weeks of age. This matter requires further investigation.

Interestingly, the LF-fed lipectomized rats developed as much total subcutaneous adipose mass as their controls by storing a somewhat greater amount of lipid in subcutaneous adipocytes. Such a hypertrophic response to lipectomy has also been observed in other experiments (*1*, *5*, *6*). However, it is now apparent that a hypertrophic response to lipectomy is usually quite limited, and only serves to accommodate new lipid stores that would otherwise have been accumulated in the excised cells. In some animals, such as the Sprague-Dawley rat, the degree of such possible accommodation is probably small, while in others, especially the potentially obese, it may be quite large (*1*).

We conclude from this study that the development of the subcutaneous adipose tissue during the first few postnatal weeks of the rat's life is a precisely regulated event. That is, the proliferation of subcutaneous adipocytes is monitored and adjusted. Subcutaneous adipocyte regulation in the rat is thus similar to the regulation seen in skin and liver, except that adipocyte regulation may terminate at some time shortly after weaning when adipocyte proliferation terminates. That regulation does indeed terminate is suggested by the failure of Kral (*8*) to observe regeneration of inguinal fat in the Sprague-Dawley rat lipectomized at 15 weeks of age. Furthermore, our previous failures to observe subcutaneous adipose tissue regeneration in the NCS/R mouse lipectomized at 12 days of age (*6*) or to observe regeneration of the epididymal fat pad in young rats and mice (*5*, *6*) suggest that there are strain and site variations in the phenomenon of adipose tissue regeneration and thus perhaps in the normal mechanisms or sequences of adipocyte proliferation and development.

This study leads to at least two observations which could be relevant to human obesity. First, assuming a degree of similarity between human and rat, the existence of an adipocyte proliferation regulatory process suggests that the hyperplastic component of human obesity may well be the result of a disorder in that process, as the hypertrophic component of obesity is very likely the result of a disorder in the process that regulates adipocyte size. Determining the nature of such regulatory disorders obviously has a high priority for future study. Second, the observation that the high-fat diet promoted a greater degree of adipocyte regeneration than did the chow diet supports the notion that dietary factors can affect adipocyte proliferation and ultimate adipocyte number (*3*).

In summary, we have observed complete regeneration of subcutaneous adipose tissue in rats which were lipectomized at 3 weeks of age and fed a high-fat diet beginning at 12 weeks of age. Rats fed only a chow diet achieved only incomplete regeneration. The restored subcutaneous adipose tissue mass of the HF-fed rats was equivalent to the subcutaneous adipose mass of controls in terms of both adipocyte number and mean lipid content per cell. Therefore, the proliferative processes which establish the adipocyte population of the subcutaneous fat tissue, and the system which determines average adipocyte size, are both active and precise in their regulation at least until the time of weaning in the rat. How long beyond weaning the regulated response of regeneration will occur and what role dietary factors play in the response are questions which remain to be answered.

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9. This work was supported in part by grant PCM76-09324 from the National Science Foundation and by a grant from the Howard M. Pack Foundation. We thank J. W. Monahan for his excellent technical assistance.

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Surgical Removal of Adipose Tissue Alters Feeding Behavior and the Development of Obesity in Rats

Abstract. *Lipectomized and sham-operated rats were fed a high-fat diet to induce hyperphagia and rapid fat accumulation. Lipectomized rats with 25 percent fewer adipocytes were less hyperphagic and accumulated less fat, but their adipocytes remained equal in size to adipocytes of controls. A role for adipocyte size in fat storage regulation and food intake control is postulated.*

The hypothesis that body weight of mammals is regulated received experimental support as early as 1939 (*1*), but the more specific hypothesis of Kennedy (*2*) that body fat mass is regulated was not directly tested until the studies of Liebelt and co-workers in 1963 and 1965 (*3*). In their studies, as well as those of others (*4*), surgical removal of adipose tissue in mice or rats resulted in enlargement of remaining fat depots relative to those of control animals. Such findings were interpreted as demonstrating compensatory growth of adipose tissue and thus that total body fat mass of mice and rats is regulated. The clearest demonstrations of such apparently compensatory growth were in brain-damaged or genetically obese animals. In contrast, in recent experiments with normal rats and mice it was found that removal of various adipose tissue depots does not result in compensatory growth of remaining depots (*5*). Thus, total body fat content of rats and mice is probably not directly regulated, as Kennedy had suggested, but regulation of some other parameter related to total body fat must be responsible for the usual stability of body fat. In this report we present evidence that body fat stability in the adult rat is achieved by means of the regulation of adipocyte lipid content (or adipocyte size), and that such regulation can operate by influencing food intake.

