Autologous fat transplants influence compensatory white adipose tissue mass increases after lipectomy

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Lacy, Eva L., and Timothy J. Bartness. Autologous fat transplants influence compensatory white adipose tissue mass increases after lipectomy. Am J Physiol Regul Integr Comp Physiol 286: R61–R70, 2004. First published October 2, 2003; 10.1152/ajpregu.00476.2003.—Direct tests of the hypothesized total body fat regulatory system have been accomplished by partial surgical lipectomy. This usually results in the restoration of the lipid deficit through compensatory increases in nonexcised white adipose tissue (WAT) masses of ground squirrels, laboratory rats, and mice, as well as Siberian and Syrian hamsters. We challenged this hypothesized total body fat regulatory system by testing the response of Siberian hamsters to 1) lipid deficits [lipectomy, primarily bilateral epididymal WAT (EWAT) removal], 2) lipid surfeits (addition of donor EWAT with no lipectomy), 3) no net change in lipid [EWAT or inguinal WAT (IWAT) lipectomy with the excised fat replaced to a new location (autologous)], 4) lipectomy with the same pad (EWAT lipectomy only) added from a sibling (nonautologous), and 5) sham surgeries for each treatment. Food intake generally was not affected. Body mass was not affected across all treatments. Grafts ~3 mo later had normal appearance both macro- and microscopically and were revascularized. The normal lipectomy-induced compensatory increases in nonexcised WAT masses surprisingly were exaggerated with autologous EWAT transplants, but not for autologous IWAT or nonautologous EWAT transplants. There was no compensatory decrease in native WAT masses with nonautologous EWAT additions. Collectively, only lipectomy triggered reparation of the lipid deficit, but the other manipulations did not, suggesting a system biased toward rectifying decreases in lipid or an inability of the hypothesized total body fat regulatory system to recognize WAT transplants.

Siberian hamsters; food intake; body weight; fat pad specific

A CENTRAL QUESTION REGARDING the development and maintenance of obesity is whether total body fat is regulated and, if so, how it is accomplished. The possibility of total body fat regulation was first articulated by Kennedy (21) in his “lipostatic theory.” Since that time, this question has been addressed primarily through indirect manipulation of body fat via over- or underfeeding (e.g., Refs. 17 and 20), manipulations that are not specific to only white adipose tissue (WAT). A direct approach for the manipulation of adiposity is to surgically remove or add fat and assess the degree to which body fat returns to initial levels (for review see Ref. 31). Surgical body fat removal (partial lipectomy, hereafter termed “lipectomy”) results in compensatory restoration of fat levels in several rodent species, including laboratory rats (e.g., Refs. 10–12) and mice (5, 18), rabbits (36), Kellakui lambs (34), Siberian hamsters (25, 28, 30), Syrian hamsters (16), and golden-mantled ground squirrels (7, 8). This ability to regulate total body fat seems especially well developed in Siberian hamsters (Phodopus sungorus) because they are responsive to both the degree and lateralization of surgically induced lipid depletion (28) and they show seasonally appropriate lipectomy-induced body fat compensations under summer-like conditions, when free-living animals would be engaging in active reproductive efforts [for review see Ref. 31]; moreover, these compensatory responses are fat-pad specific (25). That is, the pattern of reparation of the surgically induced lipid deficit is dependent on which WAT depot(s) is removed (e.g., Ref. 25). The apparent seasonality of body fat responses to lipectomy and the precision with which body fat appears to be regulated make them an ideal model to probe this apparent adiposity-sensing system.

An alternative means of probing the adiposity-sensing system is to add, rather than to subtract, body fat via WAT grafts. Isolated fat cells have been transplanted in experimental rodent models of obesity with the intent to test the morphological and physiological changes in the transplanted adipocytes vs. their native counterparts (e.g., Ref. 2). Because of the small number of grafted cells, the impact of these transplanted adipocytes on general physiology was trivial at best. The ability of larger amounts of WAT to survive after removal and insertion at another site has been repeatedly demonstrated in humans by reconstructive and plastic surgeries, termed autologous fat transplantation (for review, see Ref. 38). Unlike lipectomy, however, fat transplantation infrequently has been used to test the physiological responses to added body fat (e.g., Ref. 24). Our interest in this approach as a means of testing the apparent body fat regulatory system was stimulated by the recent reports of the successful transplant of physiologically meaningful amounts of subcutaneous WAT in mice (6, 14). Because Siberian hamsters appear exquisitely sensitive to lipectomy-induced decreases in body fat (28), we tested the effects of lipectomy, lipectomy combined with WAT grafts, and of WAT grafts alone on body fat regulation in these animals. Several predictions are apparent based on the compensatory increases in body fat that occur after lipectomy by the remaining nonexcised WAT depots. First, if the lipectomized WAT simply is grafted to a different site within the same animal, then there is no lipid deficit; therefore, the WAT pads that normally show compensatory increases in mass in response to the lipectomy should not do so. If there is no lipectomy, but instead WAT is added from a donor animal, then the WAT depots of the host should decrease in response to the inappropriate level of body fat resulting from the fat addition. These predictions, of course,
are predicated on the notion that the adiposity-sensing system of the animals can detect the grafted fat by a graft-derived humoral factor and/or by a neural signal such as from sensory nerves that normally innervate WAT (13, 33).

