Ontogenies of hypothalamic-, dietary-, and ovariectomy-induced obesities.

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ONTGENIES OF HYPOTHALAMIC-, DIETARY-, AND OVARIECTOMY-INDUCED OBESITIES

A Dissertation Presented

By

EARL L. SIMSON

Submitted to the Graduate School of the University of Massachusetts in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

September 1982

Department of Psychology
ONTGENIES OF HYPOTHALAMIC-, DIETARY-, AND OVARIECTOMY-INDUCED OBESITIES

A Dissertation Presented

By

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ACKNOWLEDGEMENTS

Numerous people have helped to make this dissertation possible. First and foremost, my thanks to my advisor and friend Dick Gold. His support and encouragement made the hard things easy. Thanks are also due to my committee, Drs. John Donahoe, Mark Friedman and Gordon Wyse, whose help, understanding and suggestions have made this a better dissertation.

The help and advice of Dr. George Wade is also acknowledged. His generous lending of equipment and supplies made many aspects of this research possible. Drs. Walter Kroll and Priscilla Clarkson are also thanked for the use of their equipment for fat cell analyses.

Many thanks are due to: Jay Alexander, for data collection and histology; Sue Anne Assimon, for the male data in Chapter III; Alan P. Jones, for teaching and performing numerous fat cell analyses; Mark J. Schwarz, for data collection, surgical assistance, fat cell analyses and letting me play advisor; and Chris Decoteau, for her expert typing of this dissertation. Thanks also to Paul Sawchenko and Ricardo Eng, whose helpful advise and lively discussions prior to and during this dissertation are greatly appreciated.
ABSTRACT

Ontogenies of Hypothalamic-, Dietary-, and Ovariectomy-induced Obesities

September 1982

Earl L. Simson, B.S., University of Massachusetts
M.S., University of Massachusetts, Ph.D. University of Massachusetts

Directed by: Professor Richard M. Gold

These studies look at the development of obesity using three animal models: hypothalamic-, dietary-, and ovariectomy-induced obesities. In Chapter I the delayed obesity seen by others in high-fat diet fed weanling rats given hypothalamic knife-cuts was explored. Young rats fed a standard low fat diet following hypothalamic knife-cuts became overweight without delay. Adult rats given knife-cuts comparable to those in the weanlings gained weight more rapidly and achieved higher weights than did those cut as weanlings. Chapter II demonstrates that as percent of calories from fat in the diet increases, the length of delay between surgery and obesity also increases, adding support to the hypothesis that dietary fat is a controlling factor in the development of weanling hypothalamic obesity.

Dietary factors in the development of obesity in non-brain-lesioned rats were investigated in Chapter III. Weanling rats given a supermarket diet in addition to lab chow showed delayed excess
weight gain. Rats started on the supermarket diet as adults gained excess weight immediately. Removal of the supermarket diet in adulthood resulted in rapid weight loss regardless of the age of diet introduction. Reintroduction of the diet resulted in even more rapid weight gains. In Chapter IV the additivity of dietary- and ovariectomy-induced obesities was investigated. Supermarket diet and/or ovariectomy administered to weanling rats produced delayed excess weight gain. But different lengths of delay, carcass and adipocyte analyses indicated separate mechanisms for the two obesities. Same treatments administered to adult rats revealed no delay in the expression of obesity, but again carcass and adipocyte analyses showed different profiles.

Chapter V examines the effect of ovariectomy on adipocyte number. Ovariectomy decreases adipocyte number in the parametrial fat pad, while disinhibiting adipocyte number in the retroperitoneal fat pad. Chapter VI discusses the hypothesis that common mediators, involving the development of lipoprotein lipase, the insulin to glucagon ratio, and thermogenesis, may be found among the three models.
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CHAPTER I
HYPOTHALAMIC KNIFE-CUT HYPERPHAGIA
AND OBESITY IN WEANLING RATS

In adult rats, electrolytic lesions of the ventral-medial, hypothalamus (VMH) produce hyperphagia (Brobeck, Tepperman and Long, 1943) and obesity (Kennedy, 1957). Parasagittal knife cuts alongside the medial hypothalamus also produce hyperphagia and obesity (Gold, 1970; Gold, Jones, Sawchenko and Kapatos, 1977; Sclafani, Berner and Maul, 1973). In weanling rats VMH lesions do not initially produce a sustained hyperphagia or excessive weight gain. Only after several weeks of delay do excessive weight gains commence (Bernardis and Skelton, 1966; Bernardis and Skelton, 1967; Han, Lin, Chu, Mu and Liu, 1965; Kennedy, 1957).

Many theories have been proposed to explain this delayed hyperphagia and weight gain. A stunting of linear growth has been noted in VMH lesioned weanlings (Bernardis and Skelton, 1966; Bernardis and Skelton, 1967; Han, Lin, Chu, Mu and Liu, 1965; Kennedy, 1957) such that for their size the weanlings have increased adiposity. Consistent with this is the finding that hypophysectomized VMH lesioned animals gain weight more rapidly than hypothalamically intact hypophysectomized animals (Kurtz, Rozin and Teitelbaum, 1972), suggesting growth hormone deficiency may hold down the weight but not the obesity of VMH lesioned weanlings. However, in weanling
rats parasagittal hypothalamic knife cuts, which do not stunt linear growth, also elicit a delayed hyperphagia and delayed excessive weight gain (Gold, Ieni and Simson, 1977; Gold and Kapatos, 1975; Ieni and Gold, 1977). The occurrence of this delayed weight gain free of stunted linear growth confirms that growth hormone deficiency does not mediate the delayed onset of adiposity in weanlings. Indeed, in adult rats parasagittal knife cuts produce both adiposity and enhanced linear growth (Gold, Jones, Sawchenko and Kapatos, 1977).

Another explanation has been that the onset of hypothalamic obesity awaits some maturational event correlated with puberty (Gold, Ieni and Simson, 1977; Gold and Kapatos, 1975). However, the delayed weight gain does not precisely coincide with puberty. Furthermore, Gold et al. (Gold, Ieni and Simson, 1977) noted that individual rats began to gain weight immediately after surgery while others showed delays. Since the rats used for that study were supplied not by a commercial breeder, but were a gift from a colleague's colony, it suggested to us that some social factor such as housing conditions might mediate the onset of hypothalamic hyperphagia and excessive weight gain.

Group vs individual housing has a wide range of physiological and behavioral effects (Brain and Benton, 1979). Group housing can also restore sexual activity in male rats brain lesioned at 28-32 days of age (Twiggs, Popolow and Gerall, 1978). We therefore decided to investigate the effects of group vs single housing conditions on the development of the hyperphagia and excess weight gains of knife
cut weanlings.

Comparing across our own studies we typically find that rats knife cut as weanlings never gain weight as rapidly or attain weights as great as do rats knife cut as adults (4.8-5.5 g/day for knife cut weanlings vs 8.8-12.0 g/day for knife cut adults). This difference in the net effect of weanling vs adult administered knife cuts requires within-study verification. Age at surgery was therefore the second manipulation of the present study.

Method. Forty-eight Charles River CD female weanling rats were received at 21 days of age. They were housed singly (n = 24) or 2 per cage (n = 24) in stainless steel wire mesh hanging cages (18 X 35 X 20 cm), with Purina lab chow pellets and tap water available ad lib. At times and for reasons noted in Results, the rats were switched to the less palatable Purina lab chow powder or a more palatable high fat diet (Corbit and Stellar, 1964). Both single and group housed rats were assigned to 3 surgical conditions; control, weanling knife-cut, or adult knife-cut. For the group-housed rats there was one of each surgical type per cage. Sham surgery was not performed upon the controls, but all rats were anesthetized for naso-anal length measures at the age at which weanling and adults surgeries were performed. Sham surgery, which would include drilling of the skull, was avoided in the weanlings in order to maintain maximum flexibility in determining which rats would be knife cut as adults. We have found that reoperation through an old skull hole is
difficult and inaccurate. We have also repeatedly found that sham surgery has at most a transient effect upon weight gain, and in a direction opposite to the overeating produced by knife-cuts.

Eight single-housed and eight group-housed 24 day-old weanlings received bilateral parasagittal retracting wire knife cuts (Gold, 1970) ("weanling-cut" rats). Under ether anesthesia the guide shaft was lowered to an estimated 1.0-1.5 mm from the base of the brain at anterior +6.0 mm (earbar zero), lateral ± 0.8 mm (mid-sagittal sinus zero). The cutting wire was then extended 2.0 mm caudally and the assembly was lowered to the base of the brain, raised 2.8 mm, and finally lowered to the point at which the wire had been extended. The wire was then retracted and the guide shaft withdrawn. Naso-anal lengths (NAL) were taken for all 48 rats at this time. Obesity indices (Bernardis and Patterson, 1968) were computed by the formula:

\[
\text{Obesity index} = \left(10^4\right) \frac{\sqrt[3]{\text{Body weight (g)}}}{\text{Naso-anal length (mm)}}
\]

Eight single housed and eight group housed rats received comparable bilateral parasagittal knife cuts as 57 day old adults ("adult-cut" rats). Their coordinates were anterior +8.4 mm, lateral ± 0.9 mm, wire length 3.0 mm, and depth of cut 3.0 mm from the base of the brain. At this time naso-anal lengths were again determined for all rats. Naso-anal length measurements were repeated at 144 and 288 days of age. To alleviate the crowding of 3 adult rats per cage the differential housing conditions were terminated
at 99 days of age. Food intakes were measured, correcting for spillage, at 4-7 day intervals for all singly housed rats from 24-99 days of age.

Frozen sections cut at 40 μ in the horizontal plane were stained with cresyl violet. Knife cut placements were reconstructed using a projecting microscope without reference to age at surgery. Analysis of variance and Newman-Keuls post-hoc comparisons were used to test for significance.

Results.

Housing conditions. Contrary to our expectations, single versus group housing did not differentially influence weight gain in knife cut or sham operated weanling or adult rats (Figure 1 and Table 1). Since there were no significant housing effects data have been collapsed for subsequent statistical purposes.

| TABLE 1 |
| WEIGHT GAIN (GRAMS/RAT) ± SEM |
|------------------|------------------|------------------|
|                  | 23-56 days old   | 57-99 days old   |
| Weanling Cut     |                  |                  |
| Single           | 180.0 ± 12.14*   | 98.1 ± 9.97*†    |
| Group            | 183.5 ± 11.60*   | 101.0 ± 12.15*†  |
| Adult Cut        |                  |                  |
| Single           | 120.6 ± 2.61     | 246.8 ± 23.38*   |
| Group            | 115.5 ± 2.42     | 218.6 ± 18.54*   |
| Control          |                  |                  |
| Single           | 124.0 ± 5.27     | 64.7 ± 3.31      |
| Group            | 117.3 ± 5.28     | 57.6 ± 2.92      |

*Differs from controls p < 0.01.
†Differs from adult-cut p < 0.01.
Fig. 1. Mean body weight after the age of surgery at weaning. Differential housing conditions were terminated on day 99.
**Weight gains.** Also unexpectedly, both weanling knife-cut groups had immediate excessive weight gains such that by 27 days of age (3 days after surgery) they had already gained significantly more weight than the control groups (22.0 ± 2.08 g vs 11.5 ± 1.38 g, p < 0.01). The differences between the weanling cut and control groups continued to increase and the early excessive weight gain was shared by all of the knife cut weanlings. Similarly, the adult knife-cut animals outweighed the control groups by 4 days after surgery (180.13 ± 4.95 g vs 126.57 ± 4.40 g, p < 0.01). The adult knife cut rats continued to gain weight rapidly, catching up to (at 68 days of age, 11 days after surgery) and then significantly exceeding (at 83 days of age) the weanling knife-cut groups (306.88 ± 12.01 g adult knife-cut vs 261.33 ± 9.90 g weanling knife-cut, p < 0.01).