In the young rat, increases in both adipocyte size and adipocyte number constitute normal growth of the adipose mass (*6*), but at about the time of weaning adipocyte proliferation usually ceases. Subsequent to weaning most adipose tissue growth occurs as the result of adipocyte enlargement alone (*7*). If adipocyte size were involved in the regulation of fat storage, one might expect that adipocytes would have a tendency to resist excess enlargement and that adipose tissue growth in rats after weaning

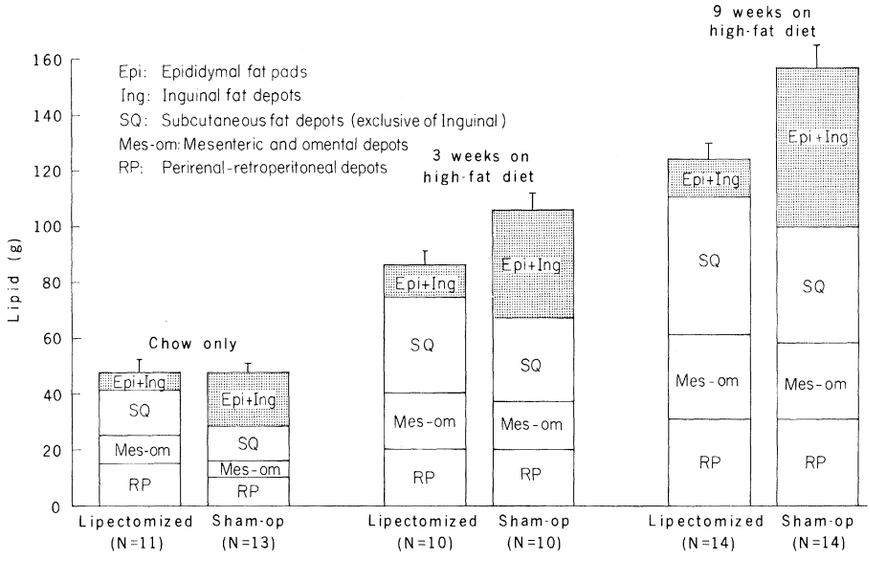

Fig. 1. Distribution of lipid among the adipose depots of 5½-month-old Osborne-Mendel rats 3 months after lipectomy or sham surgery. Rats were fed either LF (chow) exclusively, LF until age 19 weeks followed by 3 weeks on high-fat diet, or LF until age 13 weeks followed by 9 weeks on high-fat diet.

would be restrained in direct relation to the strength of that tendency.

Rats which rapidly accumulate adipose mass when fed a highly palatable diet were studied under conditions in which one component of capacity to store fat, adipocyte number, was first diminished by lipectomy. We reasoned that if adipocyte resistance to enlargement exists, surgical reduction of a rat's adipocyte number would diminish its capacity for fat storage. A lipectomized rat would thus not be able to accumulate as much fat as an intact rat.

Under certain circumstances, a deficit in fat storage capacity might be of only minor consequence to a rat. Most rats do not develop large fat stores when fed only a laboratory chow diet. Thus, the storage capacity of the remaining depots in a chow-fed lipectomized rat may be sufficient to accommodate as much lipid as is stored by nonlipectomized control rats. However, if the rat is fed a very palatable high-fat diet on which it normally becomes hyperphagic and obese, reduced fat storage capacity might serve to limit the degree to which it can become obese. Consequently, a lipectomized rat might eat as much food as an intact rat when fed only laboratory chow, but it is less likely to do so when fed a highly palatable high-fat diet.

