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Adipose Tissue Regeneration in Adult Rats[1] (40501)

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Excessive numbers of adipocytes are a major component of severe obesity in man (1, 2). Thus, the achievement of a medical solution to the problem of severe obesity probably requires that we first have knowledge of the mechanisms which control adipocyte proliferation and differentiation. Recent studies of DNA synthesis in adipose tissue (3), of the behavior of cultured cells isolated from adipose tissue (4) and of adipocyte hyperplasia following lipectomy in young rats (5) have begun to provide some of that knowledge. The present report concerns the effects of lipectomy in adult rats.

During the first few weeks of life in the normal rat, adipocytes are produced at a rapid rate in all major adipose depots. However, by the time the rat attains sexual maturity, adipocyte production in the various depots either ceases or slows appreciably. Thus, the number of lipid-filled cells in the epididymal pads of the adult rat does not increase at all with age (3, 6, 7), while the number of such cells in the retroperitoneal depots doubles over the course of about a year in at least certain rat strains (7, 8). Such cessation or slowing of the appearance of new lipid-filled cells may be the result of an age-dependent process or it may be the result of feedback inhibition from the adipose mass. If it is age-dependent, then regeneration of a depot following its complete or partial removal in the adult should not occur. On the other hand, if adipocyte production continues until it is inhibited by the presence of a certain number of adipocytes (as hepatocyte production is inhibited by the presence of a certain number of hepatocytes (9)), the surgical removal of large numbers of adipocytes should cause sufficient disinhibition to allow new adipocyte production and thus regeneration of the depot to occur (as partial hepatectomy allows new hepatocyte production and thus liver regeneration to occur (9)). In the epididymal fat pads regeneration does not occur following either complete or partial lipectomy even in very young rats (5, 10, 11). Taken alone, this observation suggests that age-related cessation, rather than feedback inhibition of adipocyte production, occurs in the epididymal fat pad. However, it has recently been shown that in most fat depots of the adult rat, including the epididymal pads, increases in adipocyte number can be induced by dietary means (12). Thus, at present, it is not clear whether one or the other of the above processes is involved in the control of hyperplastic growth in the epididymal pad.

Subcutaneous fat is like epididymal fat in that new adipocytes appear when adult rats are fed certain diets. However, unlike epididymal fat, subcutaneous fat does regenerate when it is surgically removed from the very young rat (5). If regeneration of subcutaneous depots occurs following lipectomy in the adult as well, continuous inhibition of proliferation or differentiation in those depots would be strongly suggested. On the other hand, if the subcutaneous depots fail to regenerate following lipectomy in the adult, age-related cessation of proliferation or differentiation of some critical cell type would be suggested.

It is clear from previous studies that subcutaneous adipose tissue regeneration in adult rats either is slow or does not occur. Kral reported that male Sprague Dawley rats lipectomized at 15 weeks of age showed no evidence of subcutaneous depot regeneration 3 months after surgery (13).

In the present study, subcutaneous–inguinal and epididymal fat depots were surgically removed from adult (16-week old) male rats. The rats were then maintained for 6 months before being killed and examined for evidence of regeneration. In addition,

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some of the rats were fed a high fat diet during the entire postsurgical period, since the results of previous studies (5, 8, 12) suggest that high fat feeding stimulates adipocyte proliferation. We reasoned that if regeneration of adipose tissue normally occurs in the adult rat, high fat feeding might accelerate the process, while if regeneration does not normally occur, high fat feeding might induce it to occur.

Materials and methods. Nine Osborne Mendel rats obtained from Camm Laboratories, New York, were lipectomized and 12 rats of the same age, sex, strain, and approximate body weight were sham-lipectomized. All rats were anesthetized with ether during surgery. Subcutaneous–inguinal and epididymal fat depots were bilaterally exposed in all rats, and removed in the experimental rats as previously described (11). Right and left depots were pooled for all analyses. The mean wet weights of the removed depots were: 9.09 ± .56 and 3.69 ± .21g respectively. Based on the results of other experiments in this laboratory, the respective mean fat cell numbers in these depots are estimated to be 20–30 × 10^6 and 10–13 × 10^6. Four of the lipectomized rats and five of the sham-lipectomized rats were fed *ad libitum* a highly palatable high fat diet routinely used in our laboratory (14). The other rats were fed Purina Laboratory Chow also *ad libitum*. Six months following surgery, all rats were killed by decapitation. All subcutaneous fat in the inguinal areas was dissected and prepared for analysis of lipid content and cellularity (15). Epididymal, retroperitoneal, and noninguinal subcutaneous depots were also dissected and analyzed.