Therefore, the purpose of the present study was to answer the following questions: 1) Do autologous WAT transplants block body fat compensation after partial lipectomy? 2) Do between-animal WAT transplants block lipectomy-induced body fat compensation? and 3) Does increasing total body fat by transplanting WAT between animals in the absence of lipectomy affect total body fat? This was accomplished in experiments 1 and 2 by performing bilateral epididymal WAT (EWAT) or inguinal WAT (IWAT) removal followed by replacement of the excised fat at a different location in the same individual. In experiment 3, bilateral EWAT removal was followed by either transplantation of EWAT from a male sibling or no transplantation. Experiment 3 also included animals that were not EWAT lipectomized but received EWAT transplants from a male sibling. After surgery (12 or 13 wk), a time necessary for total body fat recovery from lipectomy-induced lipid deficits (26), nonexcised WAT pad and graft masses were measured to determine the compensatory responses to the various combinations of lipectomy and fat transplantation.

**METHODS**

**Animals and Housing**

Adult male Siberian hamsters (3–4 mo of age) were group housed from birth in a long photoperiod (16:8-h, light-dark cycle; lights on 0200) and given PMI Rodent Diet no. 5001 and tap water ad libitum. The genealogy of these animals has been described recently (9) except that F2 wild-trapped hamsters were added in 1999 (donated by Stephan Steinlechner, School of Veterinary Medicine, Hanover, Germany). In each of the studies described below, hamsters were removed from group housing and housed singly for 7–10 days before surgery. At that time, they were assigned to treatments such that the groups were matched for the percent change in body mass during the initial single housing period and for absolute body mass at the time of surgery.

**Procedures**

**Experiment 1: A test of autologous EWAT transplantation.** This was a preliminary study in which we attempted to examine the feasibility of fat transplantation, so initially we only formed the transplant groups, with their age- and body mass-matched sham-operated counterparts added later.

Twenty-one hamsters were divided into four groups. For all animals receiving WAT transplants, whether autologous or between animals, in this or in all other experiments presented here, the area between the dorsal subcutaneous WAT pad and IWAT was the targeted area for the graft. More rostrally placed transplants were closer to the dorsal subcutaneous WAT, whereas the more caudally placed transplants were closer to the IWAT (see below for a thorough description of the surgery). One group underwent unilateral EWAT removal (left EWATx), two groups underwent bilateral EWAT removal (EWATx), and one group underwent sham EWATx. In the unilateral EWATx group, the EWAT pad that was removed was transplanted as two bilateral dorsal subcutaneous transplants (unilateral EWATx + 2 grafts; n = 3). In one bilateral EWATx group, one of the EWAT pads was replaced as two bilateral dorsal subcutaneous transplants (bilateral EWATx + 2 grafts; n = 3). In the remaining bilateral EWATx group, both of the EWAT pads were replaced as two dorsal subcutaneous transplants (one rostral, one caudal) ipsilateral to the side of removal (bilateral EWATx + 4 grafts; n = 4). For the control hamsters, dorsal incisions were made, but no lipectomy or transplant occurred (sham; n = 11).

**EWAT lipectomy and transplantation.** All surgeries were performed under pentobarbital sodium anesthesia (50 mg/kg) administered intraperitoneally. EWATx was accomplished by making an abdominal incision through which both EWAT pads could be accessed, as we have described previously (e.g., Refs. 25–30, and represents removal of ~5% of total body fat (28)). Briefly, fat pads were removed, with care taken to preserve the blood vessels supplying the testes. During sham surgery, the EWAT pads were exposed, but not removed. The peritoneum and abdominal muscles were sutured, and the skin was closed with wound clips. The masses of the excised pads were weighed before transplantation. For transplantation, small bilateral dorsal incisions were made, the skin and fascia were loosened using blunt tissue forceps, and the transplants were placed under the skin, which was closed with wound clips. Sham EWATx and sham transplantation consisted of exposure, but not removing, the pads and all of the procedures for actual transplantation except that no tissue was inserted under the skin.

Body mass for all hamsters was recorded for weeks 5–12. At week 12, the animals were anesthetized with pentobarbital sodium and perfused transcardially with heparinized saline followed by 4% paraformaldehyde in 0.15 M PBS for histological analysis of the WAT pads. Transplanted fat pads, residual or intact EWAT, IWAT, and retroperitoneal WAT (RWAT) were dissected, weighed, and placed in vials of 4% paraformaldehyde buffer. Testes were dissected and weighed. A subset of transplants (n = 2/animal) and intact EWAT samples (n = 1/animal) from two to three animals of each treatment group were embedded in paraffin, sliced at 4 μm on a rotary microtome, and mounted on slides. Two transplants from one to two hamsters of each group were counterstained with hematoxylin and eosin for histological examination and compared with intact EWAT from age-matched animals. Intact EWAT, two transplants from one of the unilateral EWATx + 2 grafts group, and two transplants from each of the remaining transplant groups were processed by immunocytochemistry for the specific adipose tissue membrane protein, AD-3 (e.g., Refs. 23 and 40, generously donated by Gary Hausman, USDA, Athens, GA). Tissues were deparaffinized, briefly incubated in 0.3% H2O2 followed by 1:10 normal goat serum, and then incubated with the AD-3 monoclonal antibody (mouse anti-AD-3) for 48 h in a humid chamber at 4°C. Slides were then incubated with biotinylated goat anti-mouse IgG followed by avidin-biotin complex and exposed to diaminobenzidine hydrochloride (Sigma, St. Louis, MO). Slides were rinsed with 0.15 M PBS between incubations.