To determine whether the adult knife-cut rats were more sensitive to orosensory properties of their diet, at 144 days of age all the rats were switched for 12 days from Purina lab chow pellets to Purina lab chow powder. Over the 12 day period on the powder, the adult-cut rats lost weight (2.08 ± 0.84 g/day/rat) such that their weight change on powder differed significantly from that of the weanling-cut groups which gained 0.14 ± 1.26 g/day/rat and the controls who lost only 0.03 ± 0.10 g/day/rat (p's < 0.01). Sensitivity to diet can increase with obesity (Franklin and Herberg, 1974; Peters, Luttmers, Gunion and Wellman, 1978), and the adult cut rats were more obese at 144 days (Table 2). To control for this factor we repeated
Table 2

Obesity Index (± SEM) at Four Ages

<table>
<thead>
<tr>
<th>Group</th>
<th>24 days</th>
<th>57 days</th>
<th>144 days</th>
<th>288 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weanling-cut</td>
<td>310.5 ± 2.79</td>
<td>313.9 ± 2.61*+</td>
<td>334.1 ± 3.11*+</td>
<td>377.1 ± 6.23*†</td>
</tr>
<tr>
<td>Adult-cut</td>
<td>310.2 ± 1.36</td>
<td>299.4 ± 1.76</td>
<td>360.7 ± 5.71*</td>
<td>396.0 ± 5.41*</td>
</tr>
<tr>
<td>Controls</td>
<td>310.9 ± 2.06</td>
<td>302.7 ± 2.19</td>
<td>306.81 ± 3.19</td>
<td>327.9 ± 3.38</td>
</tr>
</tbody>
</table>

*Differs from controls p < 0.01.
†Differs from adult-cut p < 0.05.
‡Differs from adult-cut p < 0.01.

The powder test when the weanling-cut group had reached the mean body weight the adult group had had at the first powder testing (438.1 g weanling knife-cut at age 233 days, 437.4 g adult knife-cut at age 144 days). All groups were placed on powder on day 233 for 28 days. Once again, the weanling-cut group gained weight (0.41 ± 0.30 g/day/rat), while the adult-cut group lost (0.50 ± 0.33 g/day/rat). The control group gained 0.32 ± 0.15 g/day. However, these differences did not reach significance and an apparent prior housing condition effect, of dubious replicability, appears at this late stage for the weanling knife-cut rats. Following the second course on the powder diet all animals were given a high-fat diet (Corbit and Stellar, 1964). For reasons not clearly understood, high-fat diets accelerate hypothalamic obesity after VMH lesions (Corbit and Stellar, 1964) or knife cuts. Over the 27 day period of access to this diet the weanling-cut group
and the adult-cut rats did not differ from each other, gaining 5.61 ± 0.41 and 6.43 ± 0.39 g/day/rat respectively. Both of these gains were significantly greater than those of the controls (2.49 ± 0.31 g/day/rat).

Food intake. Food intakes, recorded for single-housed rats up to 99 days of age (when housing conditions were changed), are shown in Figure 2. The food intakes reflect the body weight changes. At all points measured the knife-cut rats ate more than the controls (p's < 0.01), and excepting the last week, the adult-cut rats ate more than the weanling cut rats (p's < 0.01) after 57 days of age.

Naso-anal lengths and obesity indices. Consistent with the body weight data, by 57 days of age the weanling knife-cut rats were significantly longer and more obese than the unoperated rats (Tables 2 and 3). Thus, neither obesity nor enhanced linear growth were delayed. By day 144 and again at day 288 both the adult and weanling-cut rats were more obese than the controls. The adult-cut rats were more obese than the weanling-cut rats, again paralleling their weights. The two knife-cut groups did not differ from one another in naso-anal lengths at 144 or at 288 days of age.

Histology. Reconstructions of the knife cut placements of 10 rats are seen in Figure 3. No systematic differences in the location or extent of the knife cuts were seen between weanling and adult knife-cuts. The cuts of the weanling-cut rats with the greatest weight gains were virtually coextensive with those of the adult-cut rats with the greatest weight gains (Figure 3A). Similarly, the least effective
Fig. 3. Bilateral parasagittal knife cuts superimposed upon a horizontal section of the brain. A shows the placement of knife cuts in the rat with the greatest weight gain in weanling-cut (dashed line) and adult-cut groups (solid line) respectively. B shows the placement in the rat with the least (but still elevated) weight gain in the weanling-cut (dashed line) and adult-cut (solid line) groups respectively. C–E each show the placement of two histologically equivalent knife cuts, one weanling cut (dashed line) and one adult knife-cut (solid line). In each case the growth curves were representative respectively for the weanling cut and adult cut groups. Abbreviations: pom, medial preoptic nucleus; ha, anterior hypothalamic nucleus; fp, paraventricular nucleus, parvocellular; TCHL, lateral corticohabenular tract; TOHL, lateral olfactohabenular tract; F, fornix; FMP, medial forebrain bundle; fm, paraventricular nucleus, magnocellular; ZI, zone incerta; FMT, mammillothalamic tract; hdd, dorsomedial hypothalamic nucleus, dorsal.
### TABLE 3

**MEAN NASO-ANAL LENGTHS (mm ± SEM) AT FOUR AGES**

<table>
<thead>
<tr>
<th>Group</th>
<th>24 days</th>
<th>57 days</th>
<th>144 days</th>
<th>288 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weanling-cut</td>
<td>123.3</td>
<td>197.5</td>
<td>218.3</td>
<td>229.2</td>
</tr>
<tr>
<td></td>
<td>± 1.4</td>
<td>± 5.8†</td>
<td>± 1.8*</td>
<td>± 2.78</td>
</tr>
<tr>
<td>Adult-cut</td>
<td>125.2</td>
<td>187.3</td>
<td>218.9</td>
<td>227.5</td>
</tr>
<tr>
<td></td>
<td>± 1.3</td>
<td>± 1.2</td>
<td>± 1.6*</td>
<td>± 1.71</td>
</tr>
<tr>
<td>Controls</td>
<td>123.3</td>
<td>185.3</td>
<td>212.1</td>
<td>223.3</td>
</tr>
<tr>
<td></td>
<td>± 0.7</td>
<td>± 2.1</td>
<td>± 2.1</td>
<td>± 1.49</td>
</tr>
</tbody>
</table>

*Differs from controls p < 0.05.
†Differs from controls p < 0.01.

(but still in excess of control values) cuts in each group match up closely (Figure 3B). Three other pairs of cuts, one member of each pair from each group, matched up perfectly (Figures 3C, D, E). The remaining cuts were similar to one another as well.

**Discussion.** This is the fourth paper from this laboratory dealing with delayed hyperphagia and weight gain following hypothalamic knife cuts in weanling rats. The first paper reported delayed onset of hyperphagia and weight gain beginning only after 8 weeks of age in female Carworth CFE rats (Gold and Kapatos, 1975). The second paper also demonstrated a delayed weight gain, this time in Charles River CD female rats with intact pituitaries (Ieni and Gold, 1977), but in the third study we found that only 2/3 of the knife-cut weanling Charles River CD derived females showed delayed weight gain. One third of the animals demonstrated excessive weight gains from
the time of surgery at the age of 24 days (Gold, Ieni and Simson, 1977). The rats used in that study were supplied not by a commercial breeder but had been born in a rat colony at the University of Massachusetts. This led us to believe that handling or other social interactions were responsible for the lack of delay. However, handling proved not to be a cause (Gold, unpublished observations), nor, as seen in the present paper, did group vs single housing have a differential effect on the age of development of excessive weight gains.

While the main dependent variable in the present study (housing conditions) proved to be undifferentiating, there was the unexpected finding of no delay of weight gains in all knife-cut weanlings, both group and single-housed. If linear growth had been stunted by our knife cuts, then one could have suggested that these rats were functionally hypophysectomized, and hypophysectomized knife-cut weanling rats do show early obesity (Ieni and Gold, 1977). Such was not the case, but early enhanced linear growth, which was also evident at 57 days of age, does indicate that our knife cuts did modulate pituitary function. In our previous three reports increases in naso-anal length had also occurred, but they had been delayed (Gold, Ieni and Simson, 1977; Gold and Kapatos, 1975; Ieni and Gold, 1977). Even with corrections for the enhanced linear growth of the weanling knife-cut group, there was still early obesity according to the 'Lee Index' (Bernardis and Patterson, 1968). Similarly, the onset of excess food intake was delayed in the
three previous studies, but not in the present data.

An explanation for the present lack of delay (and for the previous lack of delay in 1/3 of subjects (Gold, Ieni and Simson, 1977)) in terms of subtle selective interruption (or sparing) of neurocircuitry that was not previously interrupted (or spared) defied careful comparison of the knife cuts across this series of studies. The placement and extent of the knife cuts of the present report did not differ noticeably from that which we have seen previously.

We may be seeing some genetic change over the years towards rats that grow more rapidly, especially in response to obesity producing brain surgery or palatable diets. Indeed, commercial breeders typically select breeding stock that grow rapidly. It has been just such a trend that prompted us to switch from the high fat diet of our previous studies to the present chow diet, as even the controls started to gain rapidly on the high fat diet (e.g., see Figure 1 of the present report).

One of the major differences between the present study and the previous ones is in the diet that we offered. The three earlier studies used a high-caloric high-fat diet (61% fat calories, 2 parts Purina lab chow:1 part vegetable shortening, 5.4 kcal/g) while in the present study the rats were given a standard (12% fat calories) lab chow diet (Purina pellets 3.6 kcal/g). The critical effect of the fat content of the diet on the development of hypothalamic hyperphagia has recently been verified by us within the
confines of one study (in preparation).

Despite histologically equivalent knife cuts, the adult knife-cut animals of the present study were initially over 50% more hyperphagic than the weanling-cut rats, and the adult-cut rats continued to out-gain the weanling-cut rats throughout the period of observation. The two groups also showed differential body weight responses to diet manipulations. The adult-cut rats were finicky when placed on a powdered diet, losing weight during two separate tests. The weanling-cut rats, comparable to the controls, continued to gain weight, albeit more slowly, when placed on the powdered diet. This finickiness does not appear to be weight-dependent. In contrast, the weanling-cut rats mimicked the adult-cut rats when placed on a high-fat diet. Thus, weanling-cut animals can gain weight on powder (like controls), but overrespond to a palatable diet (like adult knife-cut rats).

If, as it appears, the knife cuts were similar, yet the behavior different (weanling-cut vs adult-cut), then the weanling-cut rats may have enhanced recovery of function, and/or some brain circuitry may normally develop following the time of brain surgery. Recovery of function seems the less likely of the two, given the relatively constant but elevated food intake of weanling knife-cut rats over the course of the experiment.

The hypothesis of brain development occurring after surgery (and therefore spared by it) has more support. Brain water content decreases until 50 days of age (Himwick, 1973) as a result of
increases of cellular protein and deposition of myelin (Vernadakis and Woodbury, 1962). Brain protein levels do not reach mature levels until 40 days of age (Timiras, 1972), and myelination is not complete until 60 days of age (Jacobson, 1963).

We have shown that neither housing conditions (single vs group housing) nor the location of the obesifying knife cut can control the age of onset of obesity in knife-cut weanling female rats. We tentatively attribute the disparity between the earlier report of delayed obesity in knife cut weanlings and the present report of no delay to our switching from a high-fat diet to more conventional fare. The verification of this tentative conclusion and its implications will be the subject of a subsequent report.

We have also shown that the age of surgery can affect the degree of obesity produced, equivalent knife-cuts producing greater weight gains when placed in adults.
CHAPTER II
EFFECTS OF DIETARY FAT UPON HYPOTHALAMIC HYPERPHAGIA

Young rats are often resistant to obesity. For example, ovariec
tomy at birth or at weaning does not result in excessive food in-
take or excessive weight gain until after 6 weeks of age (Wade and
Zucker, 1970; Slob and Van der Werfften Bosch, 1975). Obesity due
to dietary manipulations is also delayed. Weanling rats fed a high-
fat diet do not begin to outweigh chow-fed controls until approxi-
mately 60 days of age (Lemonnier, 1972), and weanling rats given
access to a sucrose solution along with lab chow and water do not
begin to outweigh control animals until they are about 70 days of age
(Kanarek and Hirsch, 1977). Supermarket diet-induced obesity is sim-
ilarly delayed until after 8 weeks of age (Simson et al., in prepar-
ation). The earliest the obesity of the genetically obese Zucker
fatty rat can be externally detected is about 28 days of age, though
indicators of impending obesity can be found earlier (Bray and York,
1979).

