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Adipose Tissue Regeneration in 6-Month-Old and Adult Rabbits following Lipectomy (41734)

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Abstract. The effects of surgical ablation of adipose tissue were studied in male New Zealand rabbits. They were lipectomized or sham-operated either at 6 or 12 months, ages at which size and number of adipocytes are, respectively, stabilized in this species. The lipectomized animals were subjected to removal of about 80% of the perirenal and omental and to the totality of the dorsoscapular and inguinal fat tissues. Approximately 35 and 48% of the total body fat were, thus, surgically removed, respectively, in 6- and 12-month-old rabbits. All rabbits were killed 3 months after surgery and were carefully dissected. There was no significant difference in food consumption and body weight gain between lipectomized and sham-operated rabbits. Surgical removal of dorsoscapular, inguinal, and omental fat did not lead to regeneration whereas regeneration of the perirenal fat was substantial. At sacrifice the perirenal weight reached approximately 55% of the initial weight. Regeneration of perirenal adipose tissue in adults proceeded at roughly the same rate as after lipectomy in younger rabbits. These results suggest that adipose tissue regeneration in the rabbit is site dependent.

There is a disparity among various studies as to the regenerative response after lipectomy. In all experiments with rats and mice (1–6), except that of Taylor and Bean-Hopkins (7), surgical removal of the epididymal fat pad did not lead to regeneration, regardless of the age of the animals at the time of surgery or the extent of the lipectomy. In contrast, it has been observed by most authors (4–6, 8) that excision of the inguinal subcutaneous fat depot leads to a regenerative response even in adult animals. The absence of subcutaneous adipose tissue regeneration in adult rats reported by Kral (2) may be due to differences in surgical technique, or to strain differences, or because the time allowed for the regeneration process was not sufficiently long. The regeneration proceeds in response to lipectomy alone, but may be accelerated by a high-fat diet (8) which normally stimulates adipocyte proliferation (9, 10). Faust *et al.* (8) observed a limited hyperplastic response of subcutaneous adipocytes to lipectomy in low-fat-fed weanling rats, while in high-fat-fed animals there was a complete regeneration of subcutaneous adipose tissue, in terms of both adipocyte number and adipocyte size. However, in another experiment and in adult rats, the same authors (5) found that chow-fed rats restored almost as many adipocytes as did high-fat-fed rats.

There is also great disparity among studies as to the compensatory growth of remaining depots following a surgical reduction of adipose tissue mass. Enlargement or increased lipid content of some parts of the adipose mass, in response to the removal of some other parts, has been reported to occur (1, 3, 4, 6, 8, 11, 12), but this phenomenon was not constantly observed (2, 5, 13).

It is clear from these studies that the response to lipectomy is dependent on numerous factors: site of excision, species used, time after operation, type of diet fed to the animals.

Most of the lipectomy studies have been performed in rats and mice where adipose tissues in the later stages of life show a large site-to-site variation in hyperplastic growth (14, 15). In rabbits, there have been few studies on the cellular growth of adipose tissue (16, 17). Late perirenal and dorsoscapular adipose tissues growth was only examined by Nouguès and Vézinhet (17); they reported that, in these tissues, there is rapid adipocyte hypertrophy up to 6 months of age. After this age mean adipocyte size stabilizes and growth is due to uninterrupted increase in the number of adipocytes. Beyond the age of 10 months the cellularity is stable. In other respects, Vézinhet and Prud'hon (18) have shown that rabbit internal adipose fat develops late, whereas sub-

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cutaneous fat has an earlier development. Such information made it possible to choose different sites and ages for lipectomy.

In the present study, two subcutaneous fat depots (dorsoscapular and inguinal) and two internal fat depots (perirenal and omental) were surgically removed from young (6-month-old) and adult (12-month-old) male rabbits. The rabbits were then kept for 3 months before being killed and examined for evidence of regeneration or compensation in remaining depots.

Material and Methods. Male New Zealand rabbits were housed in individual wire cages and allowed free access to water and a commercial pelleted diet (15% crude protein and 14% crude fiber in the dry matter), with a caloric concentration of 3.7 kcal/g. The room temperature was 21 ± 1°C and the animals were subjected to a lighting schedule that consisted of 12 hr of light and 12 hr of darkness.

In this paper, data are presented on two series of rabbits: 15 6-month-old rabbits and 18 12-month-old rabbits. In the first age group, 6 animals were lipectomized, 5 were sham-operated, and 6 served as controls. In the second age group, 7 were lipectomized, 5 were sham-operated, and 6 served as controls.