Results. Substantial amounts of dissectable fat and extractable lipid were found to be present in the inguinal areas of lipectomized chow-fed rats, but the amounts were significantly less than in sham-operated controls. Adipocyte number in the inguinal areas of the lipectomized rats was also less than in controls, but the difference was not significant. When inguinal and noninguinal subcutaneous depots are combined for analysis, the lipectomized rats are found to have somewhat less total subcutaneous fat and lipid and somewhat fewer total subcutaneous adipocytes than controls, but the differences are not significant. Regeneration of the inguinal area fat thus was substantial, but perhaps not complete, in the chow-fed rats (Table I).

Regeneration was also substantial in the high-fat-fed rats. In absolute terms, more cells were restored to the inguinal areas of high-fat-fed rats than to the inguinal areas of chow-fed rats. However, relative to high-fat-fed controls, restoration of adipocyte number was only about 50% complete. Thus, removal of the inguinal depots is a sufficient stimulus for the promotion of inguinal depot regeneration in adult rats while an additional stimulus such as high-fat-feeding may accelerate the process somewhat, although not enough to produce cell number equivalence with high-fat-fed controls within 6 months. With either diet there are no differences in adipocyte size between lipectomized rats and their

TABLE I. ADIPOSE TISSUE CHARACTERISTICS OF INGUINAL AND COMBINED NONINGUINAL SUBCUTANEOUS FAT DEPOTS IN 10-MONTH-OLD MALE OSBORNE MENDEL RATS LIPECTOMIZED AT 4 MONTHS OF AGE.

	Inguinal				Combined noninguinal subcutaneous				Total subcutaneous fat		
	Wet weight (g)	Total lipid (g)	Cell size (μg lipid/cell)	Cell no. (×10^6)	Wet weight	Total lipid	Cell size	Cell no.	Wet weight	Total lipid	Cell no.
High-fat-fed											
Lipectomized	42.40[a, b, c]	31.88[b, c]	0.70[c]	47.64[b]	96.68[c]	67.23[c]	0.74[c]	91.65[c]	139.08[c]	99.10[b, c]	139.30[b, c]
(n = 4)	±6.55	±3.33	±0.08	±4.07	±11.35	±8.06	±0.09	±3.28	±15.03	±7.74	±6.39
Sham-operated	80.37	65.58	0.73	91.68	94.68	72.10	0.67	112.95	175.04	137.67	204.63
(n = 5)	±8.30	±6.71	±0.05	±12.47	±10.54	±6.32	±0.06	±19.34	±18.56	±12.92	±28.88
Chow fed											
Lipectomized	16.19[b]	9.81[b]	0.29	36.45	29.85	18.19	0.27	69.95	46.03	28.72	106.40
(n = 5)	±2.02	±1.55	±0.06	±7.77	±4.14	±2.52	±0.02	±7.12	±5.63	±3.89	±13.07
Sham-operated	25.26	15.68	0.33	48.45	30.62	18.80	0.29	64.31	55.89	34.49	112.76
(n = 7)	±2.00	±1.56	±0.03	±3.08	±3.20	±2.30	±0.04	±4.34	±5.08	±3.48	±6.05

[a] Values given are mean ± SEM.

[b] $p < .05$, Lipectomized vs sham-operated (one tailed Student's t test).

[c] $p < .05$, High-fat-fed lipectomized vs chow fed lipectomized (one tailed Student's t test).

respective controls. Thus, while regeneration is still incomplete, those adipocytes which have been newly formed are of normal size.