Electrolytic lesions of the ventromedial hypothalamus of female
weanling rats do not produce hyperphagia or excessive weight gain
until seven weeks of age (Bernardis and Skelton, 1966; 1967; Han et
al., 1965; Kennedy, 1957). Parasagittal hypothalamic knife cuts
also elicit a delayed hyperphagia and delayed weight gain when the
rats are fed the traditional Corbit and Stellar (1964) high fat diet
(Gold and Kapatos, 1975; Gold et al., 1977; Ieni and Gold, 1977).
This does not mean that it is impossible to make a young rat obese. Hypophysectomy combined with either VMH lesions (Kurtz et al., 1972) or hypothalamic knife cuts (Ieni and Gold, 1977) produces precocious obesity. Also, we (Simson and Gold, 1981) have recently shown that hypothalamic knife cut weanling rats on a low fat/high carbohydrate Purina Lab Chow pellet diet exhibit immediate hyperphagia and excessive weight gain. We suggested that the critical variable responsible for the early obesity in that study might have been the low (4.5%) fat content of the Purina Lab Chow diet fed to the knife cut weanlings. We now confirm that hypothesis and demonstrate over a wide range of dietary percent fat that the age of onset of excessive weight after hypothalamic knife cuts is a function of dietary fat; the greater the fat content, the longer the delay.

Part 1

First a direct comparison was made between rats fed the standard Purina brand laboratory chow checkers (3.33 kcal/g; 4.5% fat, 11% of calories from fat) used in our recent study (Simson and Gold, 1981) vs the original Corbit and Stellar (1964) high fat diet (5.25 kcal/g; 36% fat, 61% of calories from fat) used in our earlier studies (Gold and Kapatos, 1975; Gold et al., 1977; Ieni and Gold, 1977).

Method. Eighteen Charles River CD female rats were received from the commercial breeder at 21 days of age. They were singly housed in stainless steel wire mesh (.85 cm between wires) hanging cages. Rats were randomly assigned to two diet groups. Purina Laboratory Chow
pellets were scattered loose inside the cage (n = 9), or high fat diet was packed as a cake in a 7 cm diameter glass bowl (n = 9). Tap water was available ad lib. to both groups. After an overnight adaptation to the diet, six rats from each group received bilateral parasagittal retracting wire knife cuts as previously reported (Simson and Gold, 1981). Under ether anesthesia the guide shaft was lowered to an estimated 1.0-1.5 mm from the base of the brain at anterior + 6.0 mm (from earbar), lateral ± 0.8 mm (from midsagittal sinus). The cutting wire was then extended 2.0 mm caudally and the assembly was lowered to the base of the brain, raised 2.8 mm, and finally lowered to the point at which the wire had been extended. The wire was then retracted into the guide shaft and the guide shaft withdrawn. The three remaining rats in each diet group were only anesthetized, and naso-anal lengths were taken for all rats at this time for computation of Lee (1929) obesity indices (Bernardis and Patterson, 1968). Food intake and body weight were taken twice weekly and naso-anal lengths were taken again at the end of the experiment.

Part 2

Having verified that delayed obesity occurred with the high-fat diet but not with Lab Chow, additional levels of fat were tried. A "medium" fat diet (4.0 kcal/g, 52% fat calories) or a "higher" fat diet (5.8 kcal/g, 67% fat calories) were used to further assess the effect of dietary fat on the development of hypothalamic obesity.

Method. Sixteen Charles River CD female weanling rats were received
and housed as above. Except as noted, all procedures were as above.
The rats were randomly assigned to one of two diet groups: medium fat
diet (n = 8) or higher fat diet (n = 8). The fat for the diets, as
with the Corbit and Stellar diet, was hydrogenated vegetable shortening, which was heated to melting and mixed with Purina Laboratory Chow
powder. The medium fat diet contained (by weight) 20% shortening, 20%
non-metabolizable petroleum jelly and 60% Purina powder; the higher fat
diet contained (by weight) 40% shortening and 60% powder. The petro-
leum jelly was added to the medium fat diet to ensure that it was as
greasy as the other fat diets (Corbit and Stellar type and "higher").
Five rats from each group received bilateral parasagittal knife cuts.
All animals were anesthetized for naso-anal length measurements at this
time, at 50 days of age, and at 90 days of age at which time the ex-
periment was terminated.

Part 3

Adding fat to Purina Lab Chow increases the fat content but also
dilutes the protein. Purina Lab Chow gets 26% of its calories from
protein, whereas the 52% (medium) fat diet gets 14%, the 61% fat
(Corbit and Stellar) diet 12%, and the 67% (higher) fat diet gets only
10% of its calories from protein. Since weanlings are growing rapidly,
the delayed excessive weight gain might be due to protein malnutrition.
To test this notion we used a synthetic diet with high fat and
adequate protein. The previous diets also differed in caloric density;
11% fat calories (Purina Lab Chow) contains 3.33 kcal/gm, 52% fat
(medium) contains 4.0 kcal/gm, 61% fat (Corbit and Stellar) contains
5.25 kcal/gm and 67% fat (higher) contains 5.8 kcal/gm. Thus, high fat went with high caloric density. To control for this, the caloric density of the synthetic diet was lowered to 3.3 kcal/gm, which approximates the caloric density of Purina Lab Chow.

**Method.** Eight Charles River 21 day old females were received and housed as before. They were given a diet that consisted of 20% of calories from protein (casein and methionine) and 75% of calories from fat (hydrogenated vegetable shortening). The remaining 5% of calories came from carbohydrate (cornstarch). Vitamins and salts were added to balance the diet. A caloric density of 3.3 kcal/g was achieved by the addition of cellulose. Following an overnight adaptation to the diet five of the rats received bilateral parasagittal knife cuts, as before. All animals were anesthetized to measure naso-anal lengths at 22, 90 and 110 days of age.

**Results.** A repeated measures analysis of variance of body weight revealed a significant days x treatment x diet effect (p < .001), indicating that the length of delay of obesity is affected by the diets. A finer analysis indicated that the knife cut animals fed the lab chow (11% fat) departed from their controls immediately (Figure 4, p < .01 at 29 days of age), as was found in our previous study (Simson and Gold, 1981). Those knife cut rats fed the Corbit and Stellar (61% fat) diet exhibited the delayed excess weight gain seen in three earlier studies (Gold and Kapatos, 1975); Gold et al., 1977; Ieni and Gold, 1977). These rats did not depart from their control group until after 50 days of age (Figure 4, p < .02 at 57 days of age). That the length
Fig. 4. Effects of diet (Purina Lab Chow pellets, 11% fat by calories, vs. Corbit and Stellar type high fat diet, 61% fat by calories) upon the body weight response to obesifying parasagittal hypothalamic knife cuts in weanling rats.
of delay is a function of the percent fat in the diet is confirmed in Parts 2 and 3 by the body weight curves of the 52%, 67% and 75% fat fed animals (Figure 5). Those rats receiving the 52% fat diet and knife cuts appeared to separate from their respective control group after 40 days of age (p < .02 at 47 days of age), whereas the 67% fat fed rats did not appear to separate from their controls until after 60 days of age (p < .02 at 71 days of age). The knife cut rats fed the 75% fat diet did not appear to separate from their control group until after 70 days of age, this despite adequate protein, but even by 110 days of age their weights did not significantly exceed those of their controls.

The food intake data show a similar delayed onset of excessive intake according to dietary fat. The knife cut chow fed rats of Part 1 showed an immediate and sustained hyperphagia (averaging 33% more food than control fed rats, Figure 6). This replicates our previous findings (Simson and Gold, 1981). The knife cut rats fed the Corbit and Stellar (61%) diet, although showing a slightly elevated caloric intake after 40 days of age, had a more rapid rise in food intake after 50 days of age resulting in a 70% excess food intake. Again, the effect of dietary fat can best be seen in Parts 2 and 3 where other fat diets are included. Figure 7 shows a pattern similar to the body weight data. The food intakes of the knife cut rats fed a 52% fat diet, 61% fat diet or 67% fat diet appear to depart from their respective controls after 40, 50 and 60 days of age. The knife cut rats fed the 75% fat diet throughout the length of the study never ate more than their respective controls, mimicking the body weight data.
Fig. 5. As in Figure 4, but using 3 additional diets; 52, 67 and 75% fat by calories. The 61% fat diet data are repeated to facilitate comparisons.
Fig. 6. Food intake data for Figure 4 rats.
Fig. 7. Food intake data for Figure 5 rats.
The obesity indices also parallel the body weights and food intakes (Table 4). Obesity indices of the knife-cut rats fed the 52% fat diet were increased at 50 days of age \((p < .01\) t-tests) compared to control values. However, the knife cut rats fed the 67% fat diet, who were not heavier than controls at 50 days of age, also did not have increased obesity indices at that age. By 90 days of age all the knife cut rats, except those on the 75% fat diet, had increased obesity indices compared to controls \((p's < .01)\). The 75% fat fed rats never had elevated obesity indices.

Histology on the knife cut rats revealed placements similar to those previously reported (Simson and Gold, 1981). Knife cut placements were lateral to the paraventricular nucleus (PVN) of the hypothalamus, and extended from above the PVN to the base of the brain.

Discussion. We have replicated within one study our earlier findings that knife cut weanlings maintained on a low fat (11% of calories from fat)/high carbohydrate diet respond to the brain surgery immediately, whereas knife cut weanlings maintained on a Corbit and Stellar (1964) high fat diet (61% of calories from fat) delay excess weight gain until after 7 weeks of age (Simson and Gold, 1981; Gold and Kapatos, 1975; Gold et al., 1977; Ieni and Gold, 1977). We have also shown, through the use of the 52%, 67% and 75% fat calorie diets, that the length of delay is consistent with the percentage of fat calories in the diet. The greater the percentage of fat calories, the longer the delay. This length of delay is not dependent on the low protein content of the diet, nor the high
<table>
<thead>
<tr>
<th>Age (Days)</th>
<th>Diet (% of calories)</th>
<th>11% fat</th>
<th>52% fat</th>
<th>61% fat</th>
<th>67% fat</th>
<th>75% fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>KC Control</td>
<td>KC Control</td>
<td>KC Control</td>
<td>KC Control</td>
<td>KC Control</td>
</tr>
<tr>
<td>22</td>
<td></td>
<td>314.6 ± 2.5</td>
<td>317.6 ± 6.6</td>
<td>312.0 ± 4.6</td>
<td>312.7 ± 4.3</td>
<td>312.2 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>(116.6 (113.3 ± 1.9)</td>
<td>(111.5 (115.0 ± 1.0)</td>
<td>(118.0 (116.3 ± 1.2)</td>
<td>(114.0 (115.3 ± 3.3)</td>
<td>(111.6 (112.25 ± 3.6)</td>
<td>(111.6 (112.25 ± 3.3)</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>331.0 ± 4.0</td>
<td>304.3 ± 1.5</td>
<td>309.5 ± 4.4</td>
<td>301.7 ± 1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(181.7 (180.3 ± 6.0)</td>
<td>(180.3 ± 3.5)</td>
<td>(165.3 (175.3 ± 2.6)</td>
<td>(175.3 ± 3.3)</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>324.6 ± 11.7</td>
<td>301.0 ± 4.3</td>
<td>371.0 ± 6.2</td>
<td>309.0 ± 1.40</td>
<td>346.2 ± 10.8</td>
</tr>
<tr>
<td></td>
<td>(218.0 (220.3 ± 4.6)</td>
<td>(216.0 (215.5 ± 3.8)</td>
<td>(222.3 (217.0 ± 6.4)</td>
<td>(213.3 (212.7 ± 5.2)</td>
<td>(213.3 (212.7 ± 1.2)</td>
<td>(179.0 (184.8 ± 3.4)</td>
</tr>
<tr>
<td>110</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>311.84 ± 6.79</td>
<td>304.83 ± 4.92</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

**TABLE 4**

OBESEITY INDICES (± SEM) (NASO-ANAL LENGTH (mm) ± SEM)
caloric density, nor the greasiness. This is reflected in the long delay of the 75% fat caloric diet which has 20% protein and a caloric density of 3.3 kcal/g, and the brief delay of the extremely greasy 52% fat calorie diet which contained added non-nutritive grease.

The possibility that the delayed onset of obesity is caused by a lack of protein in the high fat diets was not supported by the findings of the 75% fat fed rats. In this diet 20% of the calories came from protein, yet this diet produced such a long delay that we finally terminated the experiment. Also, the other high fat diets, while low in protein, contained adequate amounts. For example, the rats fed the 67% fat diet, which contains the lowest percent protein (10%), consumed 46.98 kcal/day for the first week on the diet. Ten percent of those calories (4.70) were from protein. The general requirements for a female rat 26 days of age is 4.8 kcal of protein (Altman and Dittmer, 1974). Thus, on two accounts, protein malnutrition cannot account for the data. This 10% protein level is also above the concentration at which adult VMH lesioned rats decrease their food intake (Mayer and Krauss, 1963).