The lipectomized rabbits were subjected to removal of the perirenal, omental, dorsoscapular, and inguinal fat tissues. In the perirenal region, fat tissue was left intact around the kidneys to ensure adequate tissue support. In the same way the omental fat was left intact in the duodenal, pancreas, and spleen regions and great care was taken to preserve these organs and their neurovascular supply. On the contrary, the depot removal was as surgically complete as possible for the dorsoscapular and inguinal fat tissues.

The sham-operated rabbits merely had the depots exposed and handled to the same extent as the operated rabbits, without damage to the neurovascular supply.

Both groups were anesthetized by intravenous injection of pentobarbital (25 mg/kg body weight). The controls were killed and carefully dissected at the time of surgery to measure initial body composition for later comparison and to determine the percentage of tissue removed. The lipectomized and sham-operated rabbits were killed 3 months after surgery and dissected.

The dissection procedure has been reported previously by Vézinhet and Prud'hon (18). All the major fat depots: perirenal, omental, mesenteric, dorsoscapular, inguinal, epididymal, and remaining intermuscular fat depots were excised and considered as total dissectable fat. In the case of bilateral depots, right and left sites were combined.

Body weights of lipectomized and sham-operated rabbits were recorded weekly. Food intakes were calculated for individual rabbits by carefully collecting any spilled food, weighing, and subtracting it from the total food supplied. This was done for each week starting from the week before surgery. The data were analyzed using Student's unpaired t test and Mann–Whitney U test.

Results. *Extent of lipectomy.* From comparisons (Table I) between the mean weights of excised adipose tissues and the mean weights of the same fat depots in controls, it can be calculated that lipectomy removed approximately 70% of the perirenal fat in the rabbits operated at 6 months and 75% in the rabbits operated at 12 months. These percentages were, respectively, 80 and 85% for the omental fat tissue. For the dorsoscapular and inguinal fat depots which are well-individualized in the rabbit it can be considered that they were removed completely. Approximately 35 and 48% of the total body fat were, thus, surgically removed, respectively, in 6- and 12-month-old rabbits.

Weight gain and food intake. All animals in both groups lost weight during the first postoperative week. The greater weight loss observed in the lipectomized animals was due, at least in part, to the amount of adipose tissue that was removed; when correction was made for the latter, the body weight loss was similar in lipectomized and sham-operated animals. After the first postoperative week, animals of both groups gained weight at similar rates (Fig. 1). Body-weight differences between lipectomized and sham-operated rabbits were never statistically significant (Mann–Whitney U test).

Food consumption for both lipectomized and sham-operated animals was low during the first 2 weeks following surgery. The values noted before surgery were reached from the third week onward after surgery. No significant differences in weekly postsurgery food intakes

