 day old body weights. However, differences between SMD-fed and pellet-fed mothers, and between their respective offspring were evident during the post-natal period. SMD-fed mothers lost weight during the lactation period, whereas pellet-fed mothers maintained an elevated weight. How or if this maternal post-natal effect implies differential effects on pups' lactational environment cannot be directly determined from these experiments. Nevertheless, some possible mechanisms are suggested and addressed from available findings.

SMD-fed mothers' offspring had a lower survival rate for the first half of the lactation period, but not the second half. Normally, standard diet fed mother rats maintain a chronic, elevated core temperature throughout the first two postpartum weeks, making them vulnerable to acute rises in body temperature as a consequence of huddling contact with their young (35, 36). This thermal mechanism serves as a signal to induce mothers to terminate litter

contact bouts. Recently, diet-induced thermogenesis, its main site of action being brown adipose tissue (BAT), has been identified as a mechanism for enhanced energy or heat expenditure (28, 37, 38). Nursing mice have been found to have suppressed thermogenic activity in BAT, even though they exhibit the typical 'physiological hyperphagia' of lactation (39). Studies that have exposed overweight mothers to a varied palatable diet during lactation, as used here, report that dams do not exhibit even the typical nursing period hyperphagia seen in chow-fed dams (17, 18, 40). Perhaps in the present study an absence of lactational thermogenic suppression, or an alteration in BAT thermogenesis due to consumption of high energy palatable foods, resulted in excessive maternal heat expenditure. Hence, normal mother-infant temperature-dependent interactions may have been disrupted in SMD-fed mothers, in turn threatening the survival of their poikilothermic infants. Since pups are capable of thermoregulation at about 15 days of age (41), along with the ability to self-locomote and to actively seek their mother, the survival of SMD-fed mothers' offspring would, by this mechanism, not be as tenuous for the latter half of the lactational period.

Another preweaning effect of maternal SMD exposure was that by 10 days of age offspring of SMD-fed mothers weighed less than offspring of pellet-fed mothers. Despite their lower weight, these offspring had heavier fat pad weights at weaning. This effect may be due to changes in the amount or composition of SMD mothers' milk (16), in the levels of a hormone or of some micro- or macro-

nutrient essential for pups' growth, or in some other aspect of the post-natal environment. The enhanced fat deposition that had been present in their youth was not readily apparent in these animals as adults, nor was stunting of linear growth evident in the adults. Thus, the early SMD exposure does not potentiate an animal's later susceptibility to dietary obesity.

The influence of pre- and post-natal SMD exposure on other factors considered to coincide with the development of obesity also did not materialize. First, pre-weaning exposure to SMD produced no consistent effect on female or male offsprings' subsequent caloric intake.

Second, pre-weaning exposure to SMD did not alter the age of onset of excessive weight gain for the female offspring. Female offspring of both SMD-fed and pellet-fed mothers weaned onto a SMD showed a similar delayed onset beginning at 69 days of age. This was characterized by a rapid, fairly abrupt change in body weight. The onset of excessive weight gain in males with pre- and post-weaning SMD exposure was delayed beyond that of males with only post-weaning SMD exposure. SMD-fed males who had pellet-fed mothers began to outweigh pellet-fed controls at approximately 62 days of age; whereas, SMD-fed males who had SMD-fed mothers did not outweigh these controls until 125 days of age. Contrasting the onset pattern seen in females, the development of obesity in males was characterized by a gradual, more linear onset. Others have noted (2, 47) that the greater delay in males may be related to their faster baseline

growth rate. Protein intake was lower for all SMD-fed animals versus pellet-fed controls. Males, being comprised of more lean body mass than females, might be more sensitive to a low protein diet. It has been shown that males provided a low protein diet along with sucrose versus a low protein diet alone will exhibit a flattened growth curve (12). However, others find that young animals with dietary components sufficient for growth available will not choose less adequate, palatable calories at the expense of compromising growth (8, 10, 11). In the present study, all groups displayed comparable protein intakes at early stages of development, separations in intake coming later.

The nature of the delayed onset of obesity evident for both female and male weanlings is suggested by the animals' feed efficiency data. SMD-fed rats had a less efficient weight gain for the first 9 (females) or 12 (males) weeks after weaning versus pellet-fed rats. In other words, although consuming more calories from the start of weaning than pellet-fed rats, SMD-fed weanlings weighed no more than their pellet-fed counterparts. Enhanced energy expenditure by increased BAT activity is associated with low efficiency in weight gain. Other studies also suggest an age-related component to diet-induced thermogenesis, younger rats possessing an enhanced ability (43, 44). Also, some constituent mechanisms of BAT activity associated with weanling diet-induced thermogenesis diminish and approach adult levels with age (45, 46).

These age-dependent effects may explain the delayed onset of excessive weight gain seen in weanlings exposed to a high caloric diet.

Finally, maternal SMD feeding prior to weaning did not alter adult adiposity or adipose tissue morphology effects produced by post-weaning diets. For females and males offered the same diet, the offspring of SMD-fed mothers did not differ significantly in obesity index values, carcass lipid content, white adipose tissue weights, or adipocyte size and number from offspring of pellet-fed mothers at 132 days of age.

Unlike some pre-weaning dietary manipulations that potentiate post-weaning weight gain (19, 20), food intake (13, 19), or adipose tissue cellularity (21, 22, 25), pre- and post-natal exposure to a supermarket-type diet does not increase an animal's subsequent susceptibility to obesity. This implies that post-weaning nutritional factors are dominant in the development of this form of obesity.

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