Effects of white adipose tissue grafts on total body fat and cellularity are dependent on graft type and location

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Submitted 16 February 2005; accepted in final form 24 March 2005

Lacy, Eva L., and Timothy J. Bartness. Effects of white adipose tissue grafts on total body fat and cellularity are dependent on graft type and location. Am J Physiol Regul Integr Comp Physiol 289: R380–R388, 2005. First published March 31, 2005; doi:10.1152/ajpregu.00116.2005.—Surgical removal of body fat (lipectomy) triggers compensatory increases in nonexcised white adipose tissue (WAT), thus restoring adiposity levels in many species, including Siberian hamsters. In Siberian hamsters, when their lipectomized WAT is transplanted to another site (autologous grafts, no net change in body fat), healthy grafts result, but the lipectomy-induced compensatory increases in nonexcised WAT masses are exaggerated, an effect that apparently occurs only when the grafts contact intact WAT. When WAT is added to nonlipectomized hamsters to increase body fat, native WAT pads do not decrease. Thus WAT addition or removal-replacement does not induce compensatory WAT responses consistent with total body fat regulation as does WAT subtraction. Therefore, we tested whether the exaggerated response to lipectomy occurring with autologous WAT transplantation is dependent on graft site placement and whether the donor graft source [inguinal or epididymal WAT (IWAT, EWAT), sibling vs. nonsibling] affected body fat responses to WAT additions in nonlipectomized hamsters. Lipectomized hamsters received subcutaneous autologous EWAT grafts placed remotely from other WAT (ventrum) or in contact with intact WAT (dorsum), whereas intact hamsters received EWAT or IWAT grafts from sibling or nonsibling donors. The exaggerated response to lipectomy only occurred when grafts were in contact with intact WAT. EWAT, but not IWAT, additions to nonlipectomized siblings or nonsiblings increased native IWAT and retroperitoneal WAT mass but not EWAT mass compared with controls. Collectively, WAT transplantation to either lipectomized or nonlipectomized hamsters increased body fat contingent on graft contact with intact or native WAT.

transplants; white fat; food intake; obesity; lipectomy

THE NOTION THAT TOTAL BODY fat is regulated was first articulated by Kennedy (18) in his “lipostatic hypothesis” in 1953. Several other incarnations have emerged in recent years with the discovery of leptin, the largely white adipose tissue (WAT)-derived factor thought by some to convey total body fat levels to the brain (for review, see Ref. 31). Tests of the hypothesized total body fat regulatory system have primarily been indirect challenges, such as decreasing body fat via food restriction and/or fasting or increasing body fat via overfeeding, accomplished by feeding highly palatable diets or tube feeding (for review, see Refs. 13 and 26). Although much has been learned about the indirect physiological effects of these manipulations on adiposity, they are not direct tests of total body fat regulation because they alter a wide range of other physiological systems (e.g., gastrointestinal, neural, endocrinological, thermogenic). In contrast, total body fat levels can be directly manipulated by removing body fat (partial lipectomy, hereafter referred to as lipectomy) or by adding fat (grafts or transplants; for review, see Ref. 26).

Lipectomy triggers a compensatory increase in the mass of the nonexcised WAT pads across a wide range of species and conditions that ultimately (8–16 wk depending on the species) results in the restoration of total body fat, as evidenced by equivalent carcass lipid contents between the lipectomized and sham lipectomized animals (for review, see Ref. 26). We recently (19) used combinations of lipectomy and WAT grafts to challenge the total body fat regulatory system in Siberian hamsters (Phodopus sungorus). Specifically, we removed epididymal WAT (EWAT) pads and replaced them subcutaneously at a different location (dorsum) within the same animals (i.e., autologous grafts), creating no initial change in total body fat levels. Surprisingly, autologous grafts in lipectomized hamsters triggered lipectomy-like compensation by the nonexcised fat pads (i.e., the animals responded as though the WAT was actually removed), which was exaggerated twofold compared with lipectomy without WAT replacement (19). It was observed that the animals showing the exaggeration of the lipectomy-induced increases in nonexcised WAT pad masses had autologous WAT grafts that directly contacted intact WAT on the dorsum [i.e., inguinal subcutaneous WAT (IWAT)], but this was not tested explicitly. This prompted speculation that this autologous graft-host WAT interaction underlies this exaggerated response to lipectomy.