Osborne-Mendel rats were used for this study since they are known to become unusually hyperphagic and obese when presented with a high-fat diet (*8*). In the first experiment, both subcutaneous inguinal fat depots were removed from each of 35 23-day-old male rats. At 55 days of age, 75 to 80 percent of each epididymal fat pad was also removed. This particular surgical sequence was used because pilot studies had shown that removal of large portions of the rat epididymal fat pad at age 23 days induces testicular atrophy, while such surgery at age 55 days does not. Thirty-seven additional Osborne-Mendel rats, identical in age and mean body weight to the experimental rats, served as sham-operated controls.

All animals were maintained on Purina Lab Chow for at least 1 month following the second operation. Purina chow is relatively low in fat content (4.5 percent fat by weight), and is subsequently designated as LF. At 3 months of age, 14 lipectomized and 14 sham-operated rats were switched from LF to a nutritionally adequate semipurified diet, which was 55 percent fat by weight (*9*); we refer to it as HF. The daily caloric intake of HF exceeded that of LF by about 60 percent when HF was first introduced. Body fat accumulation thus proceeded rapidly. The remaining 44 rats continued on LF. Of these, ten lipectomized and ten sham-operated rats were switched from LF to HF 6 weeks later. After an additional 3 weeks all rats were killed (at 5½ months of age). Thus, there are six groups for comparison: lipectomized versus sham-operated in each of three dietary conditions: LF only, HF for 3 weeks, and HF for 9 weeks.

Figure 1 shows lipid contents in adipose depots of all rats at the end of the first experiment. It is immediately apparent that HF feeding caused a large and rapid burst of body fat accumulation. Rats fed HF for 3 weeks had twice as much depot fat as LF-fed rats, while rats fed HF for 9 weeks had three times as much depot fat as LF-fed rats. Comparison of the total mass of adipose tissue between the lipectomized and sham-operated rats for each dietary condition reveals that HF and LF feeding had different effects. The two LF groups had the same mean amount of total depot lipid at death (47.5 ± 4.6 versus 47.5 ± 3.5 g), indicating that lipectomy did not interfere with the accumulation of fat by LF-fed rats. Body weights of the two groups were also virtually identical (540 ± 17 versus 546 ± 13 g), so it is reasonable to assume that the two groups ate essentially the same amount of food during the course of the experiment. Whether the LF-fed lipectomized rats restored the small amount of lipid that was already in the depots at the time of surgery cannot be determined with adequate precision since the total lipid content of the four surgically removed depots averaged only about 3 g. However, the intact depots perfectly compensated for the absence of the excised depots by accommodating the lipid which would have normally been stored by the excised depots between the time of surgery and the time of death. Such compensation probably did not require an increase in food intake in lipectomized animals compared to controls since, for the most part, it involved accommodation of new lipid rather than replacement of surgically removed lipid.

The capacity of the fat depots for the sort of compensation seen in the LF-fed rats is clearly limited, as demonstrated by the total fat depot weights of the HF-fed rats. After only 3 weeks of HF feeding, lipectomized rats had a lipid deficit of 19.8 g compared to controls (86.0 ± 5.2 versus 105.8 ± 6.1 g), while after 9 weeks of HF feeding the deficit was 32.1 g (124.5 ± 5.6 versus 156.6 ± 8.6 g). Both of these differences in lipid content are highly significant ($P < .01$, Student's t-test), and since they were not present in rats fed LF only, they cannot be attributed to the effects of surgery alone.

Clearly, lipectomized Osborne-Mendel rats with fewer fat cells can accumulate as much lipid as control rats when only LF is available to both groups. The relatively small amount of body fat normally accumulated by the Osborne-Mendel rat on the LF diet can be easily accommodated by the remaining depots of the lipectomized rats. However, those depots cannot fully accommodate the relatively large amount of fat that is accumulated during HF feeding. The postulated adipocyte size restraints which limit adipose tissue growth apparently do not operate in the Osborne-Mendel rat when the rat is relatively lean, but when

the rat accumulates large amounts of fat, as on the HF diet, the restraints become operative. Perhaps the Osborne-Mendel rat can become so obese on a high-fat diet precisely because adipocyte size restraints are inoperative when the rat is relatively lean. In the Swiss mouse and the Sprague-Dawley rat, there appears to be far less flexibility in adipocyte size following lipectomy (*5*) and these animals are not as easily fattened by feeding of a high-fat diet.