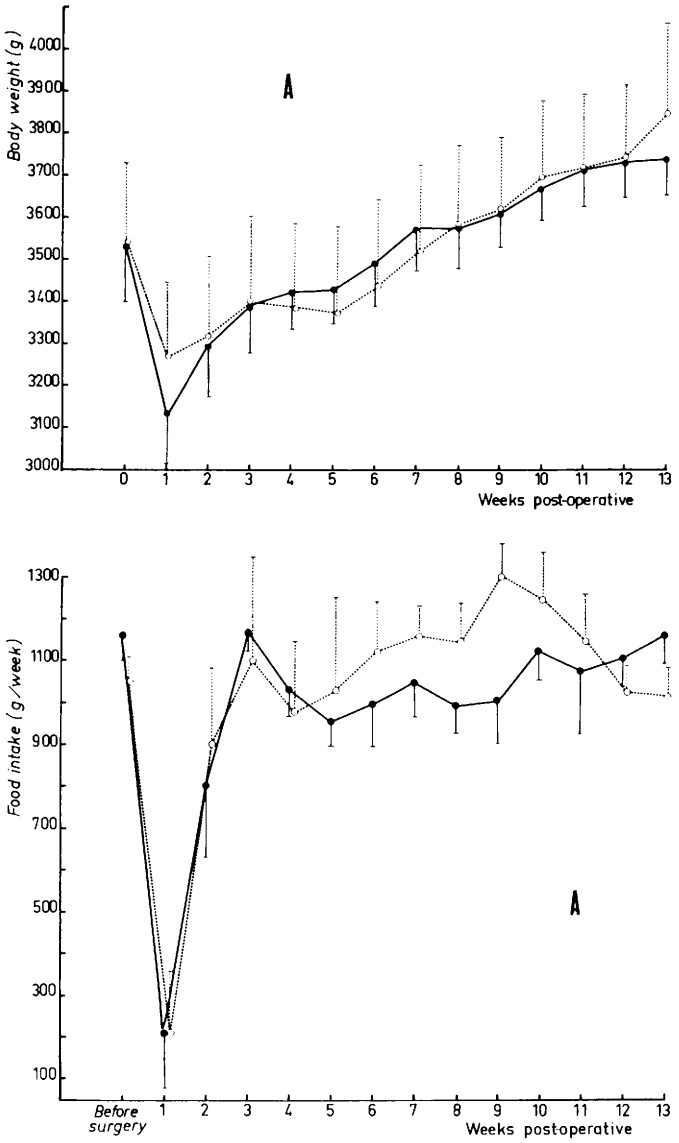

FIG. 1. Body-weight gain (±SEM) and food intake (±SEM) following lipectomy in (A) 6- and (B)12-month-old rabbits. (○) Sham-operated, (■) lipectomized.

were noticed between lipectomized and sham-operated rabbits (Fig. 1), whatever the age at the time of surgery (Mann–Whitney *U* test).

Body composition of controls. Body weight and mean fat depot weights for the major subcutaneous and internal fat depots of controls are shown in Table I. The weights of all the major fat depots were significantly higher for the 12-month-old rabbits than for the 6-month-old group, with the exception of the inguinal, epididymal, and remaining dissectable fat depots.

Body composition of lipectomized and sham-operated rabbits. Weights of dissected fat pads at sacrifice are shown in Table II. Surgical removal of dorsoscapular and inguinal fat did not lead to regeneration, regardless of the age at lipectomy. There were no inguinal or dorsoscapular fat tissues evident in the rabbits 3 months after surgery. Evidence