Palatability, however defined, can never be ruled out when diets are consumed orally. However, the high fat diets (at least the 52, 51, and 67% ones) are generally considered to be more palatable than Laboratory Chow, as they cause greater weight gains in adults. It would be a remarkable coincidence if palatability shifted dramatically through ontogeny in a manner that coinciden-
tally correlated perfectly with the percent of dietary fat calories.

Why then should dietary fat dictate food intake and body weight of knife cut weanling rats? The nursing rat, consuming mothers milk, is ingesting a high fat diet. Indeed, 63-70% of the calories in rat mother's milk come from fat (Luckey, 1963; Mayer, 1935). Pups weaned onto a high fat diet continue to show preweaning metabolic patterns including high pancreatic lipase activity (enzymes responsible for the splitting of free fatty acids and glycerol from triglycerides and phospholipids), low pancreatic amylase activity (responsible for the breakdown of starches), and slowed hepatic glucokinase activity (responsible for the conversion of glucose to glucose-6-phosphate), (Deschidt-Lankman, Robbrecht, Camus Baya, and Christophe, 1974; Robberecht et al., 1970; Walker and Eaton, 1967). In addition, on high fat diets, which increase triglyceride levels, glucagon secretion from the pancreas rises (Bottger, Dobbs, Glaoana and Onger, 1973).

Weaning onto a high carbohydrate diet yields a different profile. There are decreases in serum cholesterol and free fatty acids (Hahn and Koldowsky, 1966), and increases in hepatic lipogenic enzymes (Lockwood et al., 1970). High carbohydrate diets also stimulate secretion of insulin and inhibit glucagon release. These studies suggest that the utilization and storage of dietary fats and carbohydrates are dramatically different in our rats fed the 11% fat (chow) vs. higher fat content diets.

We have recently found (Gold and Simson, 1982) that serum
ratios of insulin to glucagon (I/G) are elevated in adult knife cut rats. In sham operated rats fed a high fat diet glucagon levels are high (low I/G ratio) compared to shams fed a high carbohydrate diet. These opposite effects in adults of knife cut surgery and high fat diet may account for the rapid weight gains observed in adult knife cut rats fed high fat diets. Low I/G ratios (especially the high glucagon) are needed to convert high fat diets into available energy, but knife cuts elevate I/G ratios. The resulting deficit of available energy may be responsible for the greater excesses of post-knife cut food intake seen with high fat diets in adults.

In weanlings the pancreas may be less responsive to the effects of the knife-cut brain surgery (perhaps due to hormonal competition), requiring synergistic stimulation from high dietary carbohydrate to elicit insulin release and inhibit glucagon release, thus effecting elevated I/G ratios. The need for this synergistic carbohydrate would gradually wane with age.

This hypothesis suggests that there are carbohydrate/fat ratios below which it is not possible to produce obesity with hypothalamic knife cuts in the young rat, and that changing the carbohydrate/fat ratio changes the age at which a rat can become obese. This notion is supported by our findings; Lab Chow, no delay, has a C/F calorie ratio of 5.73, while the other diets (52%, 61%, 67% and 75% fat calories) have progressively longer delays and progressively lower C/F ratios, respectively, of .65, .46, .34, and .07.
Many approaches to the study of human obesity require the use of animal models. For example, one needs animal subjects to directly measure body composition, or to precisely control or measure activity and caloric intake, especially when long periods of observation are involved. Of the several animal models of obesity, dietary obesity probably has the greatest similarity to human obesity, as the general availability of varied and tempting foodstuffs is often cited as responsible for the obesity seen in much of the adult human populations (Rolls, 1979). Dietary obesity in adult rats has been produced by high fat diets (Schemmel, Mickelson and Gill, 1970), by high carbohydrate diets (Kanarek and Hirsch, 1977), and by supermarket diets (Rolls, Rowe and Turner, 1980; Sclafani and Gorman, 1977; Sclafani and Springer, 1976). Of the three, the supermarket snack foods are especially relevant to human experience, and also produce the most rapid weight gains (Sclafani, 1978).

Human obesity is largely an adult onset phenomenon, typically affecting 30% of the population, only roughly 10% of children are classified as obese (Forbes, 1973). High fat and high carbohydrate diets produce obesity only in adult rats, not in weanlings, but the ontogeny of the more tempting supermarket type dietary obesity (SMD) has not previously been determined, and so is the subject of the pres-
ent report. This study includes an examination of the ontogeny of SMD obesity and the possible influence of weanling vs later exposure to SMD upon adult patterns of weight gain and weight retention, all in outbred female rats. For comparison, a group of adult female inbred rats are also tested with SMD, and the ontogeny of SMD obesity is in addition examined in outbred male weanlings.

Method. The first experimental group consisted of 29 Charles River (CD) outbred female rats weaned by the commercial breeder at 3 weeks of age and housed the same day in our laboratory, 2 per solid bottom cage. Nineteen of these female weanlings were fed only Purina Lab Chow pellets and water ad lib., while the other ten, the weanling started group, received, in addition, a supermarket diet (SMD) consisting of assorted cookies, marshmallows, chocolate bits, cheese, salami, bologna, peanut butter, and sweetened condensed milk (mixed 1:1 with water). Obesity indices (Simson and Gold, 1982a) based upon nose- anal length measurements were taken at 22 weeks of age. At this time ten of the chow fed rats were switched to chow plus SMD and became the adult started group. Nine rats remained as chow-only controls. Obesity indices were again taken at 36 weeks of age at which time the SMD (but not the chow) was removed from both weanling started and adult started SMD groups. All animals were thus left on chow and water alone. At 55 weeks of age all rats were again measured for obesity indices and the previously SMD fed animals were returned to the SMD plus chow and kept on it until they were 64 weeks old, at which time the experiment
was terminated. Due to death or tumors only 6 weanling started and 6 adult started SMD fed rats completed the entire study.

In order to evaluate the effect of genetic variability upon individual responses to SMD, eight Charles River inbred Fischer 344 adult female rats (172.0 ± 3.26 (SEM) g at start of experiment) were tested using procedures identical to those used above for the adult started outbred CD rats. Four of the inbred rats were switched from Purina Laboratory Chow pellets to Laboratory Chow plus SMD, while four rats remained with the Chow. SMD was given for 9 weeks.

In order to determine whether the delay and variability were sex linked, a third experimental group consisted of 14 male rat offspring of Charles River (CD) outbred rats (primiparous mothers) bred in our laboratory. The animals were weaned at 3 weeks of age from plastic solid bottom cages and subsequently were housed singly in hanging wire cages. Six male weanling control rats were provided only Purina Lab Chow pellets and water ad lib. The other 8 male weanlings were placed on a modified supermarket diet consisting of chocolate chip cookies, peanut butter, and a whole milk-sugar solution (1.892 liters milk: 536 g sugar), along with ad lib water and lab chow. It is important to note that the administration of this modified SMD, in addition to housing weanlings singly in wire cages, proved in other studies to successfully and sufficiently produce obesity in both weanling and adult females (Simson, Jones, Schwarz and Gold, 1982).

**Results.** For the first two weeks the outbred female weanlings on SMD plus chow gained weight even less rapidly than the chow only controls
(p < .05, t-test). Only after the age of 8 weeks did they gradually begin to outweigh the chow only group (Figure 8 and Table 5). At 22 weeks of age obesity indices confirmed that the SMD fed rats were indeed more obese (Figure 9 and Table 6).

In contrast to the delayed and gradual excess weight gained by the female outbred weanling started rats, outbred female rats started on SMD as 22 week old adults immediately began such rapid weight gains that by 25½ weeks of age their weights matched those of the weanling started rats. From this point onward both the weanling and adult started rats gained at comparable rates. Obesity indices taken at 36 weeks of age confirm the similar levels of obesity of the weanling and adult started female groups. In striking contrast to this between group similarity, there were enormous within group differences, the most obese rat in each SMD group weighing more than twice as much as the leanest, and the leanest being well within the range of the chow only controls. Individual body weights are shown in Figure 9 at 36 weeks of age. After the SMD was withdrawn at 36 weeks of age both obese groups lost weight, until at 55 weeks of age they no longer differed significantly from controls in body weight or in obesity index. Upon reintroduction of SMD both formerly obese groups rapidly regained, and then surpassed, their former weights. Individual data are again shown at 64 weeks of age, a unique symbol being used for each rat to permit individual comparisons at 36 and 64 weeks. The relative amount of excess weight gained by 36 weeks, before the SMD was withdrawn, accurately predicted the relative excess weight reached
Fig. 8. Body weights of supermarket and pellet fed female rats. This is an enlargement of the beginning of Figure 9. SEM are also shown.
<table>
<thead>
<tr>
<th>Age Group</th>
<th>3-5 weeks</th>
<th>5-8 weeks</th>
<th>8-22 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weanling SMD</td>
<td>4.87 ± .20*</td>
<td>3.97 ± .22</td>
<td>2.02 ± .20*†</td>
</tr>
<tr>
<td>Adult SMD</td>
<td>5.65 ± .14</td>
<td>3.50 ± .31</td>
<td>1.25 ± .15</td>
</tr>
<tr>
<td>Control Pellets</td>
<td>5.64 ± .37</td>
<td>3.68 ± .46</td>
<td>1.17 ± .13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age Group</th>
<th>22-25½ weeks</th>
<th>25½-36 weeks</th>
<th>36-55 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weanling SMD</td>
<td>1.48 ± .30*‡‡</td>
<td>1.41 ± .25*</td>
<td>- .82 ± .12**</td>
</tr>
<tr>
<td>Adult SMD</td>
<td>4.70 ± .74**</td>
<td>1.87 ± .41*</td>
<td>-1.03 ± .19**</td>
</tr>
<tr>
<td>Control Pellets</td>
<td>.73 ± .13</td>
<td>.38 ± .10</td>
<td>.34 ± .06</td>
</tr>
</tbody>
</table>

55-64 weeks

<table>
<thead>
<tr>
<th>Age Group</th>
<th>3.30 ± .67**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult SMD</td>
<td>3.90 ± .60**</td>
</tr>
<tr>
<td>Control Pellets</td>
<td>.16 ± .19</td>
</tr>
</tbody>
</table>

*Differs from controls, p < .05, Mann Whitney - U Test.
**Differs from controls, p < .01, "
†Differs from adult SMD, p < .05, "
‡‡Differs from adult SMD, p < .01, "

Boxed areas indicate supermarket diet availability.
Fig. 9. Body weights of supermarket and pellet fed female and male outbred groups. The supermarket diet was removed and pellets given to the weanling started and adult-started female groups at 36 weeks of age. Each of 12 SMD fed female rats is given a unique symbol to show its rank at 36 and 64 weeks of age. At 55 weeks the supermarket diet was returned to both of these groups. For the males individual data are plotted at 19 weeks of age.
## TABLE 6
### OBESITY INDICES

<table>
<thead>
<tr>
<th>Age Group</th>
<th>22 weeks</th>
<th>36 weeks</th>
<th>55 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weanling SMD</td>
<td>333.8 ± 4.78(^*)</td>
<td>357.1 ± 7.14(^*)</td>
<td>324.9 ± 6.17</td>
</tr>
<tr>
<td>Adult SMD</td>
<td>308.1 ± 4.49(^a)</td>
<td>356.3 ± 11.18(^*)</td>
<td>322.5 ± 6.88</td>
</tr>
<tr>
<td>Pellet Fed</td>
<td>307.5 ± 3.54</td>
<td>314.7 ± 1.48</td>
<td>322.3 ± 2.73</td>
</tr>
</tbody>
</table>

The obesity index is computed by the formula:

\[
\frac{\sqrt{\text{Body Weight (g)}}}{\text{Naso-anal length (mm)}} \times 10^4
\]

An obesity index in the range of 300-315 is considered normal.

\(^*\)Differs from pellet fed \(p < .01\).
\(^a\)Differs from Adult SMD \(p < .01\).
\(^a\)Adult SMD were started on SMD plus chow at 22 weeks of age.
\(^b\)SMD withdrawn at 36 weeks of age, leaving only chow.

by 64 weeks, after the SMD was re-introduced (Spearman Rank Order Correlation = .97, \(p < .001\)). Thus, the variability in susceptibility to obesity was highly reproducible.