Figure 1 also shows the site-to-site variations in fat accumulation in both LF- and HF-fed rats. Lipectomized rats fed LF achieved total body fat equivalence with control rats by significantly increasing the weights of all their remaining fat depots relative to the weights of the depots of the control rats (mesenteric and retroperitoneal: $P < .01$; subcutaneous: $P < .05$). In contrast, weights of the remaining depots were not significantly different between lipectomized and control HF-fed rats. The accumulated lipid deficit in the HF-fed lipectomized rats is thus due to a failure of their remaining depots to contain a lipid excess relative to the depots of controls. As the epididymal and inguinal depots of HF-fed control rats enlarge, the relative lipid deficit in the lipectomized HF-fed rats increases correspondingly.

Adipose cellularity of all depots depicted in Fig. 1 was measured by a previously published method (*10*). Lipectomized rats had a total of 25 percent fewer adipocytes than controls in the dissectible depots. Lipectomized rats fed LF had significantly larger fat cells than their controls (retroperitoneal: 0.99 ± 0.08 versus 0.70 ± 0.05 μg of lipid per cell; mesenteric: 0.44 ± 0.03 versus 0.29 ± 0.02 μg of lipid per cell; $P < .01$, Student's t-test). However, with ad libitum HF feeding these cell size differences were obliterated; lipectomized rats fed HF for either 3 or 9 weeks had fat cells that were equivalent in size to the fat cells of their controls (for example, with 9 weeks of HF, retroperitoneal: 1.4 ± 0.08 versus 1.5 ± 0.07 μg of lipid per cell; mesenteric: 1.4 ± 0.08 versus 1.2 ± 0.09 μg of lipid per cell). As suggested above, it indeed appears that the regulatory restraints which operate on the HF-fed rats to achieve adipocyte size equivalence between the lipectomized and control rats do not operate at the relatively small adipocyte size of the LF-fed rats.

As predicted, the differences in fat storage in HF-fed rats were closely related to changes in food intake. Figure 2a depicts the mean differences in body weight and cumulative food intake between the 14 lipectomized and 14 sham-operated rats fed HF for 9 weeks. It thus illustrates both the pattern with which a relative body weight deficit developed in the lipectomized rats in the first experiment, and the relative decline in food intake associated with the development of that deficit. The body weight difference is not significant at any particular time point, presumably because body weight variance is large relative to the body fat difference between the two groups. However, comparison of the actual daily food intakes of the two groups by means of analysis of variance reveals that the food intake decline in the lipectomized rats is significant ($F = 5.45$, 1/598 d.f., $P < .05$). Furthermore, examination of the cumulative food intake difference curve reveals that the food intake divergence does not occur until about 3 weeks after the introduction of the high-fat diet. This observation supports our view that the lipectomized rats were no more distressed by the surgery than their controls. It also suggests that restraints on lipid accumulation were no different between the two groups during the initial period of weight gain. Evidently HF was equivalently palatable to both groups of rats, but some aspect of the resultant fat accumulation affected them differently so that eventually they responded differently to the diet.