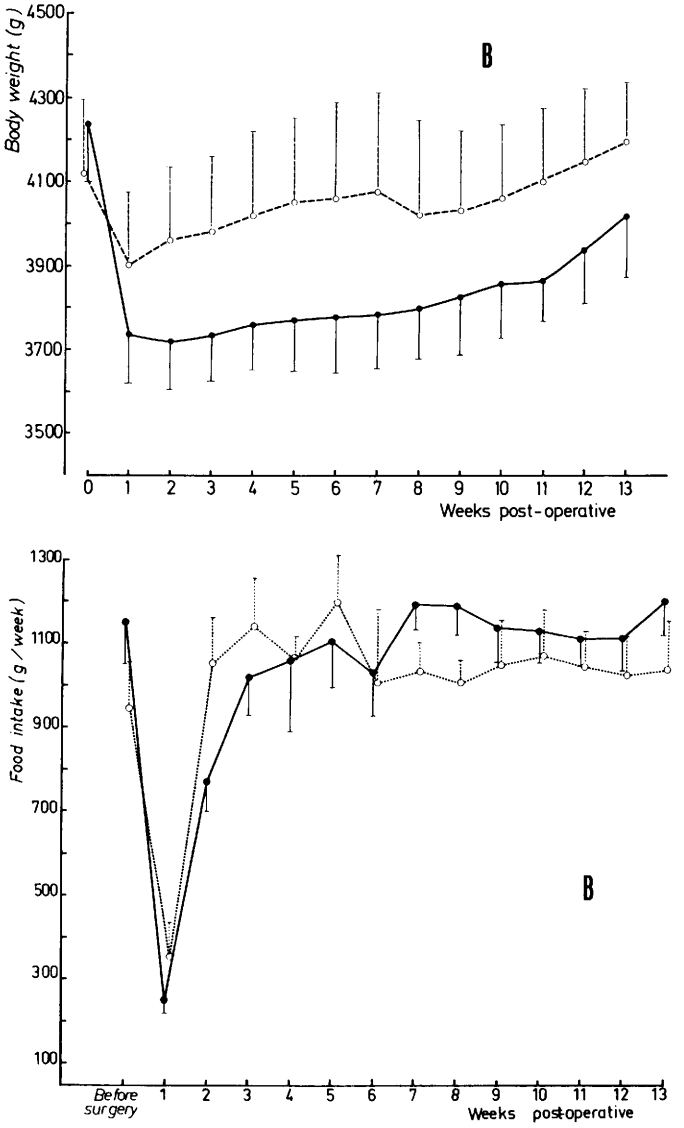

FIG. 1—*Continued.*

of regeneration of the perirenal and omental fat tissues was provided by comparing the amount of fat found at sacrifice with the amount of fat present in the controls dissected at the time of surgery. Greater amounts of dissectable fat than was left at operation were found to be present only in the case of perirenal fat. At sacrifice, no significant difference in the amounts of perirenal fat was observed between the 12-month lipectomized and sham-operated rabbits, but the amounts were significantly less in the 6-month lipectomized than in the 6-month sham-operated rabbits. The perirenal weight reached approximately 56% of the initial weight in the rabbits operated at 6 months and 53% in the rabbits operated at 12 months. Age of the animals at the time of lipectomy has not affected the capacity of the perirenal fat to regenerate. Partial regeneration of the perirenal fat occurred at areas peripheral to the initial excision site.

There was no evidence of regeneration of omental fat tissue in the 6- as well as in the 12-month lipectomized rabbits.

TABLE I. FAT DEPOTS WEIGHTS IN CARCASSES OF CONTROL DISSECTED RABBITS AND WEIGHTS OF THE VARIOUS TISSUES REMOVED IN LIPECTOMIZED RABBITS

	Controls		Lipectomized	
	6 months	12 months	6 months	12 months
Body weight (g)	3273 ± 100	4020 ± 207*	3544 ± 144	4237 ± 136
Fat pad weight (g)				
Perirenal	83.2 ± 15.7	173.4 ± 25.9*	59.5 ± 7.4	134.2 ± 20.8
Omental	16.8 ± 2.9	51.3 ± 9.3*	13.8 ± 1.1	44.6 ± 4.3
Mesenteric	36.3 ± 7.9	83.7 ± 12.4*	—	—
Dorsoscapular	15.9 ± 2.3	37.4 ± 8.9*	16.8 ± 1.8	59.2 ± 9.4
Inguinal	16.6 ± 2.2	26.2 ± 6.8	17.7 ± 2.8	31.9 ± 3.9
Epididymal	4.0 ± 0.4	4.6 ± 0.6	—	—
Remaining dissectable fat	138.4 ± 25.0	181.6 ± 36.3	—	—
Total dissectable fat	311.2 ± 53.7	558.2 ± 91.1*	—	—
	(6)	(6)	(6)	(7)

Note. Values represent means ± SEM; (*N*) number of animals.
* $P < 0.05$, 6-month-old versus 12-month-old control rabbits (one-tailed Student's *t* test).

Three months after surgery, the weight of mesenteric, epididymal, and "remaining dissectable fat depots" was not significantly different between lipectomized and sham-operated animals. There was no general compensatory enlargement of other depots following surgical removal of perirenal, omental, dorsoscapular, and inguinal fat tissues. At sacrifice, the 6-month lipectomized rabbits were significantly different from sham-operated rabbits in total dissectable fat. There was no significant difference in the case of the 12-month operated animals. In either case, there was no evidence of compensatory enlargement of other depots following surgical removal of perirenal, omental, dorsoscapular, and inguinal fat tissues. Indeed, 3 months after surgery, the weight of mesenteric, epididymal, and "remaining dissectable fat depots" was not significantly different between lipectomized and sham-operated animals.

Discussion. In this study, approximately 40% of the total body fat of New Zealand rabbits was surgically removed, which was somewhat greater than the 20–30% removed in previous studies. Moreover, two subcutaneous (dorsoscapular and inguinal) and two internal fat tissues (perirenal and omental) were removed, whereas usually the effects of adipose tissue excision have been reported only for epididymal and inguinal fat depots.

Surgically excised perirenal fat depot regenerates, whereas the dorsoscapular, inguinal, and omental fat depots do not. These results are in agreement with those of Faust *et al.* (8) and Larson and Anderson (4) which demonstrate in the rat that regenerative response is site dependent. In the rat, inguinal fat tissues regenerate after lipectomy while epididymal fat tissues do not. Regenerating fascial sheath, regrowing at the site of the inguinal fat depot and covered with unilocular adipocytes, was seen in the rat 4 weeks after surgery by Faust *et al.* (8) and as soon as Day 8 postlipectomy by Roth *et al.* (19).

Regeneration of perirenal adipose tissue in adult rabbits proceeded at roughly the same rate as after lipectomy in younger rabbits. These observations support the findings of Faust *et al.* (5) which demonstrate that in at least some depots of the adult rat regeneration occurs.