**Experiment 2: Effects of fat replacement on body fat compensation after lipectomy.** For this and all subsequent studies, bilateral lipectomy was used, and transplants always consisted of four dorsal subcutaneous grafts generated by dividing each excised WAT pad into two approximately equal-sized pieces placed two on each side, one rostrally, one caudally, and both ipsilateral to the side of removal.

**Experiment 2a: EWAT lipectomy and autologous transplantation.** Twenty-eight hamsters were divided into three groups: a bilateral EWATx group (EWATx, n = 12), a bilateral EWATx group that received the excised fat as four autologous dorsal subcutaneous transplants (EWATx + grafts, n = 9), and one sham EWATx group (sham, n = 7). Lipectomy, lipectomy plus autologous transplantation, and sham lipectomy plus sham autologous transplantation were done as described in experiment 1. Food intake and body weight were recorded for 13 wk after surgery. At week 13, the animals were killed and perfused as in experiment 1 above. The transplanted fat pads and residual or intact EWAT, IWAT, and RWAT were dissected, weighed, and placed in vials of 4% paraformaldehyde buffer. Testes were dissected and weighed.
Experiment 2b: IWAT lpectomy and autologous transplantation. Thirty-one hamsters were divided into the following three groups: a bilateral IWATx group (IWATx, n = 11), a bilateral IWATx group that received the excised fat as four autologous dorsal subcutaneous transplants (IWATx + grafts, n = 8), and one sham IWATx group (sham, n = 12). The IWATx-only and sham surgery groups also underwent sham transplantation procedures. For all IWATx groups, lpectomy was accomplished via cutaneous incisions made over the lateral thigh, as we described previously (e.g., Ref. 25), and represents ~10% of total body fat (28). Briefly, the IWAT pads were removed from overlying skin and underlying musculature via blunt dissection. Sham IWAT lpectomy consisted of exposing the IWAT pads and separating them from the overlying skin, but not underlying muscle. Transplantation and sham transplantation were performed as in experiment 1 except the grafts were inserted through the existing lateral incisions. Food intake and body weight were recorded for 13 wk after surgery. At week 13, the animals were killed and perfused as above. The transplanted fat pads, EWAT, IWAT, and RWAT were dissected, weighed, and placed in vials of 4% paraformaldehyde buffer. Testes were removed and weighed. Experiment 2b was completed by using two cohorts of animals that included all groups. There were no differences between any of the measures for the two cohorts except two cohorts of animals that included all groups. There were no differences between any of the measures for the two cohorts except two control groups were combined for statistical analysis of all experiments (EWATx and sham transplant group (sham; n = 10) and sibling graft group (sibling grafts, n = 8), a sham EWATx group that received fat excised from a male sibling consisting of four dorsal subcutaneous transplants (sibling grafts only; n = 20), and a sham EWATx and sham transplant group (sham; n = 10). Unfortunately, a small modification to the EWAT lpectomy reduced blood flow to the testes, rendering the EWATx and EWATx + sibling graft groups functionally castrated; therefore, these animals were killed and their testes were removed and weighed.

Experiment 3: Effects of sibling fat transplantation on body fat with and without EWAT lpectomy. Hamsters were divided into the following four groups: a bilateral EWATx group with sham transplant (EWATx, n = 16), a bilateral EWATx group that received fat excised from a male sibling consisting of four dorsal subcutaneous transplants (EWATx + sibling grafts, n = 8), a sham EWATx group that received fat excised from a male sibling consisting of four dorsal subcutaneous transplants (sibling grafts only; n = 20), and a sham EWATx and sham transplant group (sham; n = 10). Unfortunately, a small modification to the EWAT lpectomy reduced blood flow to the testes, rendering the EWATx and EWATx + sibling graft groups functionally castrated; therefore, these animals were killed and their testes were removed and weighed. Replacement animals for these groups and an additional sham-operated group were added (EWATx, n = 15; EWATx + sibling grafts, n = 16; and sham operated, n = 7), with the EWAT lpectomy done without the small modification. The two sham-operated groups (sham EWATx + sham graft) differed only in cumulative and weekly food intake but not in other measures; therefore, data from these two control groups were combined for statistical analysis of all measures except analyses of weekly food intake, where separate tests were done. Thus all other surgical procedures were identical to those in experiment 2a above for the autologous transplants except that, in this experiment, sibling males were used as donors of the EWAT pad transplants. This was accomplished by using the EWAT removed from the EWATx and EWATx + sibling graft males as donors of WAT to their brothers in the two groups receiving transplants. Because of this design (i.e., using brothers from the same litters), we were unable to strictly control the size of the donor EWAT pad received by their host brothers for the EWATx + sibling graft group relative to the size of the EWAT removed from these hosts, a problem obviously not encountered with autologous transplants in experiments 1 and 2 above. Food intake and body weight were recorded for 12 wk after surgery. At week 12, one-third of the animals from each group was killed and perfused as described above, and the transplanted fat pads, EWAT, IWAT, and RWAT were dissected, weighed, and placed in vials of 4% paraformaldehyde buffer. Of the remaining two-thirds of the animals, all fat pads were dissected, weighed, and either frozen in liquid nitrogen or discarded. The animals comprising each of the one-third subgroups were matched for mean percentage change in body mass from surgery to week 10. Testes were removed and weighed. The pads frozen from one-third of the animals in each treatment were analyzed for norepinephrine (NE) content as an indicator of sympathetic nervous system innervation of the WAT (3, 39) via HPLC with electrochemical detection (see below).