The 4 inbred Fischer 344 rats (adult started females) also responded without delay to the SMD (Figure 10). They gained 1.17 ± .10 (SEM) g/day, averaged over 9 weeks, vs 0.33 ± .07 g/day for the 4 Chow only controls. Presented in the same format, also over 9 weeks, the 10 female adult started outbred CD rats gained 2.85 ± .49 (SEM) g/day on SMD vs 0.47 ± .11 g/day for the 9 Chow only controls.
Fig. 10. Mean change in body weight of Fischer 344 inbred female rats given SMD and of pellet fed controls. Data are presented for the last two weeks on pellets preceding the introduction of the SMD supplement, and for the 9 weeks during which the SMD was available. Individual values are given for the final data point.
CHARLES RIVER
FISCHER 344 (inbred)

CHANGE IN BODY WEIGHT (g)

SMD Diet

Supermarket Diet

Pellet Diet

WEEKS

Figure 10
The much greater SEM for the SMD fed outbred rats is presumably due to their genetic variability. An approximate correction for the greater size and greater weight gains of the outbred rats is obtained when the SEM is presented as percent of the mean weight gain. For the SMD fed inbred rats the SEM is only 8.5% of the weight gain, as compared with 19.0% for the SMD fed outbred rats.

Like the female weanlings on the SMD, the male SMD fed weanlings did not immediately begin to outweigh the pellet fed controls (Figure 9). However, the male SMD weanling group showed more gradual onset of excess weight gain than seen in females, with a significant weight difference emerging by 10 weeks of age ($p < .05$). At ten weeks of age the pellet fed control males' weight gain began to decelerate, while the SMD fed males continued to gain in a more linearly fashion. By 19 weeks of age the weights were, respectively, $497 \pm 25.6$ and $641 \pm 41.1$ g. Obesity indices taken at 19 weeks of age confirmed that males fed a SMD at weaning were significantly obese as compared to controls (Obesity Indices: Pellets, $307.9 \pm 3.6$, vs SMD, $330.4 \pm 2.6$, $p < .02$). In addition, individual male data are plotted for this last datum. As with the females, large within group body weight differences are exhibited by the male SMD fed rats.

**Discussion.** Our observation that female and male weanling rats offered SMD do not begin to outweigh pellet fed controls until adulthood parallels the delayed obesity seen following various other manipulations. Delayed obesity is seen in response to high fat diets (Lemonneir, 1972; Peckham, Entenman and Carroll, 1972), high carbo-
hydrate diets (Kanarek and Hirsch, 1977), as well as following hypothalamic brain lesions (Kennedy, 1957) or hypothalamic knife cuts (Gold and Kapatos, 1975), and following ovarietectomy (Wade and Zucker, 1970).

Obesity is nevertheless possible in weanlings. Hypophysectomized weanlings on Metrecal or high fat diet respond respectively to brain lesions (Kurtz, Rozin and Teitelbaum, 1972) or knife cuts (Ieni and Gold, 1977) without delay, and hypothalamic knife cut weanlings with intact pituitaries respond with minimal delay if they are kept on a low fat diet (Simson and Gold, 1981). Also, high carbohydrate diets (sugar-water) produce early obesity through a stunting of linear growth (Kanarek and Marks-Kaufman, 1979).

The grouping into delayed vs early onset obesities by no means suggests that there are two underlying physiological mechanisms of obesity; one delayed, the other not delayed. For example, hypothalamic lesion and hypothalamic knife cut obesity are blocked by subdiaphragmatic vagotomy, whereas SMD obesity is not blocked by vagotomy (Gold, Sawchenko, DeLuca, Alexander and Eng, 1980), suggesting separate mechanisms. Yet, both hypothalamic and SMD obesities are delayed in weanlings.

Although here both female and male weanlings displayed a lag in excessive weight gain while maintained on a SMD, the nature of the delay pattern varied between the sexes. Female SMD weanlings' obesity onset began at 8 weeks of age as a rapid, fairly abrupt weight separation from controls, whereas the males had a longer delay,
to 10 weeks of age. Male weanlings also had a more gradual onset of weight departure from controls. These sex differences coincide with those seen after weanling knife-cuts, being a 7-8 week of age delay for females and a 9-10 week of age, more gradually established, delay for males (Gold, 1970).

A similar difference in weight gain patterns exists for adult started rats (Sclafani and Gorman, 1977). By day 5 on SMD, adult females' weights exceed controls, but, in contrast, it is not until day 40 that a significant difference is obtained for adult males (Sclafani and Gorman, 1977). Male rats' faster baseline growth rate has been noted as possibly of importance in this sex difference (Gold and Kapatos, 1975; Sclafani and Gorman, 1977).

Four mechanisms or factors that should be considered as possibly mediating the delay in the onset of SMD obesity are gonadal hormones, exercise, dietary fat content, and thermogenesis. Our data also test the notion that a critical age for adipocyte proliferation influences the ultimate level of obesity. Finally, the great individual variability that we observed raises genetic considerations.

Mediation of SMD obesity via diet-induced perturbations in gonadal hormones is suggested by the relatively low levels of these hormones in weanlings, and by the well documented effects of ovariectomy and of gonadal hormones on weight gain and food intake (Wade and Gray, 1979). If SMD obesity were mediated by gonadal hormones, then one would expect to find that ovariectomy obesity (which clearly is
gonadally mediated) and SMD obesity would share a common age of onset, and that the two obesities would not be additive. Contrary to this prediction, we have recently found (Simson, Jones, Schwarz and Gold, 1982) that SMD obesity and ovariectomy obesity do not share a common etiology. Ovariectomy obesity begins just after puberty (5 weeks of age) where SMD obesity begins 2-3 weeks later. Furthermore, SMD and ovariectomy effects upon body weight are additive, further suggesting that there are separate mechanisms.

A second potent variable in obesity is the amount of exercise, exercise tending to reduce adiposity. SMD fed adult rats given access to exercise wheels become only about half as obese as SMD fed rats that do not have access to wheels (Sclafani, 1978). It is, however, difficult to see how exercise could be a factor in the delay of SMD obesity, as female rats with access to running wheels run very little prior to puberty (Kennedy and Mitra, 1963).

A third variable to consider is the amount of fat in the diet. We have recently shown that the delayed obesity seen after hypothalamic knife cuts in weanling rats is peculiar to the use of a diet extremely high in fat content (61% of calories) (Simson and Gold, 1982b). When knife cut weanlings are given a low fat diet (Purina Lab Chow, 11% fat calories or Purina Mouse Chow, 26% fat calories), there is no delay. When we separately measure the food intakes of various components of SMD, we find that rats on SMD plus chow obtain 30-35% of their calories from fat (Simson, Schwarz and Gold, in preparation). This level of fat content is close to that of Mouse
Chow, which did produce weanling onset obesity in knife cut rats. (High vs low fat SMD's remain to be tested). The notion that the dietary fat level regulates the ontogeny of SMD obesity is also inconsistent with the observation of a temporally similar delay seen when obesity is produced by supplementing chow with sugar water (Kanarek and Hirsch, 1977), which contains 0% fat.

Having eliminated gonadal hormones, exercise, and dietary fat content as likely mediators of delayed obesity, we feel obliged to offer at least one viable mechanism to mediate the delay. The most likely candidate would appear to be thermogenesis. Rothwell and Stock (1979) have reported that young Sprague-Dawley strain rats on SMD consume 80% more metabolizable energy than controls, but only a small part of this could be accounted for in the carcass analysis, the rest of the energy being shunted into diet-induced thermogenesis. The main site of the thermogenesis has been identified as the brown adipose tissue (Foster and Frydman, 1978). Trayhurn and James (1981) have suggested that diet-induced thermogenesis is an age-related phenomenon. Our data is consistent with this idea, the young animals (under 8-10 weeks of age) being able to burn off the excess calories more than when they reach adulthood. Indeed, Hill (1981) has reported a decrease in diet-induced thermogenesis at 56 days of age in supermarket diet fed female rats. During this period (i.e., prior to 56 days old) rats on SMD in our laboratory consume 40% more calories than controls, yet weigh no more (Simson, Schwarz and Gold, in preparation).
The age of onset of obesity is of considerable theoretical importance for the adipocyte proliferation model of obesity. Briefly, that model suggests that each adipocyte has a limited fill capacity, that the proliferation of adipocytes can only occur during a critical young age, that excessive caloric intake or other factors during that period can activate or block adipocyte proliferation, and that all of the adipocytes possessed by an animal will eventually fill if highly palatable diets are offered.

While Faust, Johnson, Stern and Hirsch (1978) have shown that fat cell number can increase well into adulthood, others have not supported this finding (Louis, Vernadoe, Agoha and Laster, 1979; Simson, Jones, Schwarz and Gold, 1982). Others have reported hyperplasia only after 20 weeks on SMD (Mandenoff, Lenoir and Apfelbaum, 1982). Given that the adult started rats were on the diet only 14 weeks, differential rates of weight change (weanling SMD yielding greater obesity), with possibly hypercellular juvenile onset obese rats resisting weight loss upon removal of the SMD and/or overresponding upon its reintroduction, were anticipated, but did not materialize. Indeed, the adult started group, if anything, responded more rapidly upon SMD reintroduction. Thus, unavailability of such foods early in life does not protect rats from SMD obesity as adults.

Some investigators (Rolls, Rowe and Turner, 1980) find that the SMD fed rats maintain their elevated body weights when switched to chow and water, while others (Sclafani and Gorman, 1977) share our observation that the obesity is lost when SMD is withdrawn. Closer
examination reveals that female albino rats and brief (17-60 day) diet exposures were used when reversible obesity was found, whereas male hooded rats receiving long (90 day) diet exposures maintained elevated weights. In the present study the temporal part of the confound is resolved by the use of albino rats and long (100 and 231 day) SMD exposures. Since our rats failed to maintain elevated body weights despite prolonged access to SMD, we can conclude that the duration of the diet does not influence the ability to lose them. That appears to leave strain (and possibly sex differences) to account for the finding that dietary obesity is retained after the diet is withdrawn.

Finally, we come to strain (genetic) considerations. The extremely wide range of weight gains seen in the individual data points of Figure 9, and the .97 rank order correlation between the individual excess weights of the females on first exposure vs re-exposure to SMD, suggest that genetic variability within this outbred (i.e., genetically heterogenous) population is responsible for the phenotypic variability. The possibilities of undetected disease impairing some of the rats, of disruptive interactions from group housing, or of a one time error by the breeder seem ruled out by our recent replication at a separate facility of extreme variability of weight gains within a group of 20 individually housed SMD fed adult outbred female rats of the same strain and supplier (Gold, 1981). The genetic basis of most of the variability is further supported by our finding that in genetically uniform Fischer 344 rats the SEM of
the weight gain is only 8.5% of the weight gain, whereas for the out-
bred CD adult females the SEM is 19.0% of the gain. Also related to
strain-considerations in the development of dietary obesity is the
finding that hooded rats show precocious obesity on SMD (Sahakian,
Burdess, Luckhurst, and Trayhurn, 1982).

One cannot help but note how similar this genetically based
variable, adult onset SMD, is to the human condition. We suggest
that this similarity is more than superficial, that the physiological
mechanism of SMD obesity and of its ontogenetic delay in rats will
hold for the human condition of gradual adult-onset middle-age spread.

We have recently also investigated whether genetic variability
might not be responsible for much of the variability also seen in
several typically used psychological measures including shuttle-box
avoidance, lever pressing (FR) for food pellets, and open field ac-
tivity (McElroy and Gold, in preparation). We compared Charles
River CD outbred males with Fischer 344 inbred males, and found only
small strain differences in the variability of response rates. Thus,
the large within strain genetic variability that we observe in res-
pONSE to SMD may not widely generalize to other types of responses.
Perhaps this is because obesity is one of the relatively few traits
that are best left heterogenous within a population. It is good
 genetic strategy to always have on hand both lean and obese members
so as to be prepared both for rapid escape from predators and extreme
heat on the other hand, and famine and extreme cold on the other
hand.
CHAPTER IV
SUPERMARKET DIETARY AND OVARIECTOMY-INDUCED OBESITIES COMPARED IN WEANLING AND ADULT RATS

Weanling rats are temporarily relatively insensitive to the manipulations that produce hyperphagia and obesity in adult rats. They typically show a delayed obesity. Kennedy (1957) found that weanling rats receiving electrolytic ventromedial hypothalamic lesions did not begin to outweigh control rats until after seven weeks of age. Weanling rats receiving parasagittal hypothalamic knife-cuts show a similar delay (Gold and Kapatos, 1975). Also, the increased body weight of Zucker (fa/fa) rats is not detectable until after four weeks of age (Bray and York, 1979). Furthermore, hyperphagia and obesity are delayed until 6-7 weeks of age following neonatal (Wade and Zucker, 1970) or weanling (Slob and van der Werfften Bosch, 1975) ovariectomy. Finally, dietary obesity, induced by high fat diets (Lemonnier, 1972; Peckham, Enteman and Carrol, 1962), or high carbohydrate diets (Kanarek and Hirsch, 1977), is also delayed.

