Compensatory Growth of Adipose Tissue After Partial Lipectomy: Involvement of Serum Factors

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The regulation of body weight/fat was studied by investigating mechanisms for compensatory adipose tissue growth after removal of bilateral epididymal fat pads from male adult Wistar rats. Food intake during the first 4 weeks and energy expenditure on Days 8–10 postsurgery were not different between lipectomized and sham operated rats. During Days 29–31 post surgery, a small (2.4%) but significant ($P < 0.05$) increase in heat production per metabolic body size was detected in lipectomized as compared with sham operated rats. The carcass composition of lipectomized and sham operated rats was not significantly different 16 weeks after surgery. The compensatory growth was fat pad–specific: mesenteric, retroperitoneal, and inguinal fat pads, but not perirenal fat pads, were heavier in lipectomized rats than in sham operated rats as early as 4 weeks postsurgery. Examination of fat cell size distribution in the compensating pads indicated a shift toward larger cells in retroperitoneal fat, but not in inguinal fat of lipectomized as compared with sham operated rats. Serum from lipectomized rats, but not media conditioned by exposure to retroperitoneal fat pads from lipectomized rats, stimulated proliferation of preadipocytes *in vitro* more than that from sham operated rats. Thus, compensatory adipose tissue growth after lipectomy may be mediated, in part, by blood-borne factors that are derived from tissues other than adipose tissue. Exp Biol Med 229:512–520, 2004.

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That body fat may be regulated was first proposed by Kennedy's (1) lipostatic hypothesis, which suggested that long-term energy balance is achieved by controlling lipid energy stores. Subsequent studies using surgical removal of fat (partial lipectomy) as a means of directly reducing total fat content have demonstrated compensatory increases of body fat in lipectomized animals of many species (reviewed in Ref. 2). The mechanisms by which the total body fat level recovers after lipectomy are not well understood. Food intake has been measured in many studies but with conflicting results. In most instances, fat pad removal does not produce significant increases in food intake (reviewed in Ref. 2), prompting the suggestion that changes in the metabolic handling of ingested calories and/or decreases in energy expenditure must be responsible for the increase in lipid deposition that occurs following lipectomy (2). To our knowledge, however, energy expenditure has not been determined in animals recovering from lipectomy.

Animals typically compensate for the fat removed during lipectomy through an increase in mass of nonexcised fat depots. In general, adipose tissue expansion is characterized by adipocyte hyperplasia and/or hypertrophy and is affected by many variables including blood-borne factors that originate from both fat and nonfat tissues and paracrine factors that are secreted by adipose tissue and regulate the proliferation and/or differentiation of preadipocytes (3). Potential signals conveying the information of body weight/fat reduction of lipectomized animals and the pattern of cellularity change in the compensating fat depots have not been defined, although a recent study with mice indicated that leptin, an adipose tissue–derived hormone thought to be involved in the regulation of energy balance, is not required for the compensatory recovery of body fat after lipectomy (4). The potential involvement of other circulating and paracrine factors in the compensatory growth of adipose tissue after lipectomy has not been explored.

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Thus, the present study in Wistar rats addressed the following questions: (i) Do alterations in food intake and/or energy expenditure account for the increase in lipid deposition following lipectomy? (ii) What is the pattern of cellularity changes in specific fat pads during compensatory growth? (iii) Are blood-borne factors involved in the compensatory growth? (iv) Do paracrine factors secreted from adipose tissue stimulate the compensatory growth? To address these questions, food intake was monitored and indirect calorimetry measurements were performed on sham operated and lipectomized rats during the first 4 weeks postsurgery. Fat depot weight and cell size distribution profiles were determined at 2, 4, or 16 weeks. Primary preadipocyte cultures were used as a bioassay system to demonstrate the presence of proliferative factors in serum and adipose tissue conditioned medium collected from the 2- and 4-week animals.

Materials and Methods

Animals and Surgical Procedures. Seventy-four adult male Wistar rats (300–325 g) were obtained from Harlan Sprague Dawley (Indianapolis, IN). The rats were single housed in plastic cages with Tek-fresh bedding from Harlan and had free access to tap water and chow diet (LabDiet 5012; PMI Nutrition International, LLC, Brentwood, MO) throughout the experiment. The room temperature was kept between 22° and 23°C. The light:dark period was 12:12 hrs. There was 1 week for the rats to adjust to the new environment before surgery. Experimental protocols were approved by the University of Georgia Animal Care and Use Committee and conducted according to the National Institutes of Health and U.S. Department of Agriculture guidelines.

Prior to surgery, the rats were distributed into two groups (lipectomy or sham) matched for body weight (320–330 g). Isoflurane (IsoFlo; Abbott Laboratories, North Chicago, IL) was used as anesthesia for all surgeries. For lipectomy surgery, rats were positioned on their backs; a single, small, longitudinal incision of about 1.5 cm in length was made in the skin of the abdominal area, and a second incision was made in the peritoneal wall. The epididymal fat pads were gently pulled out of the peritoneal cavity and dissected away from the testes taking care not to damage the spermatic artery or vein. Following the lipectomy, the intact testes were returned to the peritoneal cavity, and both incisions were sutured with silk sutures. The sham operation was similar to that for the lipectomized rats except that the fat pads of rats in the sham surgery group were not excised.