HPLC analysis of WAT NE content. The samples were processed according to our published procedures (41, 42) that were adopted from the method of Melford (32) with the exception that separate subsamples of each minced tissue sample were processed to determine protein content. Samples were assayed for NE content using an ESA (Chelmsford, MA) HPLC system with electrochemical detection (Coulson II, ESA 501 software). A 50-μl aliquot of each sample was injected in the system from a refrigerated (4°C) autosampler using Cat-a-phase II (ESA) mobile phase and separated with an HR-80 column (3-μm particle size). The detector settings were as follows: guard cell, 350 mV; cell 1, 50 mV; and cell 2, −300 mV. All treatment groups were represented in each of five separate runs, and standard solutions (25, 12.5, 6.25, and 3.13 ng/ml) were assayed as unknowns with each set of samples; the average accuracy of the HPLC system across the five runs and four standard levels was 99.9%. Calculated values were corrected for sample recovery on the basis of the internal standard (dihydroxybenzylamide) added at the start of tissue processing, and final NE content was expressed as nanogram NE per milligram protein.

For protein determination, 20–50 mg tissue were sonicated in 0.1 M PBS and centrifuged exactly as samples for HPLC. The infranatant EWA, IWAT, and RWAT were centrifuged at −80°C until analyzed for protein content by the Bradford (4) method modified to a microassay with a detectability range of 0.5–0.0156 mg/ml.

**Statistical Analysis**

Initial and terminal tissue and body masses, cumulative food intake, and the percentage change in body and tissues masses were analyzed by one-way ANOVA (SPSS v. 11.5; SPSS, Chicago, IL) followed by post hoc testing using Duncan’s Multiple-Range tests when appropriate. Pairwise t-tests were used to compare the initial vs. the final graft masses. Weekly body mass and food intake were analyzed using ANOVA for repeated measures. Body mass was analyzed using the following ANOVA designs: experiment 1, for weeks 5–12 (treatment × time, 4 × 9); experiments 2a and 2b, from surgery to week 13 (treatment × time, 3 × 14); and experiment 3, from surgery to week 12 (treatment × time, 3 × 13). The ANOVA design for analysis of the weekly food intake for experiments 2a and 2b was treatment × time, 3 × 13. For experiment 3, separate ANOVAs were used to analyze weekly food intake for groups with their appropriate sham controls: for sham 1 and sibling grafts only, treatment × time (2 × 12); and for sham 2, EWATx, and EWATx + sibling grafts, treatment × time (3 × 12). For all repeated-measures ANOVAs, if treatment or the week × treatment interaction was significant, post hoc one-way ANOVA was done next, followed by Duncan’s New Multiple Range tests, when appropriate, to determine differences between treatments for each week. Two-tailed Pearson Product Moments were used to determine covariation among variables. For all tests, differences among means or correlations were considered significant at P < 0.05; in most cases, exact probabilities and test values have been omitted for simplification and clarity of the presentation of the results.

**RESULTS**

**Experiment 1: A Test of Autologous EWAT Transplantation**

Lpectomy deficits and fat replacement. This experiment combined unilateral and bilateral EWAT lpectomy and partial and full replacement of removed fat. The mass of fat removed from the groups with bilateral lpectomy was significantly greater (1.8–2.3 times) than that of the unilateral lpectomy group, as would be expected from bilateral vs. unilateral lpectomy. In addition to the varied lipid deficit, we also...
created varying levels of replacement with the two grafts (one pad divided in half and replaced) and four grafts (two pads divided in half and replaced).

**Graft mass, appearance, and histology.** All grafts appeared visibly healthy, and the two EWAT halves in the bilateral EWATx + 4 grafts group often merged together to various degrees in their subcutaneous positions (Fig. 1A). Grafts decreased in mass by 27–34% across the 12-wk experiment (Table 1). This overall decrease in mass was not significant, and grafts in each treatment group lost similar mass. Histological appearance of intact EWAT and grafts was similar, with the exception of more regions devoid of adipocytes in the grafts than in the intact EWAT (Fig. 1, B and C). Nonetheless, the grafts were viable with requisite vascularization occurring for the tissue to survive during this 3-mo period. Indeed, immunocytochemical staining for the adipose membrane-specific protein AD-3 was similar between intact EWAT and grafted EWAT, with adipocytes for the two tissues having cells of similar size and shape (Fig. 1, D and E).

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Fig. 1. Gross anatomic and histological views of autologous white adipose tissue (WAT) transplants in experiment 1. EWAT, epididymal WAT; IWAT, inguinal WAT; RWAT, retroperitoneal WAT; iBAT, interscapular brown adipose tissue. A: line drawing depicting the position of the grafts from a dorsal view. Arrows indicate grafts. Typical grafts from a bilateral EWATx + 4 grafts animal after 12 wk. Note that 2 grafts have merged on each side. B: hematoxylin and eosin (H&E)-stained intact EWAT. C: H&E-stained transplanted EWAT. Note regions of both normal adipocytes and acellular spaces. D: adipose-specific AD-3 immunostaining in intact EWAT. E: adipose-specific AD-3 immunostaining in transplanted EWAT.
Weekly body and tissue mass. Body mass did not differ significantly across treatments (data not shown). Although total body mass did not differ across the groups, there were significantly different responses among the fat pad masses resulting from lipectomy and/or grafting (Fig. 2). Specifically, and as we have reported previously (28), the greater the surgically induced lipid deficit, the greater the compensatory response of the remaining nonexcised WAT pads. Thus IWAT mass was significantly greater when both EWAT pads were removed (the two bilateral EWATx groups) compared with single EWAT pad removal (unilateral EWATx) or sham lipectomy (sham; P < 0.05; Fig. 2). A similar relation between the amount of excised fat and the compensatory increases in the remaining fat were seen for RWAT. That is, RWAT mass was significantly larger than that of the sham-operated hamsters in both bilateral EWATx groups compared with the unilateral EWATx group (P < 0.05; Fig. 2).