Surgical removal of epididymal fat did not lead to regeneration or general compensatory enlargement of other depots, regardless of which diet the rats were fed. There was practically no epididymal fat tissue evident in the rats 6 months after surgery, and there were no differences in retroperitoneal depot weight, lipid content or cell number between lipectomized and nonlipectomized rats (Table II). However, in contrast to the response to lipectomy, cell number increased substantially in the epididymal fat pads in response to the feeding of the high fat diet.

Discussion. Regeneration of subcutaneous adipose tissue thus occurs in adult rats, and proceeds at roughly the same rate as after lipectomy in weanling rats (5). On the other hand, as in earlier studies (10, 11, 13) no regeneration of epididymal fat occurred in the rats in the present experiment. It is possible that the previously reported failure to observe subcutaneous adipose tissue regeneration in adult rats (13) was due to strain differences or differences in surgical technique, but it seems more likely to have been due to the fact that an inadequate amount of time was allowed for the regeneration process to proceed: (Three months (13) vs 6 months in the present study).

The present findings support the hypothesis that the process by which new adipocytes are produced is inhibited in the subcutaneous adipose depots of adult rats, but that it can be disinhibited by lipectomy or high-fat-feeding. It remains to be determined whether it is proliferation, differentiation of a continuously proliferating cell, or differentiation of a static population of preadipocytes formed early in life that is normally inhibited. The finding that chow-fed rats restored almost as many adipocytes during the 6 months following lipectomy as did high-fat-fed rats suggests that there may be a maximum rate of subcutaneous adipocyte proliferation or differentiation which limits the rate of regeneration. Alternatively, the findings may be an indication of a fixed population of preadipocytes which is insufficient to accommodate full responses to both high-fat-feeding and lipectomy.

Our continued inability to find any degree of regeneration in the epididymal fat pad suggests that different growth restraining processes may operate in different depots; i.e. the normal termination of cell production in the epididymal pad may be the result of an age-dependent cessation of some growth process, whereas cessation of cell production in subcutaneous depots appears to be the result of feedback inhibition. The dramatic difference between the two fat depots in their regrowth response to lipectomy (particularly in conjunction with the large site-to-site variation in hyperplastic growth seen in aging rats (7) and rats fed a highly palatable diet (8, 12)) suggests that site-to-site generalizations of any sort ought to be avoided whenever possible.

Summary. Adipose tissue in subcutaneous,

TABLE II. ADIPOSE TISSUE CHARACTERISTICS OF RETROPERITONEAL AND EPIDIDYMAL FAT DEPOTS IN 10 MONTH OLD MALE OSBORNE MENDEL RATS LIPECTOMIZED AT 4 MONTHS OF AGE.

	Retroperitoneal				Epididymal			
	Wet weight (g)	Total lipid (g)	Cell size (μg lipid/ cell)	Cell no. ($\times 10^6$)	Wet weight	Total lipid	Cell size	Cell number
High-fat-fed								
Lipectomized	69.25[a]	60.16	1.08	56.58	2.32	—	—	—
($n = 4$)	±8.37	±7.41	±0.07	±7.85	±0.44	—	—	—
Sham-operated	64.29	58.51	0.95	62.08	28.14	25.18	1.05	23.93
($n = 5$)	±5.57	±5.34	±0.02	±6.31	±1.93	±1.67	±0.03	±1.37
Chow fed								
Lipectomized	16.05	13.85	0.62	22.53	1.30	—	—	—
($n = 5$)	±3.03	±2.76	±0.12	±2.11	±0.14	—	—	—
Sham-operated	15.83	13.93	0.63	22.84	9.81	8.98	0.65	13.67
($n = 7$)	±1.90	±1.65	±0.07	±2.86	±1.21	±1.17	±0.07	±0.80

[a] Values given are mean ± SEM.

but not epididymal, depots of adult rats is restored following lipectomy. The restoration process proceeds in response to lipectomy alone but may be somewhat accelerated by a diet which normally promotes large increases in adipocyte number. That adult rats can both regenerate subcutaneous adipose tissue following lipectomy and increase adipocyte number when fed certain diets suggests that in at least some depots of the adult rat adipocyte precursor cells can proliferate and differentiate, and that some element of the proliferation/differentiation sequence is normally inhibited. Whether these same processes occur in man is unknown.

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