In our study, during the 3-month postlipectomy recovery period, restoration of surgically removed inguinal, dorsoscapular, and omental fat pad tissues does not appear to occur. This might be explained in different ways.

One possible explanation is that more time is needed for regrowth of these depots. Indeed, Kral (2) failed to see regeneration of inguinal depots in the rat after a 3-month postlipectomy recovery period, while Faust *et al.* (5, 8) observed such a regeneration 6 months after lipectomy. Another possibility in the case of inguinal and dorsoscapular fat depots is that

TABLE II. BODY WEIGHTS AND FAT DEPOT WEIGHTS AT SACRIFICE IN THE 6- AND 12-MONTH-OLD LIPECTOMIZED AND SHAM-OPERATED RABBITS

	Age at surgery			
	6 Months		12 Months	
	Lipectomized	Sham-operated	Lipectomized	Sham-operated
Body weight at sacrifice (g)	3741 ± 89	3880 ± 211	4010 ± 148	4195 ± 140
Fat pad weight at sacrifice (g)				
Perirenal	47.0 ± 3.5	118.8 ± 33.4*	91.9 ± 19.9	123.4 ± 24.2
Omental	5.1 ± 1.4	49.1 ± 5.5*	6.8 ± 1.7	45.1 ± 9.0*
Mesenteric	63.8 ± 7.0	62.1 ± 14.1	73.8 ± 13.6	59.0 ± 6.4
Dorsoscapular	n.r.	42.4 ± 18.3	n.r.	33.6 ± 10.0
Inguinal	n.r.	16.3 ± 3.6	1.5 ± 0.7	21.2 ± 3.6*
Epididymal	4.3 ± 0.8	4.5 ± 1.2	4.3 ± 0.9	5.6 ± 1.1
Remaining dissectable fat	137.1 ± 21.1	133.8 ± 27.3	192.9 ± 23.6	136.5 ± 16.9
Total dissectable fat	257.4 ± 29.5	426.9 ± 99.7*	362.8 ± 52.2	424.4 ± 47.3
	(6)	(3)	(7)	(5)

Note. Values are means ± SEM; (*N*) number of animals; n.r., nonregenerated.
* $P < 0.05$, One-tailed Student's *t* test between lipectomized and sham-operated rabbits.

interruption of the rich neurovascular supply, essential to fat metabolism, impairs or completely stops the growth of these particular depots in the rabbit where it can be considered that they are completely removed.

Nevertheless, there is an impressive difference between omental and perirenal fat depots in their regrowth response to lipectomy, even though they have been removed approximately in the same proportion. In this particular case there clearly seemed to be a regional difference and this might indicate the existence of different fat cell populations as suggested by Krotkiewski *et al.* (20).

The present findings are in agreement with current knowledge of the perirenal depot in the rabbit and in the rat. The studies of Bailey *et al.* (21) on Sprague–Dawley rats and of Vézinhet and Prud'hon (18) on New Zealand rabbits, show that perirenal is one of the latest maturing depots. The high growth coefficient seen in the perirenal is indicative of fat tissue which would be the last to lose its property to undergo hyperplastic growth. Lemonnier (9) and Faust *et al.* (22) found that the perirenal fat depot is the first depot to show an increase in adipocyte number due to high-fat feeding. In culture, adipocyte conversion of isolated cells from the epididymal fat decreases with age. This phenomenon is less pronounced with isolated cells from the perirenal fat, where adipocyte conversion seems to remain essentially unchanged in older rats, as reported by Björntorp *et al.* (23). The whole of these results help to explain the regenerative ability of the perirenal fat depot following lipectomy, even in the adult rabbit. This ability might be due either to inherent differences of the tissue itself, or to differences in local environment. From our study it is not possible to choose between these hypotheses. The results of Björntorp *et al.* (23) are more in favor of the first hypothesis while the results of Ashwell *et al.* (24) and Meade and Ashwell (25) suggest that differences in location or internal environment might be important.

Lipectomy did not seem to influence food intake or weight gain in this study. The results are in agreement with those of Schemmel *et al.* (1), Kral (2), Bailey and Anderson (6), and Enzi *et al.* (13) who used a less extensive method of lipectomy.

In summary, regeneration of perirenal fat tissues was observed in rabbits regardless of the age of the animals at the time of surgery. However, there was a lack of regeneration of dorsoscapular, inguinal, and omental fat tissues after 3 months postlipectomy. These results suggest that adipose tissue regeneration in the rabbit is site dependent.

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