Following surgery, food intake and body weight were measured on a subset of rats daily at the same time each day for 4 weeks. Energy expenditure was measured on two sets with 12 rats per set (six lipectomy and six sham) on Days 8–10 and Days 29–31 postsurgery using a computer-controlled indirect calorimeter with 12 open-circuit respiration chambers (Oxymax; Columbus Instruments, Columbus, OH) as described previously (5). Average oxygen consumption, carbon dioxide production, respiratory quotient (RQ, carbon diaoxide produced/oxygen consumed), and average heat production per metabolic body size (kilojoules per kilogram$^{0.75}$) were determined. The rats adjusted to the chambers for 24 hrs before energy expenditure measurements were used as experimental data.

At 2, 4, or 16 weeks postsurgery, lipectomized rats and their sham controls were sacrificed by decapitation (n = 10, 12, and 16 rats/treatment at 2, 4, and 16 weeks, respectively). Inguinal, retroperitoneal, mesenteric, perirenal, and epididymal fat pads and testes were dissected and weighed. Small samples (~50 mg) of inguinal and retroperitoneal fat were fixed in osmium tetroxide for fat cell size and number determination by Coulter counter as described previously (6). Adipose tissue conditioned medium was prepared from the retroperitoneal fat pads. The gastrointestinal tract was cleaned and returned to the carcass, and carcasses from rats sacrificed at 16 weeks after surgery were analyzed for body composition as described previously (7). In brief, the frozen carcass (including gastrointestinal tract, but not the inguinal and retroperitoneal fat pads) was autoclaved at 140°C for 40 mins and homogenized with an equal weight of water. Triplicate aliquots of homogenate were analyzed for lipid content by chloroform:methanol extraction. Water content was determined on triplicate aliquots dried at 70°C for 7 days to a constant weight. Ash content was determined on the same samples held at 500°C overnight. Protein content was calculated by difference.

Conditioned Media Preparation. Retroperitoneal and inguinal fat pads were quickly removed, further dissected to remove visible blood vessels, finely minced, rinsed three times in 37°C Hanks' balanced salt solution, blotted on P8 filter paper, and weighed. Adipose tissue conditioned media were prepared as described previously (6). Ten milliliters DMEM/F12 Ham's medium containing 72 mM gentamicin sulfate, 120 mM cefazolin, and 27 mM amphotericin B was added per 1 g tissue, and samples were incubated for 4 hrs at 37°C in a humidified 5% CO_2 atmosphere, after which the adipose tissue conditioned media were filtered from the minced adipose tissue through P8 filter paper and stored frozen at –80°C.

Bioassay System: Primary Cell Culture. Inguinal fat pads were excised aseptically from pentobarbital-anesthetized, male young Sprague-Dawley rats (80–100 g body weight). Adipose tissues from two rats were pooled, and stromal-vascular cells and preadipocytes were isolated as described previously (8). Briefly, tissues were minced and incubated with 5 ml/g tissue of digestion buffer (0.1 M N-[2-Hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] [HEPES] with 1000 U/ml collagenase) for 2 hrs in a 37°C shaking water bath (110 rpm). Undigested tissue was removed by filtering through 240 and 20 μm nylon mesh. Filtered cells were resuspended in Dulbecco's modified Eagle's medium (DMEM)/F-12 Ham's (containing 72 mM

gentamicin sulfate, 120 mM cefazolin, and 27 mM amphotericin B) and centrifuged at 600 *g* for 10 mins to separate the fat cells from the pelleted stromal-vascular (S-V) cells. Aliquots of S-V cells were stained with Rappaport's stain and counted on a hemocytometer. Cells were seeded on 12.5 cm^2 tissue culture flasks (with canted neck and 0.2 μm vented seal cap) with 2 ml of plating medium (DMEM/F12 Ham's medium, 10% fetal bovine serum, antibiotics) at a density of 4.8×10^3 cell/cm^2 and cultured at 37°C in a humidified 5% CO_2 atmosphere.

Bioassay System: Proliferation Assay. On Day 1 after seeding, the plating medium was removed, and cultures were rinsed and replaced with DMEM/F-12 Ham's with antibiotics until treatment media were applied on Day 2. Proliferation of preadipocytes and S-V cells in response to test media was determined by monitoring [^{3}H]-thymidine incorporation during the exponential growth phase (9). Cultures were treated with basal control medium (DMEM/F-12 Ham's with antibiotics, 0.5% porcine serum) or test media (for proliferation assay testing conditioned media [Ref. 6]: 25% adipose tissue conditioned media, 75% DMEM/F12, 0.5% porcine serum; for proliferation assay testing rat serum: 0.5% rat serum, 0.5% porcine serum, remainder DMEM/F12 Ham's) containing 0.50 μCi/flask [^{3}H]-thymidine from Days 2 through 5 of culture. On Day 5, the flasks were rinsed and re-fed with lipid-filling medium (10% porcine serum, 1.0 nM porcine insulin, and 10 U/ml heparin in DMEM/F12 Ham's with antibiotics) to promote lipid accretion in the preadipocytes. Lipid-filling medium was changed every other day through Day 13. On Day 15, the cells were enzymatically harvested using Hanks' balanced salt solution containing 0.5% bovine serum albumin (BSA), 0.5 mg/ml trypsin, and 125 U/ml collagenase. The lipid-filled mature adipocytes (preadipocytes now fully differentiated) and the non–lipid-filled S-V cells were separated by density gradient centrifugation through Percoll as described by Novakofski (9). The incorporation of [^{3}H]-thymidine in both cell fractions was determined by scintillation counting.