Recently, we (Simson, Gold, Schwarz and Assimon, in preparation) found, in addition, that supermarket dietary obesity is delayed. Despite adequate protein in the diet, the rate of weight gain for supermarket diet fed weanlings was even less than that of chow fed controls during the first two weeks on the diet. Only after 5 weeks of age did the rate of weight gain for the supermarket
diet fed group exceed that of the pellet fed group such that by 8 weeks of age the supermarket diet fed group began to outweigh the chow fed controls. This time frame roughly corresponds to the age of puberty (as indicated by vaginal opening) in this strain of rats (Gold, Ieni and Simson, 1977; Ramirez, 1973), and to the period during which excessive body weight begins for ovariectomized weanlings (Wade and Zucker, 1970; Slob and Van der Werfften Bosch, 1975). A common etiology (possibly involving ovarian hormones) underlying both delayed supermarket dietary obesity and delayed ovariectomy obesity was thus suggested. However, these between study comparisons require within study verification. Furthermore, if a mechanism is shared, then superimposition of ovariectomy and diet should prove redundant. The ontogeny of supermarket dietary and ovariectomy obesities were therefore explored within one study in both weanlings and adults.

Methods. Thirty (30) weanling (twenty-one-day-old) female Charles River (CD) rats were received from the commercial breeder. On the day of arrival they were individually housed in hanging wire mesh cages and were randomly assigned to one of four groups; ovariectomized/supermarket diet (OVEX/SMD, n = 10), sham operated/supermarket diet (SHAM/SMD, n = 5), ovariectomized/lab chow pellet (OVEX/pellet, n = 10) or sham operated/lab chow pellet (SHAM/pellet, n = 5). At a later date thirty (30) adult female Charles River (CD) rats, approximately 98 days old, were individually housed in
hanging wire mesh cages and were randomly assigned to the same four groups; OVEX/SMD (n = 8), SHAM/SMD (n = 7), OVEX/pellet (n = 8) or SHAM/pellet (n = 7). The supermarket diet (SMD) consisted of Purina Lab Chow pellets, water, sweetened whole milk (28.3% sucrose), chocolate chip cookies, and peanut butter. After overnight adaptation to its diet and cage, each rat received bilateral ovariectomy or sham surgery under ether anesthesia.

Body weight was taken twice weekly. The milk was changed daily. The weanlings were sacrificed at 148 days of age. The adults were sacrificed 105 days into the experiment (203 days of age). Completeness of ovariectomy was verified on all animals. Those rats in which ovarian tissue was suspected were not further analyzed, and their body weight and food intake were not used for calculations. These included two weanling OVEX/SMD rats, one weanling OVEX/pellet rat and one adult OVEX/pellet rat. The remaining rats were prepared for fat cell and carcass analysis.

Adipocyte size and number. The retroperitoneal and parametrial fat pads were removed from the rats and weighed. A portion (≈ 1 g) was processed according to the procedures of Rodbell (1964), Lavau, Susini, Knittle, Blanchette-Hirst and Greenwood (1977) and Clarkson, Katch, Kroll, Land and Kamen (1980). One hundred cells were digitized per pad. Cell volumes were computed and mean cell weights were calculated using the density of Triolein. Cell number was determined as a function of fat pad weight and mean cell weight. This method has been shown to be reliable in repeated measurement studies and appears
to be free of investigator bias (Clarkson, Kroll, Wai and Kamen, 1981).

**Nose-anal length and obesity index.** Under deep ether anesthesia mid-day nose-anal lengths were determined using outside calipers, and were used to determine Lee Obesity indices as described previously (Gold and Kapatos, 1975).

**Carcass analysis.** Since the Lee (1929) Obesity Index (Bernardis and Patterson, 1968) has been shown to correlate poorly with percent body fat in dietary obese animals (Stephens, 1980), direct measurement was required. The carcasses were prepared for analysis by a method (Gray and Wade, 1981) modified from Leshner, Litwin and Squibb (1972). Briefly, the shaved eviscerated carcass was placed in a disposable aluminum baking pan and dried in an 80°C oven to determine percent water. The dried carcass was then homogenized in a blender and an aliquot taken for the determination of percent fat. Fat content is reported as percent of wet weight.

Two way analyses of variance and post-hoc Bonferroni-t tests were used to determine significance.

**Results - weanlings.** Despite comparable mean body weights and obesity indices at the time of sacrifice (Table 7), the single-treatment groups (SHAM/SMD and OVEX/pellet) differed radically from one another in age of onset of excessive weight (Figure 11), percent body fat and water (Figure 12), adipocyte size, and adipocyte number (Figure 13).
TABLE 7

MEAN ± SEM OBESITY INDICES (OI) AND NASO-ANAL LENGTHS (NAL) IN mm OF GROUPS IN WHICH TREATMENTS BEGAN AT WEANING

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age</th>
<th>57 days</th>
<th>Age</th>
<th>148 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>01</td>
<td>Nal</td>
<td>01</td>
<td>NAL</td>
</tr>
<tr>
<td>OVEX/SMD</td>
<td>311.4 ± 3.6</td>
<td>202.9 ± 1.2</td>
<td>*331.9 ± 4.8</td>
<td>223.1 ± 1.3</td>
</tr>
<tr>
<td>SHAM/SMD</td>
<td>307.6 ± 4.6</td>
<td>194.8 ± 1.8</td>
<td>320.2 ± 4.8*</td>
<td>219.4 ± 3.1</td>
</tr>
<tr>
<td>OVEX/Pellet</td>
<td>312.1 ± 2.6</td>
<td>203.7 ± 2.5*</td>
<td>*317.0 ± 1.5</td>
<td>221.2 ± 2.9*</td>
</tr>
<tr>
<td>SHAM/Pellet</td>
<td>300.8 ± 2.0</td>
<td>197.8 ± 2.0*</td>
<td>*305.2 ± 1.7</td>
<td>218.0 ± 2.1*</td>
</tr>
</tbody>
</table>

Note: *p < .05.

Body weight. As seen in Figure 11, the effect of ovariectomy on body weight began after 50 days of age (p's < .01 at 57 days of age for both OVEX groups vs both SHAM groups). This is 20-27 days after puberty occurred in the sham operated rats (Vaginal opening between days 30 and 37, x̄ = 34.1 days, see Figure 11). The effect of the SMD on body weight began later, after 85 days of age (both SMD groups vs respective pellet groups, p's < .01 at 89 days of age). Nose-anal lengths and obesity indices at 57 days of age (after the ovariectomy effect on body weight begins and prior to the SMD effect) reveal that the SMD tended to slow linear growth while the OVEX tended to enhance linear growth such that at 57 days of age the single treatment groups differed from one another in nose-anal length (p < .01) but not in obesity index. Thus, the differences in age of onset of excessive body weight may have been due to effects of ovariectomy upon lean body growth.

When the rats were sacrificed at 148 days of age, the single treat-
Fig. 11. Mean body weight of weanling treated rats. Vaginal opening of the sham-ovariectomized rats is indicated by X.
Fig. 12. Carcass composition of 148-day-old rats, ovariectomized and/or started on SMD as weanlings.
Fig. 13. Adipocyte size and number of 148-day-old rats, ovariectomized and/or started on SMD as weanlings.
ment groups were at equivalent weights and obesity indices (Table 7), but there was an apparent additive effect in the OVEX/SMD group as indicated by body weight (Figure 11), (p < .05 vs all other groups) and obesity index (Table 7, p < .05 vs all other groups) which suggests separate mechanisms for OVEX and SMD obesities.

Carcass analysis. Direct measurement of body fat at 148 days of age revealed a radically different picture. The SHAM/SMD group had a much higher percentage of body fat than the OVEX-pellet group (p < .01, Figure 12), this despite the fact that their body weights and obesity indices did not differ. While the OVEX/pellet group had a slightly higher percentage body fat than the SHAM/pellet group, this did not reach significance. As expected from the higher fat content, water content was significantly decreased in both SMD fed groups compared to both pellet fed groups (p's < .01). The OVEX/SMD group had only a slightly higher percentage body fat than the SHAM/SMD group (p < .01, Figure 12).

Adipocyte size and number. In the parametrial (adjacent to ovaries) fat pad, adipocyte number was decreased by ovariectomy (p's < .05), and tended to be increased by SMD (if the ovaries were intact). In the retroperitoneal fat pad there was a tendency of fat cell number to increase in the OVEX group.

Consistent with the carcass analysis, the SMD groups had significantly larger adipocytes than their respective pellet fed controls (p's < .01), while OVEX alone had no effect upon cell size (Figure 13).
Results - adults. In adults the body weight (Figure 14) and obesity index (Table 8) again overestimated the amount of obesity (fat) following ovariectomy (Figure 15), although this time there was a small increase in percent fat. In adults fat cell number was not affected by OVEX/and/or SMD (Figure 16), which is consistent with the critical period notion for adipocyte proliferation. However, the failure of SMD to elicit adipocyte proliferation in adults did not appear to restrict weight gain (Table 9) or adiposity (Figure 12 vs Figure 15) as compared with weanlings.

**TABLE 8**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age 203 days</th>
<th>OI</th>
<th>NAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVEX/SMD</td>
<td>333.8 ± 4.9</td>
<td>228.0 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>SHAM/SMD</td>
<td>332.1 ± 6.3*</td>
<td>226.0 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>OVEX/Pellet</td>
<td>317.3 ± 2.2*</td>
<td>226.3 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>SHAM/Pellet</td>
<td>300.0 ± 2.0</td>
<td>222.9 ± 3.4</td>
<td></td>
</tr>
</tbody>
</table>

Note: *, p < .05.

Body weight. Both ovariectomy and supermarket diet induced immediate and rapid weight gains (Figure 14). Ovariectomy and supermarket diet were only partially additive in eliciting weight gain such that at the end of the experiment the OVEX/SMD group did not differ significantly from the SHAM/SMD group (444.0 ± 25.9 g vs 425.71 ± 27.25 g). The OVEX/
Fig. 14. Mean body weight of rats ovariectomized and/or started on SMD as adults.
Fig. 15. Carcass composition of rats ~ 203-days old, ovariectomized and/or started on SMD as adults.
### TABLE 9

**MEAN ± SEM BODY WEIGHTS (g) AT 147-148 DAYS OF AGE FOR GROUPS IN WHICH TREATMENTS BEGAN AT WEANING VS. AT AGE 98 DAYS**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Begun at weaning</th>
<th>Begun at 98 days of age</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVEX/SMD</td>
<td>407.0 ± 19.6</td>
<td>NS</td>
</tr>
<tr>
<td>SHAM/SMD</td>
<td>348.8 ± 24.8</td>
<td>NS</td>
</tr>
<tr>
<td>OVEX/Pellet</td>
<td>346.3 ± 14.6</td>
<td>NS</td>
</tr>
<tr>
<td>SHAM/Pellet</td>
<td>294.6 ± 7.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

Note: NS, non-significant.

Pellet group was heavier and had a greater obesity index than did the SHAM/Pellet group. The SMD groups were significantly heavier and had higher obesity indices than the respective pellet groups (p's < .05). Unlike the weanlings, naso-anal lengths were not significantly increased in either ovariectomized group.

**Carcass analysis.** As with the weanlings, the greatest body fat increases were produced by the SMD, with a lower than expected (based upon weight and obesity index) percent body fat for the OVEX groups. The SMD groups had higher percent body fat and lower percent water than their respective controls (p < .01). A small effect of OVEX upon body fat was significant only for the OVEX/pellet group (p < .05, Figure 15).