As noted above, the effects of increased WAT mass on the hypothesized total body fat regulatory system can be tested by WAT additions to otherwise intact animals from donor animals. The addition of WAT to Siberian hamsters (19) or to laboratory mice (28) results in species-specific compensatory responses. Thus Siberian hamsters receiving additional WAT do not compensate for their elevated total body fat by reducing their native fat pad masses despite the normal macro- and microscopic appearance of the grafts 12 wk later (19). By contrast, laboratory mice respond to WAT additions by decreasing their native fat pad masses (28). Because WAT addition or removal-replacement in Siberian hamsters does not induce compensatory WAT responses consistent with total body fat regulation as does WAT subtraction, the present study was designed to answer the following questions. 1) Does the effect of autologous WAT transplantation vary depending on the location of graft placement? 2) Does the source of the
donor graft, i.e., WAT pad [WAT vs. epididymal WAT (EWAT)], sibling vs. nonsibling, affect compensatory body fat responses? This was accomplished in experiment 1 by placing autologous EWAT grafts in direct contact with IWAT on the dorsum or not in contact with IWAT on the ventrum and in experiment 2 by transplanting EWAT or IWAT from male sibling or male nonsibling donors to otherwise intact hamsters.

METHODS

Animals and Housing

All experimental procedures were approved by the Georgia State University Institutional Animal Care and Use Committee in accordance with the National Institutes of Health and United States Department of Agriculture guidelines. Adult male Siberian hamsters (3–4 mo old) were obtained from our breeding colony, the lineage of which has been described recently (3). Hamsters were housed in a long “summerlike” photoperiod (16:8-h light-dark cycle, lights on at 0200) and given PMI rodent diet no. 5001 and tap water ad libitum. In experiments 1 and 2 below, group-housed animals were singly housed for 2 wk before surgery. At that time, they were divided into groups matched for the percent change in body mass during the initial single housing period as well as for the absolute body mass after the 2 wk of single housing.

Experiment 1: Does the Effect of Autologous WAT Transplantation Vary Depending on the Location of Graft Placement?

It appeared previously that exaggerated body fat compensation occurred in lipectomized hamsters when the graft was in contact with IWAT, but this was not tested (19). To test whether the direct contact occurred in lipectomized hamsters when the graft was in contact with IWAT, the following experiment was performed.

Sixty-five hamsters were divided into the following six groups: 1) bilateral EWAT lipectomy with sham dorsal transplant (EWATx + dorsal sham; n = 10), 2) bilateral EWAT lipectomy with sham ventral transplant (EWATx + ventral sham; n = 10), 3) bilateral EWAT lipectomy with implantation of this excised EWAT as dorsal subcutaneous transplants (EWATx + dorsal grafts; n = 12), 4) bilateral EWAT lipectomy with implantation of this excised EWAT as ventral subcutaneous transplants (EWATx + ventral grafts; n = 11), 5) sham lipectomy with sham dorsal transplant (sham EWATx + dorsal sham; n = 11), and 6) sham lipectomy with sham ventral transplant (sham EWATx + ventral sham; n = 11) (Fig. 1).

EWAT lipectomy and transplantation. All surgeries were performed under pentobarbital sodium anesthesia (50 mg/kg ip). EWATx was accomplished by making an abdominal incision through which both EWAT pads could be accessed, as previously described (20–25), and represents removal of ~10–20% of dissectible body fat (23). 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 were 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 all transplants, the EWAT pads from each side were bisected and replaced as two subcutaneous transplants, ipsilateral to the side of removal. For dorsal transplantation, small bilateral dorsal incisions were made, the skin and fascia were loosened with blunt tissue forceps, and the transplants were placed under the skin after which the incision was closed with wound clips. For ventral transplantation, small bilateral incisions were made along the lower border of the ribcage, the skin and fascia were loosened rostrally, and the transplants were placed along the ribs under the skin. The incision was then closed with wound clips. Sham EWATx and sham transplantation consisted of exposing but not removing the pads, as well as all of the procedures for actual transplantation; however, no tissue was inserted under the skin (Fig. 1). Nitrofurazone powder was applied to all wounds to minimize infection.