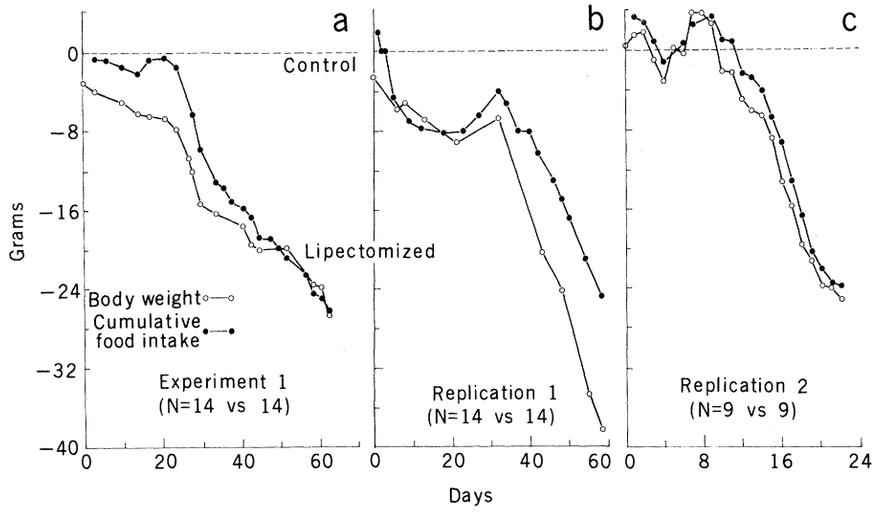

Fig. 2. Body weight and cumulative food intake differences between lipectomized and control Osborne-Mendel rats fed a high-fat diet. Control values have been set to zero. Note that the major food intake decline begins after 3 to 4 weeks of high-fat feeding in (a) and (b) but after only 10 days in (c). Relative declines in cumulative food intake were significant in each of the three experiments ($P < .05$, analysis of variance). There were no significant correlations between food intake decline and adiposity or adipose cellularity in the lipectomized rats, but total food intake and total body fat were significantly correlated in both lipectomized and sham-operated groups (in experiment 1 the correlation coefficient $r = .54$ for lipectomized rats and .94 for sham-operated rats).

To be assured of the consistency and replicability of these findings we repeated the experiment twice: once under exactly the same experimental conditions as given above (Fig. 2b), and once with rats begun on HF at 5½ months of age (Fig. 2c). In each replication the results were essentially indistinguishable from those of experiment 1, except that in replication 2 the food intake and body weight divergences occurred after only 10 days of HF feeding. The rats in replication 2 were older and thus presumably had larger adipocytes than the rats in the other experiments at the time HF was introduced, which supports our view that regulatory restraints in adipose tissue are more effective when adipocytes are relatively large.

The experiments reported here demonstrate that when lipectomized Osborne-Mendel rats are fed a highly palatable diet, they accumulate less body fat and consequently eat less food than sham-operated controls. Capacity for fat storage is thus reduced by lipectomy unless the lost tissue can regenerate (*11*). The finding that adipocyte size is equal in rats showing restrained and relatively unrestrained hyperphagia strongly suggests that adipocyte size is an important element in the regulation of energy storage and the control of food intake.

Adipocyte hypertrophy is a uniformly observed characteristic of obesity in man as well as in rats. Our findings suggest that such hypertrophy may not be a completely passive occurrence. It is likely that adipocyte resistance to enlargement was responsible for restraining the development of obesity in our lipectomized Osborne-Mendel rats, and such resistance may normally operate in rats, and perhaps in man, to restrain excessive feeding and weight gain. The hyper-

trophic component of at least some forms of obesity may thus be due to an abnormality in the mechanisms which constitute adipocyte resistance to enlargement. Whether there is some neural or humoral mediation between adipocyte size and feeding behavior is not known, but some such association now seems likely.

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9. The high-fat diet was composed (percentages by weight) of Crisco (55), casein (25), dextrose (13), salts (4), Nutritional Biochemicals Vitamin Mix (2.82), L-cystine (0.12), and L-cysteine-HCl (0.06).
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11. We have found that when inguinal depots are removed from 3-week-old rats, complete regeneration will occur over a period of about 7 months, if the rats are fed a high-fat diet [I. M. Faust, P. R. Johnson, J. Hirsch, *Science* **197**, 391 (1977)]. At the time the rats in the present experiment were killed (3 months after inguinal depot removal) inguinal depot regeneration was already clearly noticeable.
12. Supported in part by NSF grant PCM 76-09324 and a grant from the Howard M. Pack Foundation. The Osborne-Mendel rats were generously donated by C. Reader of the National Institute of Cancer Research. We thank J. Monahan and R. Kava for their excellent technical assistance.

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A Critical Period for Acoustic Trauma in the Hamster and Its Relation to Cochlear Development

Abstract. *Young hamsters pass through a developmental stage during which they are unusually susceptible to acoustic trauma. This sensitive period occurs after apparent structural and functional maturation of the ear and appears to be dependent on unidentified developmental changes within the cochlea.*

Exposure to loud sound can produce permanent damage in human and animal cochleas (*1, 2*). Recent research on the phenomenon of priming for audiogenic seizures (*3, 4*) and on acoustic trauma in young guinea pigs (*5*) suggests that young mice from certain inbred strains and young guinea pigs are particularly susceptible to acoustic trauma. The data in these studies do not provide any evidence on the locus of the developmental changes presumed to underlie the observed changes in susceptibility. The object of this study was to examine susceptibility of the hamster ear to acoustic trauma as a function of age. The data show that (i) young hamsters pass through a critical period of susceptibility to noise trauma and (ii) the developmental events underlying this phenomenon appear to occur in the cochlea.