**Adipocyte size and number.** Adipocyte number was not changed significantly by any of the adult manipulations, which supports the notion that there is a critical age beyond which adipocyte number is
resistant to modification (Figure 16). Not surprisingly, then, adipocyte size was increased in obesity, that is by SMD and to a lesser extent by OVEX (p's < .05).

**Discussion.** The suggestion that supermarket dietary obesity and ovariectomy obesity might have a common etiology is not supported by our findings. First, the temporal expression of excess weight following ovariectomy and supermarket dietary excess weight do not coincide in the weanlings, ovariectomy-induced excess weight beginning at 50 days of age and SMD excess weight not beginning until 85 days of age. Second, the two treatments are additive in weanlings and partially additive (a possible ceiling effect) in adults. Third, carcass analysis done on the same weight single treatment groups (weanling/OVEX/pellet and SHAM/SMD) reveal that carcass composition is drastically different in terms of percent body fat. Fourth, carcass analysis reveals the SHAM/SMD groups to contain much higher percentage body fat than the OVEX/pellet groups. Fifth, ovariectomy and SMD in weanlings have different effects from one another on fat cell size and number. Ovariectomy in weanlings blocks parametrial fat pad adipocyte proliferation, whereas SMD accelerates proliferation.

In contrast to the delayed excess weight gain seen in the weanlings, the adults showed immediate and rapid responses to both the SMD and ovariectomy, confirming that the delays seen in the weanlings were age dependent. Both the weanling OVEX/pellet group and the adult OVEX/pellet group maintained the typical 20-25% elevation of body weight previously seen in ovariectomized rats (Wade and Gray, 1979).
Fig. 16. Adipocyte size and number of rats ~ 203-days old, ovariectomized and/or started on SMD as adults.
However, the carcass analysis of the weanling OVEX/pellet group did not show a significant increase in percentage body fat, just a heavier rat. On the other hand, the adult OVEX/pellet group exhibited a small but significant elevation in percent body fat. The lack of increased percentage body fat despite increased body weight in the weanling OVEX/pellet group is partially attributable to enhanced linear growth. The naso-anal length measurements revealed enhanced linear growth for both weanling OVEX groups (OVEX/pellets, OVEX/SMD) at 57 days of age. This increased growth was still evident at 148 days of age. The loss of estrogens probably encouraged a growth of lean body mass more characteristic of male rats. The tendency for enhanced linear growth was also seen in the adult OVEX groups, although it did not reach significance. However, enhanced linear growth has been reported in adult ovariectomized rats (Mueller and Hsiao, 1981).

For weanlings, fat cell diameters in both fat pads were increased by SMD but were not affected by OVEX, which follows the carcass analysis. Similarly, Hendrikx, Boni and Kieckens (1980) found no differences in fat cell size in the parametrial, perirenal or inguinal fat pads of rats fed a commercial rat pellet diet following ovariectomy at birth compared to controls. However, in mice, ovariectomy at weaning has been found to increase fat cell size in parametrial and perirenal fat pads and in subcutaneous fat (Pallier, Aubert and Lemmonier, 1980).

In the parametrial fat pad SMD tended to increase and ovariectomy
to decrease fat cell number, while in the retroperitoneal pad there was what could be a compensatory tendency to increased number in the OVEX groups. This bidirectional change has been reported previously (Pallier et al., 1980). The parametrial fat pad contains the greatest number of estrogen receptors of those fat pads studied (Wade and Gray, 1978), so it was not unexpected that ovariectomy might effect it. Indeed, Roncari and Van (1978) report that estrogen stimulates subcutaneous human preadipocyte division in vitro.

Note that the severe restrictions that weanling OVEX placed upon parametrial fat cell proliferation in the OVEX/SMD group, relative to the SHAM/SMD group, did not limit their excesses in both weight or percent body fat.

In adults fat cell number was unaffected by SMD or by ovariectomy. Similarly, Hendrikx et al. (1980) have reported that ovariectomy at six weeks of age does not effect cell number in either the parametrial or perirenal fat pads of female rats sacrificed at 17 weeks of age. In comparison to our findings with weanlings, these adult data support the notion of a critical age after which it is difficult to influence adipocyte proliferation. Our findings do not, however, agree with those of Faust, Johnson Stern and Hirsch (1978) who found increased cell number in retroperitoneal and epididymal fat pads of adult male rats fed a high fat diet.

The difference between our study and Faust et al., might be attributed to the accuracy of the method used to determine adipocyte number. The Coulter Counter Technique may be more reliable for fat
cell number, whereas the Lavau et al. Technique (1977), which we used, may be more accurate in determining adipocyte size. However, Louis, Varnedoe, Agoha and Laster (1979) using the Coulter Counter Technique did not find increased cell number in adult rats fed a high fat diet. However, other differences including sex (they used males), length of time on the diet, and the strain of animals may also be important.

Body weights of the adult groups were equivalent at the time of their surgery to those of the SHAM/pellet weanling group at 99 days of age, thus making weight gain comparisons possible. At 148 days of age (when the weanlings were killed) the adult groups were 49 days into their treatments. At this time the body weights of the respective adult vs weanling started groups show remarkable similarity (Table 9, all p's = n.s.). This lack of difference between weanling started and adult started SMD rats has recently been replicated (Simson et al., in preparation). Thus, the relatively lower adipocyte number in the adult SMD groups did not limit their obesity. The carcass analysis shows the adult treatment groups to have a greater percentage fat than the weanling groups, but this may be attributed to their extra 8 weeks of age at sacrifice. The age differential at sacrifice may also explain why the combined treatments appear to have an additive effect when given to weanlings, but not when given to adults. For example, the initial response of the adults to the combined treatment is approximately 4.4 g/day, which is considerably in excess of the 2.6 g/day initial response of both single treatment groups. Even when the adult-started rats reach the age (49 days into treatments)
at which the weanling-started rats were sacrificed, the combined treatment group still shows a considerable additive effect. Only subsequent to this age (and weight) do the adult started rats appear to be getting into a ceiling effect.

We have recently (Simson et al., in preparation) found that rats started on a SMD at weaning did not begin to outweigh chow fed controls until after 56 days of age. In the present report, in contrast, the effect of the supermarket diet does not begin until 85 days of age. This difference might be a social effect; the present rats were individually housed, whereas the rats in the first study were housed two per cage. However, the supermarket diet offered the present rats was also less varied and had a higher percentage of calories available from fat than did our earlier study. Since we have previously reported that housing conditions do not differentially effect the development of hypothalamic obesity in weanling rats (Simson and Gold, 1981) we suspect that the second explanation, dietary fat, accounts for the difference seen in the delays. Also, no difference has been found between group and isolation reared rats in the development of SMD obesity (Sahakian, Burdess, Lockhart and Trayhurn, 1982). We have previously reported that the onset of hypothalamic obesity is delayed as a function of the fat content of the diet, the more calories from fat, the longer the delay (Simson and Gold, 1982). In further support of a dietary fat mechanism controlling delay, precocious obesity occurs in weanling rats fed a high carbohydrate diet (Kanarek and Marks-Kaufman, 1979).
We have shown that supermarket dietary obesity and ovariectomy induced obesity do not share a common etiology. The profiles of these obesities differ in age of onset, body fat content, and adipocyte size and number. While the manipulations given to weanling rats and adult rats produce equivalent body weights, the manner in which this is achieved in terms of adipocyte size and number is not the same, suggesting that, at least within the obesity ranges reached in the present study, high fat cell number does not predict obesity, and low fat cell number does not prevent obesity.
CHAPTER V
ESTROGENIC CONTROL OF ADIPOCYTE NUMBER

In adult female rats ovariectomy (OVEX) has been shown to lead to rapid weight gain, much of the excess weight consisting of fat (Leshner and Collier, 1973). In adults most of the increase in fat content is due to an increase in fat cell size, fat cell number remaining normal (Simson, Jones, Schwarz and Gold, 1982). The replacement of estrogen in OVEX rats partially reverses the increased weight gain (Mueller and Hsiao, 1981).

The profile shown by weanling rats is markedly different. Ovariectomy in weanlings does not lead to an immediate excess weight gain. Instead, excess weight gain is delayed until after the age of puberty (Wade and Zucker, 1970). Furthermore, the excess body weight is incompletely reflected in the carcass fat content. Rats ovariectomized as weanlings and sacrificed in adulthood do not consistently show a significant increase in percentage body fat even though there is a delayed body weight increase (Simson et al., 1982). Also, unlike the adult, ovariectomy at weaning does not change fat cell size in adulthood in either the parametrial or retroperitoneal fat pads (Simson et al., 1982). Similarly, no increases of fat cell size are observed in adulthood in the parametrial, perirenal or inguinal fat pads of rats ovariectomized at birth (Hendrikx, Boni and Kieckens, 1980).
This is not to say that ovariectomy in weanlings has no effect on adipose tissue. Once again unlike the adult, weanling ovariectomized rats show a change in fat cell number. However, the direction of the change depends on the fat pad studied. Following ovariectomy at weaning the number of adipocytes in the parametrial fat pad, which lies adjacent to the ovary and uterus, is decreased suggesting that estrogen increases fat cell number; while the number of adipocytes in the more distal retroperitoneal fat pad is increased, suggesting that estrogen suppresses fat cell number (Simson et al., 1982). Pallier, Aubert and Lemonnier (1980) noted similar bidirectional changes in mice following ovariectomy at weaning.

We hypothesize that ovariectomy at weaning has two independent and opposing effects upon adipose tissue. The first is a localized disruption of the neural and/or vascular integrity of the parametrial fat pad, leading to a suppression of adipocyte number that is limited to this one fat pad. This effect should not be reversible with estrogen replacement. The second effect of ovariectomy is hypothesized to be a hyperplasia of adipocytes in the non-surgically traumatized fat pads. This effect is presumed to be estrogen mediated and should be reversible.

To test these hypotheses we have looked at the adipocytes of ovariectomized, ovariectomized with estrogen replacement and sham operated rats. Our findings tend to confirm our hypotheses that estrogen restricts fat cell number and that the bidirectional change can be explained by surgical insult to the parametrial fat pad.
Method. Thirty-eight Charles River CD female weanling rats (21 days old) were received from the commercial breeder and housed individually in hanging wire mesh cages. The rats had ad lib access to Purina Lab Chow pellets and tap water. After an overnight adaptation to the cages and food, the rats were randomly assigned to one of three groups: ovariectomy (OVEX, n = 12) or sham surgery (SHAM, n = 12). Ovariectomies were performed under ether anesthesia using a single mid-sagittal abdominal incision. Estradiol benzoate (EB) was delivered at the rate of 2 µg/24 hours (Schwartz and Wade, 1981) via 2 week osmotic minipumps (Alza) implanted subcutaneously. The minipumps were replaced at two week intervals up to the time of sacrifice. OVEX and SHAM operated rats received equivalent lengths of silastic tubing. Body weights were measured twice weekly.

At 36 days of age (before the normal age of puberty) half of each group was sacrificed for fat cell analysis. This is an age at which there are few estrogen receptors in adipose tissue (Schwartz and Wade, 1981) and is before vaginal opening would occur in an intact animal. The remaining 19 rats were sacrificed at 70 days of age, well into adulthood, also for fat cell analysis.

Adipocyte size and number analysis. The retroperitoneal and parametrial fat pads were removed and weighed. A portion (~ 1 g) was prepared for analysis using the methods of Rodbell (1964), Lavau, Susini, Knittle, Blanchette-Hirst and Greenwood (1977) and Clarkson, Katch, Kroll, Lane and Kamen (1980). One hundred cells were measured in each pad. Cell volumes were computed and mean cell weights were
calculated using the density of Triolein. Cell number was determined as a function of fat pad weight and mean cell weight.

Carcass analysis. The carcasses of the 70-day-old rats were prepared for analysis by a method (Gray and Wade, 1981) modified from Leshner, Litwin and Squibb (1973). The fat contents are reported as percent of wet weight.

Post-hoc T tests were used to determine significance.

Results.

Body weight. As previously reported (Wade and Zucker, 1970) the onset of ovariectomy-induced excess weight gain was delayed until after 40 days of age (Figure 17, p < .01 at 46 days). EB replacement prevented the excess weight gain, the weight of the OVEX/EB group not differing significantly from that of the sham operated controls.