Statistical Analyses. Daily body weight and food intake measures were modeled separately by repeated-measures analysis of variance (ANOVA) using presurgical body weight as a covariate and *post hoc* Student's *t* tests at individual time points. Differences in single time point measures of body composition and fat pad and testes weights were determined by Student's *t* test. Differences in energy expenditure, cellularity parameters, and proliferation activity were determined by multivariate ANOVA (MANOVA) and Duncan's multiple range test using Statistica software (Statistica; StatSoft, Tulsa, OK). For all statistical analyses, differences were accepted as significant at the $P < 0.05$ level.

Results

The weight of surgically excised epididymal fat pads was 2.96 ± 0.32 g (means±SE), which accounted for 0.876% ± 0.093% of total body weight. Surgery caused a transient weight loss that was greater for the lipectomized as compared with the sham operated rats (Fig. 1). Both groups rapidly recovered, and there were no significant differences in body weight between the groups from Day 7 postsurgery through the end of the experiment.

Lipectomized rats ate less compared with the sham rats during the first 2 days after surgery, but food intakes were not different from Day 3 through the remainder of the period monitored (data not shown). Cumulative food intake over the first 4 weeks after surgery was not different between the sham operated and lipectomized rats (Table 1). Heat production, adjusted for metabolic body size (kilojoules per kilogram$^{0.75}$ per min) and RQ were not significantly different between the lipectomized and sham rats on Days 8–10 postsurgery (Table 1). At 4 weeks postsurgery (Days 29–31), a small (2.4%) but significant ($P < 0.05$) decrease in heat production adjusted for metabolic body size was detected in the lipectomized rats (Table 1).

Carcass composition was not significantly different between lipectomized and sham operated rats at the end of 16 weeks postsurgery (Table 1), which suggests that the lipectomized rats had compensated for the removal of fat.

Two weeks after surgery, there were no significant differences in the weights of the inguinal, retroperitoneal, mesenteric, or perirenal fat pads of lipectomized and sham operated rats (Fig. 2A). There was, however, a tendency ($P = 0.07$) toward lipectomized rats having relatively heavier mesenteric fat pad weights than sham rats (per 100 g body weight; data not shown). Four weeks postoperatively, lipectomized rats had significantly heavier inguinal, retroperitoneal, and mesenteric fat pads than the sham rats ($P < 0.05$); however, the weight of the perirenal fat pads was similar between lipectomized and sham rats (Fig. 2B). Sixteen weeks after surgery, weights of the inguinal, retroperitoneal, and mesenteric fat pads remained numerically higher in the lipectomized as compared with the sham rats, although they were not statistically different as the result of a greater intragroup variability at this time point (Fig. 2C). As expected, the weights of the epididymal fat pads were markedly less in lipectomized rats than in sham rats 2, 4, and 16 weeks postsurgery (Fig. 2). Testes weights were also significantly less in lipectomized rats than in sham rats at the end of 2, 4, and 16 weeks postsurgery (Fig. 2).

To determine what accounted for compensatory growth in adipose tissue mass, cell size distribution profiles were determined for two of the compensating fat pads, the inguinal and the retroperitoneal. As shown in Figure 3A, the percentage of inguinal adipocytes in each of five discrete size ranges between 30 and 230 μm in diameter was similar between lipectomized rats and sham rats at all time points studied. In the retroperitoneal fat pad there was no