IWAT mass in the unilateral EWATx + 2 grafts group increased 13%, which is less than the normal lipectomy-induced increase in IWAT mass when only one EWAT pad is removed (~30–35%; see Ref. 28). Surprisingly, IWAT mass increased by 67 and 77% in the bilateral EWATx + 2 grafts and bilateral EWATx + 4 grafts groups, respectively, and these increases were more than what is typically observed with bilateral EWATx alone (~30–40%; see Ref. 28). RWAT mass from unilateral EWATx + 2 grafts animals was not significantly greater than in the sham-operated animals but was increased by 26%, which is similar to the ~30% increase observed previously (28). RWAT mass from bilateral EWATx treatments did not differ significantly but was significantly larger than that of the sham-operated hamsters (P < 0.05; 115 and 215% increases compared with 40% increases seen normally; see Ref. 28). Surprisingly, EWAT mass from the lipectomized groups was not significantly decreased compared with that of the sham-operated hamsters, as we repeatedly have shown previously (e.g., Refs. 25 and 27–30), despite its removal, although all three lipectomy groups tended to have less EWAT than the intact sham-operated group. Thus we are confident about the excision of EWAT and, as discussed below, attribute this to stimulated growth of remnants of the EWAT after its removal that was trivial in our other studies.

Carcass composition could not be done because of the histological processing of the WAT tissue, but total dissected WAT was calculated and was significantly greater in bilateral EWATx + 4 grafts animals than in the sham lipectomy group (P < 0.05; Fig. 2), with the other treatments intermediate between these two extremes. This finding likely reflected the exaggerated compensatory response of the IWAT pads and, to a lesser degree, RWAT pads of hamsters receiving the grafts (tested and discussed below). Indeed, when the mass of the grafts was removed from this calculation, there still was a tendency (P = 0.087) for dissected WAT mass to be greater in the bilateral EWATx + 4 grafts hamsters, suggesting that the effect was based on more than the contribution of the graft alone (Fig. 2). Finally, despite the attempt to spare the testicular vasculature during lipectomy, combined testis mass was significantly greater in the sham-operated animals [P < 0.05; 0.889 ± 0.048 (SE) g] than in the unilateral EWATx group and in the unilateral EWATx group than in the bilateral EWATx + 2 grafts animals (P < 0.05), but not in the bilateral EWATx + 4 grafts group [0.620 ± 0.040 vs. 0.546 ± 0.055 and 0.390 ± 0.067 (SE) g, respectively]. We have shown previously that testes of these sizes are still functional in terms of testosterone production (e.g., Ref. 26 and H. Shi and T. J. Bartness, unpublished observations). Moreover, in this species, testosterone removal through castration produces decreases in WAT pad mass and total carcass lipid (e.g., Ref. 26). Thus a reduction in testis mass and a likely decrease in testosterone production would counteract the restoration of the lipectomy-induced lipid deficit.

Correlational analysis. To determine whether larger grafts or grafts that decreased least in size during the study led to larger increases in nonexcised fat mass, we examined the data for correlations among graft size and the percent change in graft mass. IWAT mass, EWAT mass, and total dissected WAT were not correlated with final total graft mass, unlike RWAT mass (r = 0.658, P = 0.039). None of the measures of fat mass correlated with the percentage change in graft mass across the 12 wk.

Experiment 2: Effects of fat replacement on body fat compensation after lipectomy. The surprising increase in growth in IWAT and RWAT in our initial study suggested that autologous transplants might exaggerate the body fat response to lipectomy. Experiments 2a and 2b tested this directly and also determined if fat removal and/or replacement affected food intake.

Experiment 2a: EWAT lipectomy and autologous transplantation. Graft mass and appearance. Grafts appeared healthy, and often the grafts on one side merged with each other. Grafts also typically became embedded in surrounding IWAT but remained distinct in appearance such that dissection from the adjacent WAT was accomplished easily (Fig. 3A). Photographs from nonperfused animals clearly show the presence of blood vessels associated with autologous EWAT grafts (Fig. 3B). The EWAT grafts within an individual significantly decreased in mass (~40%) from their initial size to that at the end of the study (P < 0.05; Table 1).

Weekly food intake and body mass. Food intake was increased significantly in animals with autologous fat transplants compared with sham and EWATx-only animals for weeks 2–4 (P < 0.05; data not shown). This group consistently had the highest food intake for the remainder of the study and on weeks...
6, 7, and 10 ate significantly more than lipectomized animals ($P < 0.05$; data not shown). Cumulative food intake also differed significantly across groups, with EWATx + grafts animals eating significantly more overall than EWATx-only animals ($P < 0.05$); sham-operated animals had intermediate food intakes [495 ± 26, 408 ± 10, and 446 ± 17 (SE) g, respectively]. Neither body mass at week 13 nor the change in body mass from surgery to week 13 differed significantly across treatments (data not shown).