Carcass analysis. Ovariectomy significantly increased percentage body fat (Figure 18, p < .01 compared to SHAM operated). EB replacement, while holding down the body weight gain was less successful in holding down increases in body fat. The OVEX/EB group had a percent fat intermediate between the SHAM and the OVEX groups such that they did not differ significantly from either group.

Fat cell number. At 36 days of age the cell number was reduced in the OVEX and OVEX-EB groups in the parametrial fat pad (marginally significant, p < .1, Figure 19). In the retroperitoneal pad cell number was increased, but not significantly, in the OVEX and OVEX-EB groups.
Fig. 17. Mean body weight.
Fig. 18. Mean (± SEM) percent body fat (***p < .01).
Fig. 19. Fat cell size and number at 36 and 70 days of age (*p < .05 vs SHAM; **p < .01 vs SHAM; +++p < .01 vs OVEX).
The fat cell analysis at 70 days of age replicated our earlier finding of a trend toward reduced cell number in the parametrial pad following OVEX (although not a significant reduction compared to SHAM), with an increased cell number in the retroperitoneal pad (p < .05). EB replacement surprisingly reduced cell number even further in the parametrial fat pad (p's < .01, compared to SHAM and OVEX) and reduced cell number in the retroperitoneal pad (p < .01 versus OVEX, n.s. versus SHAM).

Fat cell size. At 36 days of age there were no significant cell size differences between groups in either the parametrial or retroperitoneal fat pads. By 70 days of age there was a modest increase in the size of the parametrial cells of the OVEX group (p < .01 compared to SHAM). EB replacement mitigated this increase such that the OVEX-EB group did not differ significantly from either the OVEX or SHAM groups. At 70 days of age there were no significant cell size differences in the retroperitoneal fat pad.

Discussion. EB replacement to OVEX animals prevented excess weight gains. However, it did no partially at the expense of lean body mass. This increase in body fat despite the lack of increase in body weight has been reported in other models of obesity, including weanling VMH lesioned rats (Bernardis and Skelton, 1966, 1967; Han, Lin, Chu, Mu and Liu, 1965), sucrose-water fed rats (Kanarek and Marks-Kaufman, 1979), and MSG treated rats (Kanarek, Meyers, Meade and Mayer, 1979). Unlike our previous finding (Simson et al., 1982) OVEX at weaning did produce a significant increase in percent body fat.
We have replicated other earlier findings (Simson et al., 1982) of a decreased number of adipocytes in the parametrial fat pad with an increased number in the retroperitoneal pad. We believe this bi-directional change is best explained by the surgical trauma imposed on the parametrial fat pad during ovariectomy surgery or by compromise to its blood supply or innervation. This effect can be seen in the cell number data of the parametrial fat pad. Conversely, the increased number of fat cells after estrogen removal can be clearly seen in the non-traumatized retroperitoneal pad.

From this interpretation, removal of estrogen should generally enhance fat cell number. This increase was masked in the parametrial pad because of the surgical intervention. In the parametrial fat pad both ovariectomy and estrogen appear to be cumulative, yielding far fewer fat cells than in SHAMs. Our interpretation is further supported by the suppression of adipocytes in both fat pads at 70 days of age by EB replacement. Based on these findings we conclude that a normal function of estrogen is to limit fat cell number.

This conclusion is not in agreement with the findings of Roncari and Van (1978) who found that estrogen promotes adipocyte precursor replication. However, such a direct comparison between studies can be faulted on three points: species (rat vs human), tissue (deep fat vs subcutaneous fat), and medium of study (in vivo vs in vitro).

Estrogen (or lack of estrogen) does not seem to affect fat cell number before 5 weeks of age. However, the duration of hormone treatment (2 vs 7 weeks) confounds this issue. Further parametric
investigations are needed before one could reasonably suggest that there is a "critical" age for estrogenic effects on fat cell division. Wade (1976) has suggested that it is not the estrogen itself that is critical, but rather that prepubertal rats are unresponsive to estrogen. Schwartz and Wade (1981) found significantly fewer estrogen receptors in adipose tissue of prepubertal rats than in adults, possibly accounting for the unresponsiveness. Still unanswered however, is why fat cells do not increase in size compared to controls following ovariectomy at an early age. In adults ovariectomy results in increased lipoprotein lipase (LPL) activity (Hamosh and Hamosh, 1975) resulting in an increase in fat cell size (Simson et al., 1982). Early ovariectomy may interfere with LPL. However, we are unaware of any developmental studies dealing with LPL in either normal or ovariectomized female rats.

Note also that, despite the increase in fat cell number in the retroperitoneal pad (and we assume in other fat depots), only marginal obesity is produced in weanling ovariectomized rats. Our data are therefore inconsistent with the notion of adipocyte hyperplasia leading to obesity.
CHAPTER VI
GENERAL SUMMARY

All three animal models of obesity studied in this dissertation often do not produce obesity in weanling rats. While it appears that dietary-, knife-cut, and ovariectomy-induced obesities are distinct phenomena, a common final mediator may be found among two or all three models. In this context I will now discuss the enzyme lipoprotein lipase (LPL), the insulin to glucagon (I/G) ratio, and thermogenesis.

As mentioned in Chapter II, knife-cut adult rats have a high insulin to glucagon ratio (Gold and Simson, 1982). This is due to an increase in insulin levels combined with a decrease in glucagon. Insulin has been shown to raise adipose tissue LPL levels in adult rats while glucagon decreases LPL levels (Murase, Tanaka, Inamoto, Akanuma and Kosaka, 1981). Thus, in the adult knife-cut rat, high insulin combined with low glucagon levels would allow for very high LPL activity resulting in an obese rat, as LPL clears circulating lipids from the blood into adipose tissue.

In weanlings the knife cuts are also assumed to elevate the I/G ratio, but this is not sufficient, as unlesioned young rats (18 day old pups) receiving a high fat diet show low insulin levels along with high glucagon levels (Hahn, Sakal and Hassanali, 1980). The circulating levels of insulin increase with body weight (age).
while those of glucagon decrease. Thus, in the normal rat weaned onto a high fat diet, insulin levels are low but rise with age, stimulating LPL activity, and glucagon levels which start out high, drop as the rat ages, and thereby allow LPL levels to rise. In the knife-cut weanling it is assumed that the knife cut disinhibits the vagally mediated release of insulin. However, insulin levels of high fat fed weanlings are initially low, and glucagon levels are initially high. As a result, in weanlings on a high-fat diet, the changes in insulin and glucagon following knife cuts are alone not sufficient to elevate LPL levels. After the additional normal developmental increase of the insulin to glucagon ratio LPL levels begin to rise and the incremental effect of the knife cut becomes sufficient even with a high fat diet to rapidly shunt lipids into adipocytes.

In the normal weanling a high carbohydrate diet raises insulin levels and lowers glucagon levels (Hahn, Girard, Assan, Erolich and Kervran, 1977). Combine this with the knife-cut disinhibition of insulin secretion and the resulting insulin to glucagon ratio is sufficiently large to elevate the LPL level resulting in precocious hyperphagia and obesity. In support of this notion Simson and Gold have recently shown that a highly preferred, modestly high-fat (11% by weight)/high-carbohydrate diet (Purina Mouse Chow) produces the most rapid and sustained precocious obesity ever reported in knife-cut weanlings.

Adult rats made obese through diet also have high insulin to
glucagon ratios (Nosadini, Ursini, Tessari, Garotti, deBiasi and Tiengo, 1980). This is also due to an increase in insulin with a concomitant decrease in glucagon levels. When weanling rats are given a similar diet hyperphagia is immediate (~40% more calories consumed than controls) yet no excess weight gains occur until 7-10 weeks of age. It is possible that SMD fed weanlings have elevated thermogenesis and burn off the calories rather than store them. SMD is actually a high carbohydrate/moderate fat diet. Thus, the SMD rats could be expected to be similar to rats weaned onto a high carbohydrate diet. This would result in an increased insulin level and a decreased glucagon level. Insulin levels will become higher with age and glucagon levels will decrease. Only at this point will LPL activity result in obesity.

However, prior to this the SMD rats are hyperphagic, probably not due to the "pull" of LPL, but rather to the "push" of variability and palatability. This "push" does not result in obesity because brown adipose tissue actively burns off the excess calories. Presumably, if the LPL level were elevated, as it is at a later age, obesity would occur instead. Hahn (1977) has shown that when rats are weaned onto a high carbohydrate diet fatty acid synthetase (an enzyme that converts carbohydrates into fatty acids) levels in brown adipose tissue are elevated. These fatty acids are then oxidized in brown adipose tissue, generating heat.

Another mechanism involved in thermogenesis in young rats is stimulated when they are weaned onto a high carbohydrate diet.
Hahn, Skala and Hassanah (1980) have reported that cyclic adenosine monophosphate (cAMP) contents of brown adipose tissue rise in response to weaning. Triacylglycerol (triglyceride) lipase is then activated by cAMP, converting triglycerides into fatty acids which are oxidized, generating heat. Thus, the carbohydrate of the diet enters thermogenesis via fatty acid synthetase and the fat enters via triacylglycerol lipase.

Why then does not the thermogenesis continue such that the rat never becomes obese? Fatty acid synthetase levels in rats weaned onto a high carbohydrate diet drop to lower adult levels by 38 days of age (Hahn, 1977). Thus, one mechanism for thermogenesis is decreased. All the while the insulin to glucagon ratio is becoming larger, stimulating LPL activity, producing a delayed "pull" obesity.

This hypothesis suggests that the length of delay in dietary obesity, like hypothalamic obesity, is related to dietary fat content. While intakes were not measured in Chapter III, the types of food available in that study were of higher carbohydrate content than those offered the rats in Chapter IV. A higher carbohydrate content should produce an earlier dietary obesity, and this was indeed the case. Those rats fed a SMD in Chapter III began to outweigh controls after eight weeks of age, while those fed a slightly lower carbohydrate diet in Chapter IV did not begin to outweigh controls until eleven weeks of age.

The SMD fat cell size and number data can also be discussed in
terms of the insulin to glucagon ratio. If the effect of elevated insulin to glucagon ratios is to stimulate LPL activity then one would predict fat cell hypertrophy without hyperplasia. Indeed this was found in Chapter IV in both weanling-started rats and adult-started rats.

The third model of obesity studied was ovariectomy-induced obesity. This model does not fit as well into insulin/glucagon and LPL mediation, but then again, the obesity produced by ovariectomy is far surpassed in magnitude by the previous two models. All ovariectomized rats in these studies were weaned onto a high carbohydrate diet (SMD or pellets). Thus, insulin levels should be elevated and glucagon levels should be decreased. However, estrogen has been shown to produce large decreases in glucagon levels in adult ovariectomized rats along with slight decreases in insulin levels (Mandour, Kissebah and Wynn, 1977), although others have reported increases in insulin following estrogen treatment (Bailey and Matty, 1972). The removal of estrogen (by ovariectomy) may produce increases in glucagon with small increases in insulin, which is not a combination that should produce obesity. Only with the normal ontogenetic rise in insulin and drop in glucagon might obesity begin to occur.

However, there are some problems with this scheme for ovariectomy-induced obesity. Estrogen directly decreases adipose tissue LPL, decreases glucagon, and increases insulin levels, yet insulin increases LPL and glucagon decreases LPL. Thus, the in-
increased insulin levels and decreased glucagon levels in response to estrogen do not result in the anticipated increase in LPL. Weaning rats onto a high carbohydrate diet elevates the insulin to glucagon ratio, while the removal of estrogen reduces the ratio. These opposing influences may account for the mild obesity produced by ovariectomy.

Another hypothesis concerning the delayed obesity seen after weanling ovariectomy is that young rats do not possess sufficient estrogen receptors. Removal of estrogen in young rats has no effect since there are insufficient estrogen receptors to take note of the loss. There would therefore be no change in insulin or glucagon due to estrogen removal. Only with the development of estrogen receptors does ovariectomy-induced obesity express itself. This obesity would then be mediated through increased LPL activity in ovariectomized rats.

The additivity of SMD and ovariectomy-induced obesities in adulthood may represent a double (additive) stimulation of LPL activity, one from the altered insulin to glucagon ratio induced by the SMD, the other from estrogen removal.

The above hypotheses are as yet unproven. Sequential ontogenetic monitoring of insulin, glucagon, and LPL levels are needed in knife-cut, SMD fed, and ovariectomized rats in order to support these hypotheses.
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