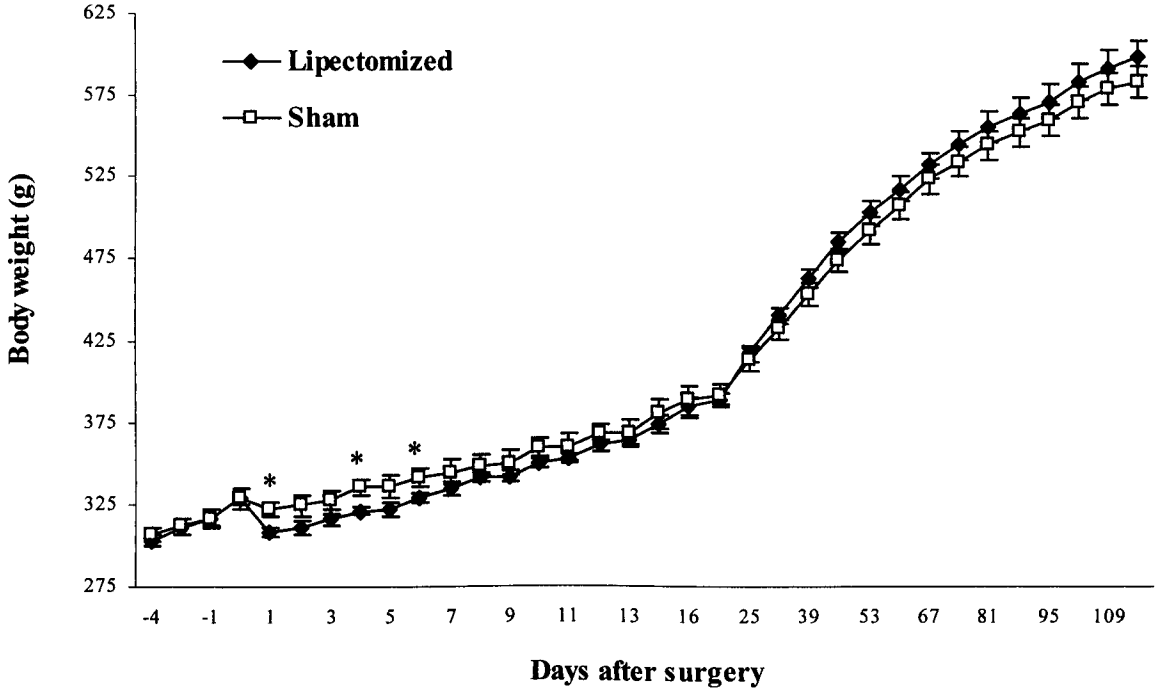

Figure 1. Body weight of sham operated and lipectomized Wistar rats through 16 weeks postsurgery. Data are means ± SE for groups of 13 rats per group. Asterisk (*), significant difference ($P < 0.05$) between groups.

difference in cell size distribution between the lipectomized and sham rats at 2 weeks postsurgery (Fig. 3B). From 2 to 4 weeks, there was a reduction in the percentage of cells in the smallest size range (30–60 μm) for both groups of rats, with the concomitant increase detectable at a larger size range for the lipectomized (110–140 μm) as compared with the sham (70–100 μm) rats. At 16 weeks postsurgery, the percentage of cells in the 110–140 μm size range remained significantly greater ($P < 0.05$) for the lipectomized rats as compared with their sham counterparts. Overall, this suggests that cellularity changes associated with compensatory growth of adipose tissue following lipectomy are fat pad–specific, with hypertrophy being a predominate feature for the retroperitoneal fat pad.

The potential contribution of blood-borne and adipose-derived factors to the hyperplasia observed in the lipectomized rats was tested in an *in vitro* preadipocyte proliferation assay. Samples tested were from the rats sacrificed 2 or 4 weeks after surgery, as they were expected to have a more active response to the lipectomy. A main effect of treatment was observed as serum from lipectomized rats stimulated the proliferation of preadipocytes to a higher degree than

Table 1. Food Intake, Energy Expenditure, and Body Composition of Sham Operated and Lipectomized Rats[a]

	Sham operated	Lipectomized
Cumulative food intake, Days 1–28, g	691.2 ± 15.2	686.4 ± 10.8
Heat production, kilojoules/kg$^{0.75}$/min		
Days 8–10	361.8 ± 9.4	370.2 ± 11.0
Days 29–31	334.4 ± 3.4*	342.6 ± 3.4**
Respiratory quotient		
Days 8–10	0.93 ± 0.03	0.94 ± 0.06
Days 29–31	0.94 ± 0.03	0.95 ± 0.03
Carcass composition, 16 weeks		
Weight, g	512.0 ± 10.7	520.0 ± 9.7
Fat, %	12.3 ± 0.6	11.4 ± 1.0
Water, %	60.9 ± 0.5	61.6 ± 0.8
Protein, %	23.8 ± 0.4	24.2 ± 0.5
Ash, %	3.01 ± 0.24	2.95 ± 0.26

[a] Data are means ± SEM for groups of 12–16 rats. For determination of energy expenditure, animals were housed in the respiration chambers for 24 hrs before experimental data were collected. Values shown are the average from the second through fourth days that the rats were in the respiration chambers during each of the two measurement periods. Chemical carcass composition was determined on the animals used for energy expenditure measurements 16 weeks after sham operation or lipectomy.

*, ** Values with different superscripts are significantly different ($P < 0.05$).