**Terminal measures of WAT.** Although body mass did not differ statistically significantly across groups, specific WAT pad masses were affected by treatment. IWAT mass from EWATx + grafts animals was significantly larger than from EWATx-only and the sham-operated control groups ($P < 0.05$, Fig. 4). Specifically, IWAT mass increased 76% over the sham-operated group in the EWATx + grafts group compared with 12% in EWATx-only animals. RWAT mass was not different between these groups. EWAT mass, however, varied significantly across treatments and was largest in the intact sham-operated animals vs. the lipectomized groups ($P < 0.05$; Fig. 4). Total dissected WAT was significantly greater in EWATx + grafts animals than EWATx ($P < 0.05$; Fig. 4), as seen in **experiment 1**. This significant difference between these groups persisted after the mass of the grafts was subtracted from the total dissected WAT ($P < 0.05$; Fig. 4); thus, the graft mass alone did not account for this increase. As in **experiment 1**, testes from lipectomized animals were significantly smaller than intact animals [$P < 0.05$; 0.375 ± 0.015 g].

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**Fig. 3.** Gross anatomic view of transplants in lipectomized animals from **experiments 1–3**. Arrows indicate grafts in A, C, and D and blood vessels in B. A: **experiment 2a:** autologous EWAT grafts after perfusion. B: close-up of an autologous EWAT graft in a nonperfused animal. Note that the grafted tissue is well vascularized. C: **experiment 2b:** autologous IWAT grafts after perfusion. D: **experiment 3:** between-sibling transplants after perfusion.
and 0.441 ± 0.059 (SE) g in EWATx and EWATx + grafts, respectively, vs. 0.711 ± 0.051 g in sham-operated animals], but, again, all testes would be considered functional in terms of testosterone secretion for this species based on previous findings (Ref. 26 and H. Shi and T. J. Bartness, unpublished observations).

**Correlational analysis.** The terminal mass of IWAT, EWAT, and RWAT was not significantly correlated with absolute terminal graft size or change in graft mass. These latter two variables were highly positively correlated, however (r = 0.820, P = 0.007); thus, initially larger grafts also were the largest grafts after 13 wk, again suggesting that even large grafts become sufficiently revascularized to support tissue viability.

**Experiment 2b: IWAT lipectomy and autologous transplantation.** *Graft mass and appearance.* As seen in the previous experiments, the grafts appeared healthy, with frequent merging of grafts on the same side as implantation (Fig. 3C). The autologous IWAT transplants decreased significantly in mass (~50%) across the 13 wk (P < 0.05; Table 1).

**Weekly food intake and body mass.** Neither weekly body mass nor weekly food intake differed significantly across groups (data not shown).

**Terminal measures of WAT.** EWAT and RWAT mass did not differ significantly across treatments at the end of the experiment, but there was a tendency for both of those pads to be larger in IWATx animals. The inability of IWAT removal to generate a robust compensatory response from the remaining fat pads (i.e., EWAT and RWAT) has been noted previously (e.g., Ref. 25) and contrasts with the robust compensatory response after EWATx (experiments 1 and 3 in the present study and Refs. 25 and 27–30). No residual tissue remains after IWAT lipectomy (e.g. Refs. 25, 28, and 30); thus, total dissected WAT in the IWATx and IWAT + grafts animals was significantly less than in sham-operated animals (P < 0.05; Fig. 5). Although IWATx animals tended to have larger EWAT and RWAT than animals with grafts, total dissected WAT did not differ across the lipectomized groups (Fig. 5).

**Correlational analysis.** The mass of IWAT, EWAT, and RWAT was not significantly correlated with absolute graft size or change in graft mass; these latter two variables also were not correlated. Although body mass change over 13 wk was relatively small (2–4%) and was not significantly affected by treatment, animals where graft mass decreased the least gained more body mass than animals with grafts that decreased more in mass (r = 0.717, P = 0.046), suggesting that transplants may have size-specific effects on some variables.

**Experiment 3: Effects of sibling fat transplantation on body fat with and without EWAT lipectomy.** *Graft mass, appearance, and NE content.* Transplanted tissue from siblings appeared similar to autologous transplants in all respects (Fig. 3D). Transplants from male siblings decreased significantly in mass (24–30%) over 12 wk (P < 0.05; Table 1), similar to the decreases seen in experiment 1. Sibling EWAT transplants contained NE, but at levels significantly less than for intact EWAT, IWAT, or RWAT (P < 0.05; see Fig. 6). Furthermore, the NE content of grafts was not affected by the presence of

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**Table 1. Initial graft mass, final graft mass, and percent change in graft size for experiments 1–3**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Initial Graft Mass</th>
<th>Total Final Graft Mass</th>
<th>Change in Total Graft Mass, %</th>
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<tr>
<td><strong>Experiment 1</strong></td>
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<tr>
<td>Unilateral EWATx + 2 grafts</td>
<td>0.43 ± 0.06*</td>
<td>0.32 ± 0.09†</td>
<td>−27.77 ± 19.22</td>
</tr>
<tr>
<td>Bilateral EWATx + 2 grafts</td>
<td>0.38 ± 0.07*</td>
<td>0.27 ± 0.10†</td>
<td>−34.46 ± 17.25</td>
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<tr>
<td>Bilateral EWATx + 4 grafts</td>
<td>0.99 ± 0.22</td>
<td>0.69 ± 0.10†</td>
<td>−27.50 ± 6.75</td>
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<tr>
<td><strong>Experiment 2a: Bilateral</strong></td>
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<tr>
<td>EWATx</td>
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<tr>
<td>Bilateral EWATx + grafts</td>
<td>0.72 ± 0.07</td>
<td>0.42 ± 0.06†</td>
<td>−40.93 ± 5.84†</td>
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<td><strong>Experiment 2b: Bilateral</strong></td>
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<tr>
<td>EWATx</td>
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<tr>
<td>Bilateral IWATx + grafts</td>
<td>1.03 ± 0.13</td>
<td>0.53 ± 0.07†</td>
<td>−48.86 ± 3.61†</td>
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<td><strong>Experiment 3: Bilateral</strong></td>
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<tr>
<td>EWATx</td>
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<tr>
<td>Bilateral EWATx + sibling</td>
<td>0.83 ± 0.05</td>
<td>0.62 ± 0.04†</td>
<td>−24.53 ± 3.56†</td>
</tr>
<tr>
<td>Sibling grafts only</td>
<td>0.90 ± 0.05</td>
<td>0.64 ± 0.06†</td>
<td>−30.63 ± 4.89†</td>
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</table>