Hamsters (*Mesocricetus auratus*) were obtained from a commercial dealer (*6*) at specific ages or were bred in the laboratory. Animals were exposed to an octave-band noise (5 to 10 khz, 125 db re 20 μN/m^2) for 2.5 minutes at one of the following ages: 11, 15, 19, 23, 27, 31, 40, 48, 55, 62, or 75 days after birth. Five days after noise exposure, cochlear microphonic (CM) responses were measured in exposed animals and in control animals of the same ages. The procedures used for noise exposure and CM recording have been described (*4*) and will be mentioned briefly here.

Hamsters were anesthetized with urethane (1.5 mg per gram of body weight, injected intraperitoneally) and placed in a head holder after tracheal cannulation. The ear canal was excised at the level of the tympanic ring, and the auditory bulla was exposed. The tip of a sound speculum containing a calibrated probe tube was sealed over the tympanic ring, and a silver ball electrode was placed onto the round window through a fenestra in the bulla. The intensity of sound (in decibel sound pressure level) necessary to produce a criterion CM response of 1.0 μv was determined at frequencies between 0.5 and 20.0 khz. All surgery and CM recording were conducted using appropriate double-blind procedures.

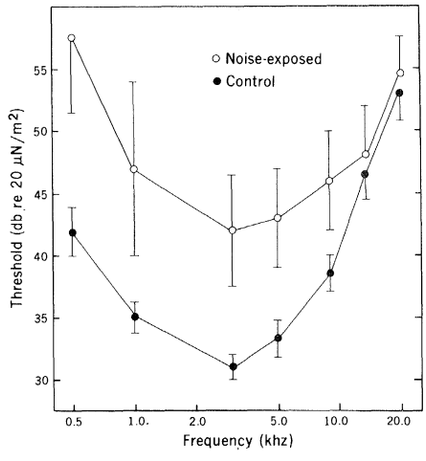

Fig. 1. Mean (± S.E.) 1.0 μv threshold versus frequency for six 45-day-old control animals and nine 45-day-old animals exposed to noise at 40 days of age.

Mean thresholds at each frequency were determined for control and noise-exposed animals in each age group. Examples in one age group are presented in Fig. 1. These data typify the pattern of threshold loss seen in all age groups in which significant threshold losses were observed (*7*). The difference between means for control and noise-exposed animals at a given frequency provided a measure of threshold loss at that frequency in a particular age group. These measures of threshold loss (in decibels) were averaged over all test frequencies (0.5,1,3,5,9,13.5, and 20 khz) in each age group. Threshold loss, measured 5 days after noise exposure, was dependent on developmental age (Fig. 2A). Although the amount of threshold loss within a given age group depended on frequency, the general relationship between threshold loss and age at noise exposure was similar at all frequencies tested (*7*).

It seems unlikely that a critical period of susceptibility to acoustic trauma has any adaptive advantage for the young hamster. Rather, we interpret the existence of the phenomenon as indicating that some developmental change is occurring between 27 and 55 days of age, a correlate of which is enhanced susceptibility to noise-induced CM deficit, a deficit that presumably reflects permanent damage to the organ of Corti (*8*).

It might be expected that a given noise exposure would not be maximally effective in producing cochlear damage before about 20 days after birth. Structural development of the hamster ear has been studied by Stephens (*9*), who reported that the middle and inner ear appear mature by light microscopy at about 30 days after birth. At 15 days, the malleoincudal joint has not yet ossified, and mesenchyme continues to be abundant in the middle ear. Thus, there is no reason to expect the middle-ear transmission system fully to function before 20 days of age. However, given the apparent structural maturation of the ear by 20 days, there is no obvious reason to predict that the effectiveness of noise exposure in inducing threshold loss should decrease