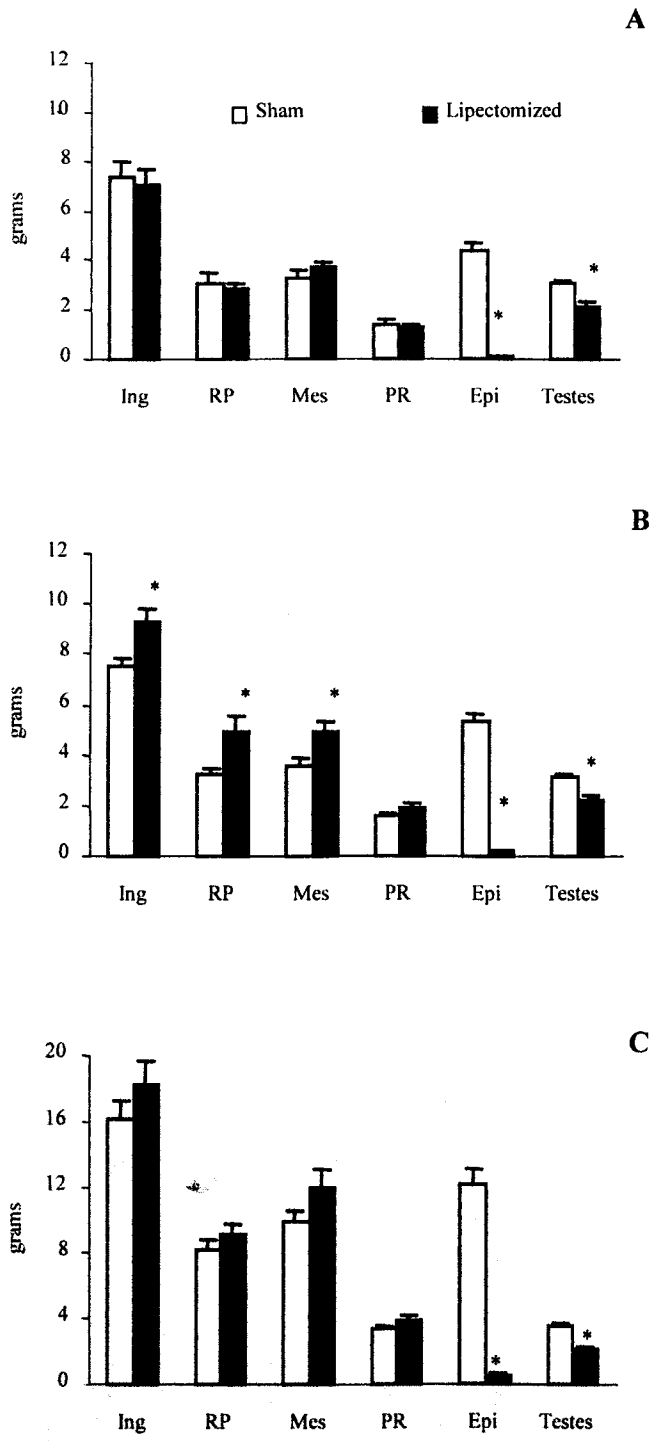

Figure 2. Weights of different fat depots and the testes 2 (A), 4 (B), and 16 (C) weeks after sham operation or lipectomy surgery. Data are means + SE for groups of 10–16 rats per treatment. Asterisk (*), significant difference ($P < 0.05$) between sham operated and lipectomized rats. Ing, inguinal; RP, retroperitoneal; Mes, mesenteric; PR, perirenal; Epi, epididymal.

serum from sham rats (Fig. 4A). This response was slightly higher with serum from the lipectomized rats at 2 weeks as compared with 4 weeks postsurgery, 22% versus 17%, respectively. In contrast, serum from the lipectomized and sham rats, at either 2 or 4 weeks, stimulated the proliferation of stromal-vascular cells to a similar extent (Fig. 4B). Likewise, media conditioned by exposure for 4 hrs to retroperitoneal fat from lipectomized rats exhibited similar proliferative activity as that prepared from the sham operated rats at either 2 (Fig. 5A) or 4 (Fig. 5B) weeks after surgery. Overall, conditioned media collected from retroperitoneal fat 4 weeks after surgery increased the incorporation of thymidine into preadipocytes (Fig. 5A) to a higher degree than that collected 2 weeks after surgery. Conditioned media collected from retroperitoneal fat of the sham rats 4 weeks after surgery also increased the incorporation of thymidine into stromal vascular cells (Fig. 5B) to a higher degree than that collected 2 weeks after surgery.

Discussion

This study examined changes in food intake, energy expenditure, and regulatory factors that may mediate the compensatory growth of adipose tissue following partial lipectomy. Food intake and energy expenditure measurements focused on the early period up to 4 weeks postsurgery and did not detect measurable increases in food intake or decreases in energy expenditure that could account for the lipid deposition associated with compensatory growth. Serum collected from lipectomized rats 2 or 4 weeks after lipectomy stimulated thymidine incorporation into proliferating preadipocytes more than that collected from sham rats. To our knowledge, this is the first study to show that circulating factors may contribute to the compensatory growth by stimulating the proliferation of preadipocytes. The identity and source of the circulating proliferative factor(s) have not been determined. Conditioned media prepared using adipose tissue of the lipectomized rats failed to stimulate preadipocyte proliferation, which suggests that circulating factors released from tissues other than adipose tissue are associated with the compensatory growth following lipectomy. The fat pad–specific nature of the compensatory response, however, indicates that the regulation of body fat following lipectomy is complex and not mediated solely by changes in circulating regulatory factors.

Body fat was restored in the lipectomized rats by 16 weeks; therefore it is logical to expect a positive energy balance as the compensatory growth develops. Both food intake and energy expenditure were measured during the early recovery period. The amount of food consumed by the lipectomized rats was not different compared with that of sham rats during the first 4 weeks after surgery. This is in agreement with the majority of studies that suggest that food intake after lipectomy is not increased during the period when the compensatory growth occurs (reviewed in Refs. 2, 10, 11). As food intake is generally not different between lipectomized and sham rats and there is an excess of calories accumulated in the form of adipose tissue, in theory, energy expenditure must be decreased to have positive energy balance for the compensatory growth. To our knowledge,