Values are means ± SE in g, except percent change. EWATx, epididymal white adipose tissue (WAT) lipectomy; IWATx, inguinal WAT lipectomy. *P = 0.051, comparison vs. bilateral EWATx + 4 grafts; †P < 0.05, paired t-test comparisons: initial vs. final mass.

**Fig. 4.** Mean ± SE WAT pad mass expressed as a percentage of sham-operated values for experiment 2a. *P < 0.05 vs. sham-operated group. †P < 0.05 vs. EWATx group.

**Fig. 5.** Mean ± SE WAT pad mass expressed as a percentage of sham-operated values for experiment 2b. *P < 0.05 vs. sham-operated group.
absence of concurrent lipectomy. NE content did not vary significantly across treatment groups in any of the native WAT pads (Fig. 6). There was a strong suggestion, however, that IWAT NE content may be decreased in animals with grafts ($P = 0.058$), suggesting in our experience that sympathetic drive may be decreased given the high correlation between NE content and NE turnover in WAT (e.g., Ref. 41 and H. Shi, R. R. Bowers, and T. J. Bartness, unpublished observation).

Weekly food intake and body mass. Weekly body mass and food intake were not statistically significantly different among the groups across the experiment (data not shown).

Terminal measures of WAT. IWAT and RWAT mass from the EWATx and EWATx + sibling grafts animals was not different between the groups but was significantly increased compared with that of the sham-operated hamsters ($P < 0.05$, Fig. 7). IWAT and RWAT mass from sibling grafts-only animals was not significantly different from sham-operated hamsters but was significantly decreased compared with EWATx + sibling grafts animals ($P < 0.05$; Fig. 7). Total dissected WAT in EWATx animals was not different from intact sham-operated animals, suggesting that they fully compensated for lipectomy-induced lipid loss (Fig. 7). Total dissected WAT from EWATx + sibling grafts and sibling grafts-only animals was significantly greater than that of the sham-operated group ($P < 0.05$; Fig. 7), an effect apparently only due to the addition of the grafts because, if their contribution to the total dissectible WAT was removed, the remaining total dissected WAT mass was not different from one another (Fig. 7).

Correlational analysis. The masses of IWAT and EWAT were not significantly correlated with absolute graft size, but RWAT mass and total dissected WAT were positively correlated with graft mass ($r = 0.335$, $P = 0.046$ and $r = 0.528$, $P = 0.001$, respectively). The change in graft mass was positively correlated with IWAT mass ($r = 0.421$, $P = 0.011$), RWAT mass ($r = 0.353$, $P = 0.035$), and total dissected WAT both with and without the graft mass included ($r = 0.565$, $P < 0.0001$ and $r = 0.420$, $P = 0.011$, respectively).

DISCUSSION

By using fat transplantation both within and between animals, an attempt was made at challenging the hypothetical body fat regulatory system by producing a fat surfeit rather than fat deficit, the latter usually resulting in accurate compensation for the fat deficit through increases in the mass of the nonexcised fat pads, as others and we have shown (for review, see Ref. 31). In the present study, we found that 1) autologous grafts (transplantation within the same individual) and nonautologous grafts (transplantation between related animals) were quite normal, exhibiting adipocyte size and shape similar to adipocytes from native pads, and became vascularized and perhaps reinnervated by the sympathetic nervous system; however, their mass was significantly reduced across the ~3 mo of the experiments, 2) EWAT-lipectomized hamsters with autologous grafts appeared to exhibit an exaggerated compensatory increase in IWAT and RWAT masses compared with the compensatory increases after EWAT lipectomy-only, but, when IWAT was analogously manipulated, this did not occur, 3) WAT additions (two EWAT pads represent ~5% total body fat) were not compensated by decreased mass of the native fat pads, 4) EWAT lipectomy plus sibling EWAT grafts (nonautologous) resulted in normal, but not exaggerated, significant compensation in the mass of the other pads, and 5) food intake and body mass were largely unaffected by any of these treatments.

The present experiments demonstrated the feasibility of using autologous and nonautologous fat transplantation to test total body fat regulation. Specifically, both types of grafts appeared macro- and microscopically near normal, were not rejected, and became revascularized. In terms of the latter, failure to become revascularized would have resulted in complete tissue necrosis, and this was not seen in any of the 240 grafts across these experiments. One microscopic characteristic of the grafts in the present study, and in other experimental fat transplant studies (22), was areas devoid of adipocytes or adipocytes devoid of lipid, but these areas did not appear necrotic. These areas likely contributed to the often significant...
decreased graft mass across these and other experiments [e.g., rabbits (22)] as well as in humans receiving autologous transplants to correct soft tissue defects and deformities. In the latter case, reductions are so common that more fat is implanted than needed to counter the eventual 10–50% decreases in volume (for review, see Ref. 38).