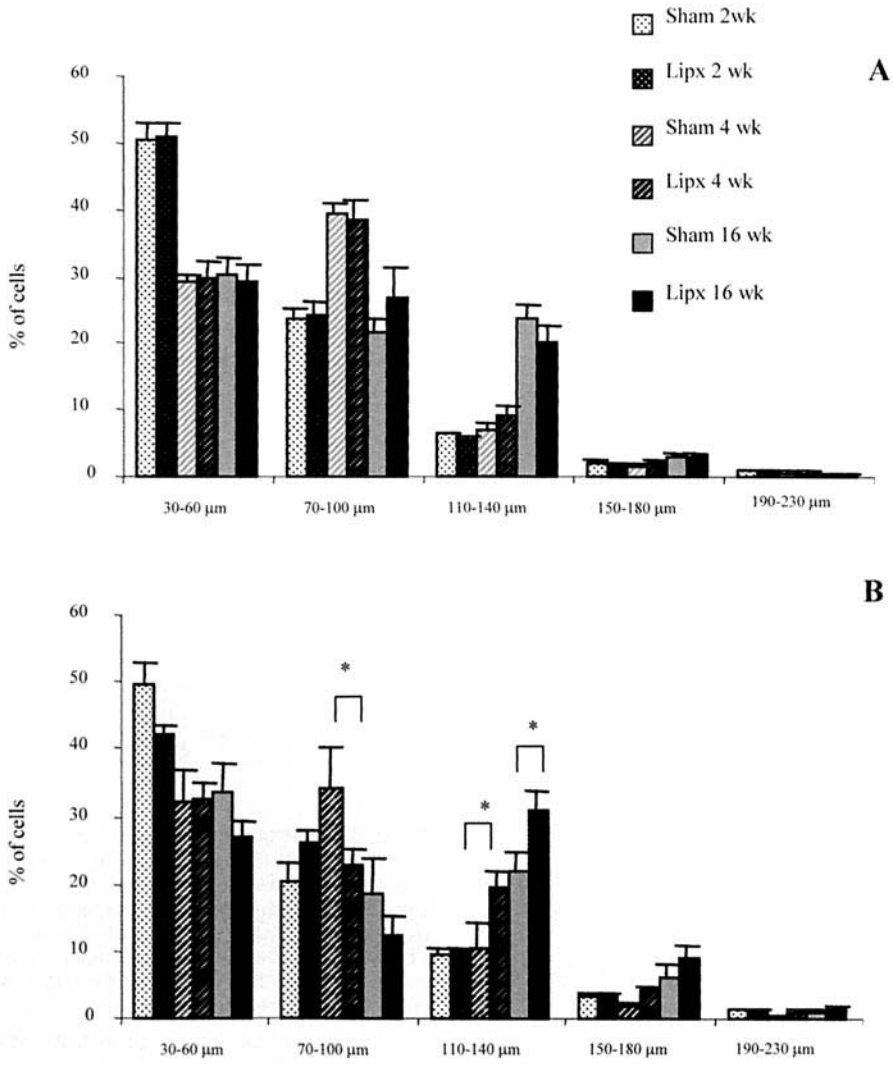

Figure 3. Cell size distribution of adipocytes in (A) inguinal and (B) retroperitoneal fat pads of sham operated and lipectomized rats 2, 4, and 16 weeks after surgery. Data are means + SE of 8–10 rats in each group. Asterisk (*),significant difference ($P < 0.05$) between sham operated and lipectomized rats within a fat pad and cell size range.

this is the first study to attempt energy expenditure measurements on lipectomized animals, and we had anticipated a compensatory decrease in heat production during the early weeks postsurgery. The predicted reduction in energy expenditure was not observed, however, during the two periods monitored, Days 8–10 and Days 29–31. In fact, there was a slight, but significant, increase in body weight–adjusted heat production in the lipectomized as compared with the sham operated rats during the second measurement period, owing perhaps to a slightly greater lean mass in recovering lipectomized rats. There may be several reasons why we failed to detect changes in food intake or energy expenditure that could account for the increased lipid deposition associated with the compensatory growth of adipose tissue in the lipectomized rats. First, we only determined food intake for the first 4 weeks postsurgery and energy expenditure at two discrete time points for 6 days total. Several fat pads were larger in the lipectomized rats at 4 weeks, indicating that some compensation had occurred. However, body composition was not determined for the animals sacrificed at 2 and 4 weeks postsurgery. Thus, we do not know whether compensatory growth of adipose tissue had been completed during the time the energy balance measurements were taken. Second, the changes in food intake and energy expenditure may have be too small to be detected with measurements taken only during a small portion of the 16 weeks during which the compensatory growth occurred. It is likely that a small positive energy balance each day will accumulate to a large amount of excess energy over 16 weeks. The energy cost of depositing the 3 g of fat removed from the lipectomized rats is 159 kJ metabolizable energy (12), which is equivalent to 12.17 g chow diet. If distributed over 16 weeks, this would represent an increase of only 0.11 g of diet per day. Such a small increase in food intake and/or reduction in energy expenditure may be too small to detect.

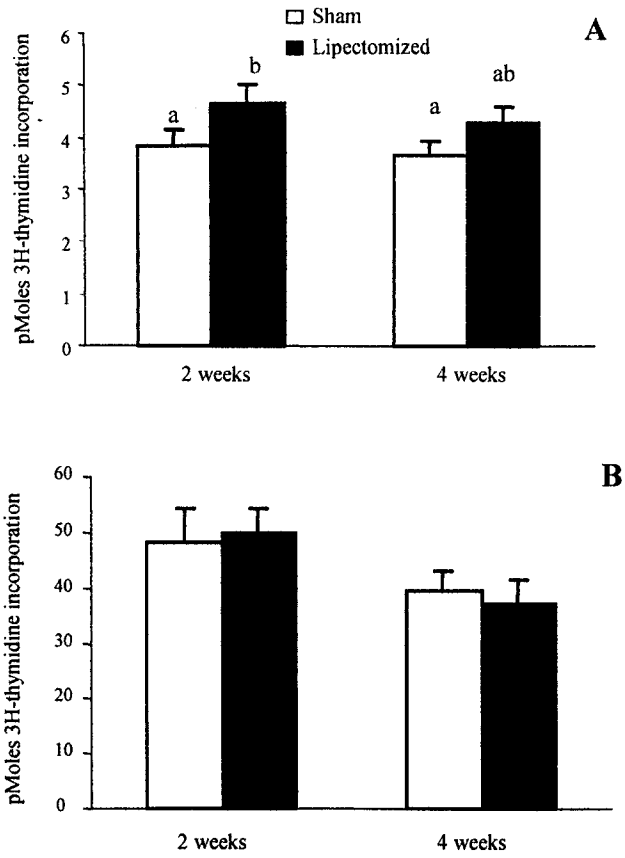

Figure 4. [^{3}H]-thymidine incorporation into the preadipocyte (A) and stromal-vascular (B) fractions of rat primary cell culture treated with 0.5% serum collected from sham operated and lipectomized rats 2 or 4 weeks after surgery. Data are means + SE for groups of 9–12 rats. Values not sharing a common superscript are significantly different ($P < 0.05$).

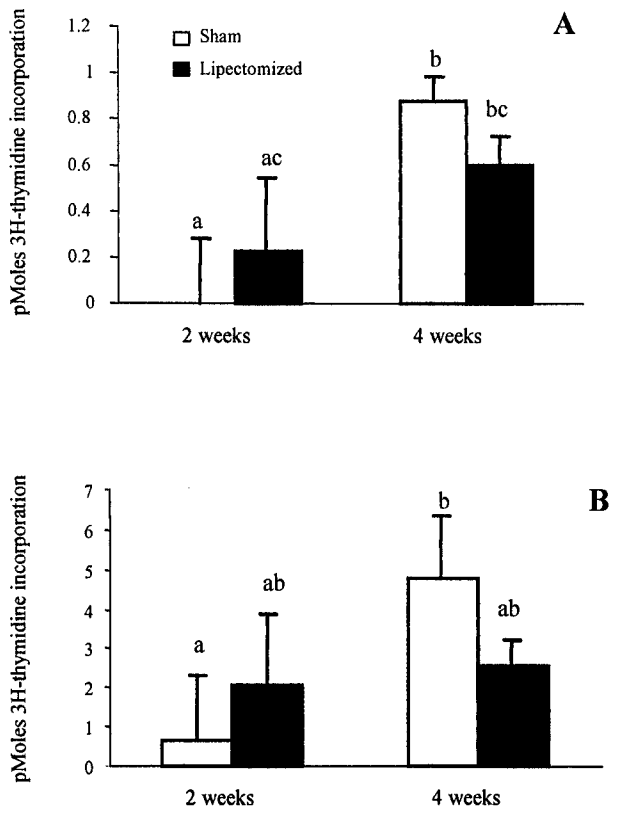

Figure 5. [^{3}H]-thymidine incorporation into the preadipocyte (A) and stromal-vascular (B) fractions of rat primary cell culture treated with 25% adipose tissue conditioned media collected from sham operated and lipectomized rats 2 or 4 weeks after surgery. Data represent stimulated activity per 10^6 adipocytes present in the fat tissue used for preparation of the conditioned media. Data are means + SE for groups of 9–12 rats. Values not sharing a common superscript are significantly different ($P < 0.05$).

Finally, the compensatory increase in lipid deposition following lipectomy may be due to a repartitioning of ingested nutrients, rather than to changes in food intake or energy expenditure *per se*. Additional studies are required to explore this possibility.

Carcass components were not significantly different between lipectomized and sham rats at the end of 16 weeks postsurgery, which suggests that the lipectomized rats had fully compensated for the excised fat. Compensatory growth after lipectomy has been reported in many previous studies in rats (11, 13–17); hamsters (18–22); mice (4); and pigs (23). In contrast, some studies with rats failed to show a restoration of body fat following lipectomy (15, 24, 25). These studies were either conducted using much younger animals (15, 25) than were used in the present study or in most other lipectomy studies, or else a greater amount of body fat was removed (24). In the later study, Kral (24) excised both inguinal and epididymal fat pads, which accounted for 24% of the total body fat from 15-week-old rats and found that the reduction persisted for at least 12 weeks. Thus, several factors may affect the compensatory response to lipectomy, including animal age at surgery, location and amount of fat removed, and study duration.

Fat depot–specific increases in the mass of nonexcised fat pads following partial lipectomy have been reported in several previous studies; however, these observations were made primarily as end point measures taken at a time of complete compensation 3–4 months after lipectomy (4, 13, 16, 18–20). In the present study, we sought to identify potential factors stimulating the compensatory response to lipectomy and thus examined rats at either 2 or 4 weeks postsurgery, time points considered to be during the dynamic compensatory phase, or at 16 weeks postlipectomy, a time point at which compensation was expected to be complete. We found that the compensatory increases in fat mass following lipectomy in our Wistar rats were detectable considerably earlier than 10 weeks as noted in a previous time-course study in Siberian hamsters (21). Thus, we observed a trend for an increase in the relative weight of the mesenteric fat pad at 2 weeks following lipectomy and significant increases in the weights of the mesenteric, inguinal, and retroperitoneal fat pads, but not perirenal fat pads, at 4 weeks postsurgery. The mesenteric and inguinal

fat pads remained 12%–21% larger in the lipectomized rats at 16 weeks postsurgery, although this difference was no longer statistically significant owing to the within group variability at this time point. Taken together, the results of the present and previous studies indicate that animals of several species are able to regulate body fat, that they do so in a fat pad–specific manner with the pattern and timing of the response in turn dependent on the species being investigated.