We found that autologous transplantation of EWAT after lipectomy appeared to exaggerate the typical lipectomy-induced compensatory response, resulting in significantly increased masses of the nonexcised fat pads (RWAT and IWAT) and a marked and unusual increase in the mass of the remnants of the excised EWAT pads that exist as a consequence of attempts to keep testicular vascularization intact (i.e., ~25% of sham-operated control EWAT mass in present study, <10% in previous studies, e.g., Refs. 25, 28). Autologous IWAT transplant after lipectomy did not result in a similar exaggeration of the nonexcised EWAT pads (i.e., EWAT and RWAT), however, nor did it attenuate the reparation of the surgically induced lipid deficit, keeping in mind that compensatory increases in WAT mass after IWAT lipectomy often fall somewhat short of being complete (25, 27, 28, 30). This finding suggests that the impact of WAT transplantation within the same individual is fat pad specific. Nonautologous (between-sibling) additions of EWAT in nonlipectomized animals did not trigger a compensatory decrease in mass of the native fat pads like a regulatory system for total body fat might yield, thereby resulting in significantly greater total dissected WAT in hamsters receiving the EWAT donation from their male siblings. Thus transplants of EWAT per se do not automatically stimulate WAT pad growth because these nonautologous EWAT grafts did not do so. Instead, a lipid deficit seems necessary for this fat growth-promoting effect of EWAT to be realized. This latter notion is reminiscent of the likelihood of survival of intraperitoneally inserted WAT grafts in laboratory mice. In this case, a surgically induced lipid deficit is needed to significantly increase the probability of successful WAT transplantation (24).

Use of WAT transplantation as a means of challenging the hypothesized total body fat regulatory system [e.g., lipostatic hypothesis of Kennedy (21)] is predicated on the condition that the WAT graft is incorporated in this system; that is, it is sensed in some manner. Fat transplants significantly impact the physiology of the host, as recent studies in laboratory mice have shown (6, 14), but whether this is largely a peripheral physiological response without central “sensing” of the added lipid cannot be discerned from those experiments. In the present experiments, we did not find that hamsters with fat additions-only (nonautologous grafts between brothers) had decreased masses of the native WAT pads, as would be predicted by Kennedy’s (21) total body fat regulation scenario. One interpretation of our data suggests that the WAT grafts did not become incorporated in this regulatory system because their presence went undetected by the central nervous system. The sensing of peripheral lipid stores by the brain is thought to occur in intact animals via humoral (e.g., leptin) or neural inputs [i.e., sensory innervation of WAT (13, 15)]. The ability of these grafts to secrete humoral signaling proteins, such as leptin, or to possess sensory nerves has not been tested to date. The revascularization apparent in the present experiment and the report of some type of innervation of a WAT graft (14) suggest the possibility for sensory nerve or sensory humoral communication from the grafts to the brain. Another interpretation of our findings is that the fat additions were sensed but that the resulting increase in total body fat was too small to trigger compensatory responses by the native fat pads [EWAT grafts represent ~5% increases in total body fat (28)]. Yet another possible interpretation of these results is that the fat additions were sensed, but, when body fat is increased directly by the grafts, there is no compensation by the native WAT, suggesting that Kennedy’s lipostatic theory only is supported for body fat increases that occur via overfeeding (17). Finally, in an analogous manner to the suggestion by Ahima et al. (1) that leptin’s ability to trigger compensatory energy responses primarily occurs with lipid deficits, but not surfeits, it may be that graft-induced energy surfeits do not trigger compensatory energy responses but lipectomy-induced lipid deficits do, as we have seen previously (for review, see Ref. 31).

The origin of the low concentrations of NE in the grafts of the present study suggests sympathetic innervation, and we suspect this likely reflects the sympathetic innervation associated with blood vessels to the tissue, rather than the reinervation of the adipocytes per se (35, 41). This hypothesis is based on the angiogenesis in the WAT transplants that must have occurred because they survived ~3 mo and the associated establishment of the sympathetic control of blood flow to the tissues (for review, see Ref. 37). The precise nature and role of this innervation requires both histological and functional studies.

The mechanism underlying the surprising exaggerated response to lipectomy in EWATx hamsters with autologous transplants is not known at this time but may be one or more of several factors secreted by WAT either humorally or as paracrine factors that stimulate fat pad growth (e.g., IGF-II; for review of potential factors, see Ref. 19). Regardless of the mechanism, it appears that not all autologous WAT grafts are capable of stimulating the growth of other pads (i.e., IWAT) and neither are all EWAT grafts capable of doing so (i.e., nonautologous EWAT in nonlipectomized hamsters).

Collectively, these studies demonstrate that total body fat usually is recovered after fat removal (for review, see Ref. 31), but their ideological counterpart of making animals inappropriately fat via WAT transplants alone does not trigger compensatory decreases in the native fat pad masses. Because lipectomy results in body fat compensation, lipectomy combined with fat replacement should result in blockade of subsequent increases in body fat, but this appears to be a fat pad-specific response (i.e., IWAT blocks the increases, whereas EWAT does not). Moreover, not only was a lipectomy-like response triggered by EWATx despite its replacement at a different location, a seemingly and surprisingly exaggerated response to the lipectomy occurred. Whether this outcome represents a single mechanism, such as increased sensitivity to lipectomy with autologous EWAT grafts, or whether it is the result of a combined normal lipectomy response with an additional stimulation of WAT growth resulting from relocation of the excised EWAT remains to be tested.

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GRANTS

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REFERENCES