The cell size distribution of adipocytes of our lipectomized rats suggests the mode of compensation varies by fat depot. An increased fat cell number has been reported in the compensating fat pad of ground squirrels (10); Siberian hamsters (19, 22); Sprague-Dawley rats (16); and C57BL/6J db^{3J}/db^{3J} mice (4), whereas an increase in average fat cell size has also been reported in some studies (16, 21). In their early work, Larson and Anderson (16) noted that the cellular nature of the compensatory response to lipectomy seemed dependent on the location of the fat depot, with internal fat depots compensating by an increase in fat cell size and subcutaneous depots compensating through an increase in fat cell number. In the present study, we observed a decrease in the percentage of cells in the retroperitoneal fat pad that were in the size range of 70–100 μm and an increase in the percent of cells in the 110–140 μm size range in the lipectomized rats at 4 weeks postsurgery. Therefore, the increase in adipose tissue mass at this time point may be due, at least in part, to hypertrophy. In contrast, no detectable changes in cell size distribution were observed for the inguinal fat pads of the lipectomized rats although fat pad mass increased. Cellularity determinations were made by a Coulter counter, which used a 30-μm lower threshold. This lower threshold eliminates debris and cellular fragments and gives an accurate representation of cell size distribution for cells greater than 30 μm. It eliminates, however, the very smallest cells; therefore changes in the size distribution of cells less than 30 μm and total cell number were not determined. Nonetheless, hypertrophy in the retroperitoneal fat pad but not in the inguinal fat pad at 4 weeks is consistent with end point measurements reported by Larson and Anderson (16) and with the regional differences in growth modality outlined by DiGirolamo *et al.* (26). The latter study, in spontaneously obese aging male Wistar rats, demonstrated that different adipose tissue regions develop at varying rates and by specific growth modalities that are region-specific. As noted previously, the fastest compensatory response in the present study was observed for the mesenteric fat pad where a nonsignificant increase in relative size was detected by 2 weeks after lipectomy, with this increase becoming significant by 4 weeks. Unfortunately, we had elected not to determine the cellularity profile for this fat depot. However, we would predict that the compensatory response observed in the mesenteric fat pad was largely due to cellular hypertrophy. DiGirolamo *et al.* (26) observed that hypertrophy was the predominant growth modality for the mesenteric depot, an intermediate modality for the retroperitoneal depot, and a lesser modality for the inguinal depot.

To investigate the other mechanisms by which compensatory adipose tissue growth following lipectomy may occur, we also tested the activity of serum and conditioned media from lipectomized or sham operated rats on stimulating proliferation of preadipocytes in primary culture. Interestingly, we found that serum from the lipectomized rats stimulated preadipocyte proliferation more than serum from sham operated rats. This suggests that a factor(s) circulating in the blood may be involved in compensatory adipose tissue growth. One potential source of such proliferative factors is adipose tissue. Our previous studies indicated that conditioned media prepared by 4 hrs of exposure to adipose tissue from obese Zucker (6) or high-fat fed (27) rats stimulate preadipocyte proliferation to a greater extent than conditioned media prepared from corresponding control animals. In the present study, however, conditioned media prepared from the lipectomized rats did not stimulate proliferation of preadipocytes *in vitro* more than that from the sham operated rats. This suggests that the proliferation factors present in serum from the lipectomized rats may not be derived from adipose tissue, or at least are not from the retroperitoneal fat depot. We chose to determine the proliferative activity of conditioned media from the retroperitoneal fat pads because previous studies indicated a more consistent compensatory response across several species and strains of lipectomized animals in this fat pad compared with other fat depots (4, 19, 22). Adipose tissue is not a unitary organ, however, and does not always grow or respond uniformly to regulatory stimuli (reviewed in Ref. 3). As noted previously, both the rate and degree of compensatory growth as well as the pattern of cellular response was fat pad–specific in our lipectomized rats. Thus, the proliferative activity of conditioned media prepared from the retroperitoneal fat pads may not be representative of all compensating depots. Therefore, the circulating proliferation factors may have been secreted by fat depots that exhibit hyperplastic growth in response to lipectomy such as the inguinal fat pad, or may have originated from a nonadipose organ.

There are many blood-borne factors that can modulate the proliferation of preadipocytes such as thyroid hormones, glucocorticoids, insulin-like growth factor-1, angiotension II, tumor necrosis factor-α, macrophage colony-stimulating factor, and transforming growth factors (reviewed in Ref. 3). Serum concentrations of these regulatory factors were not determined in the present study; thus it is unknown if circulating levels of these, or other, regulatory factors are altered by lipectomy. Furthermore, changes in circulating levels of regulatory factors would not be sufficient to explain the depot-dependent response to lipectomy observed in this and other studies. Such depot specificity may be conferred through variable rates of blood flow through the tissues or by varying degrees of local growth factor receptor

expression or activity. In addition, other regulatory pathways such as those of the sympathetic and/or sensory nervous systems may also be involved in mediating the compensatory response. Further study is warranted on the specific factor(s) in serum that stimulates the proliferation of preadipocytes and the specific mechanism by which this and other regulatory elements influence the compensatory growth of adipose tissue after lipectomy.

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