Food intake and body mass were measured weekly for 12 wk post surgery to the nearest 0.1 g, the former corrected for pouching and spillage. At week 12, all animals were killed by pentobarbital sodium overdose and transplanted fat pads, and intact EWAT, IWAT, and retroperitoneal WAT (RWAT) were harvested and weighed.

Fat cell size and number. EWAT, IWAT, and RWAT pads and all transplants were prepared for measurements ofcellularity [fat cell number (FCN) and fat cell size (FCS)] from a subset of animals in each group reflecting the mean and standard error of the mean of the body mass change across the experiment for the entire group (n = 5–9 per group). Briefly, each pad was blotted dry and finely minced. A sample of each minced pad was further processed. For small tissues (RWAT, the residual EWAT from EWATx animals), aliquots of between 20 and 100 mg were used; for larger tissues, ~250 mg of minced sample were used. Samples were fixed in osmium tetroxide according to the method of Hirsch and Gallian (17) for determination of FCN and FCS by a Coulter counter equipped with a channelizer (Beckman Coulter, Fullerton, CA), as previously described (3).

Experiment 2: Does the Source of the Donor Graft [i.e., WAT Pad (IWAT vs. EWAT), Sibling vs. Nonsibling] Affect Compensatory Body Fat Responses?

Eighty-eight hamsters were divided into the following seven groups: 1 and 2) WAT donors (siblings, n = 12; nonsiblings, n = 13),
3) sibling EWAT (sibling EWAT graft received; n = 12), 4) nonsibling EWAT (nonsibling EWAT graft received; n = 13), 5) sibling IWAT (sibling IWAT graft received; n = 13), and 6) nonsibling IWAT (nonsibling IWAT graft received; n = 13), and 7) Sham (sham incision and transplantation). All transplants were placed dorsally and in contact with native IWAT.

**EWAT and IWAT transplantation.** The animals that gained the most mass during the period of single housing were used as donors of EWAT or IWAT. All surgeries were performed under pentobarbital sodium anesthesia (50 mg/kg ip). A fat donor and the two animals assigned to receive transplants from that donor were anesthetized at the same time to reduce the interval between fat removal and transplantation. Right and left side EWAT and IWAT pads were removed from the donor and weighed. Each pad was bisected, and the four smaller pieces of each WAT type were placed separately in cold (4°C) 0.15 M NaCl until transplantation into the appropriate recipient. 

Transplantation procedures were as in experiment 1 except that all transplants and sham procedures occurred dorsally. The incisions were closed with wound clips, and nitrofurzone powder was applied to minimize infection.

Food intake and body mass were measured weekly for 12 wk postsurgery to the nearest 0.1 g, the former corrected for pouching and spillage. At week 12, all animals were anesthetized with isofluorane (Pittman-Moore, Mundelein, IL) and bled from the retro-orbital sinus using Natelson tubes (250 µL) between 0800 and 1100 during test day. Blood was stored overnight at 4°C, and serum was separated by centrifugation at 1,000 g for 20 min and stored at −20°C until assayed for serum leptin (see below). After the blood sample was obtained, subgroups of animals were killed as described below, matched for the mean percent change in body mass from surgery to week 10. Animals from each treatment were killed with an overdose of pentobarbital sodium, and the transplanted fat pads, as well as the native EWAT, IWAT, and RWAT, were dissected and weighed. Testes mass (an integrated measure of reproductive status) was determined after harvesting.

**Leptin radioimmunoassay.** Serum leptin concentrations were determined in a single assay using a commercial kit according to manufacturer specifications (multispecies kit, Linco Research, St. Charles, MO). This assay kit has been previously validated for use with human, rat, mouse, and monkey serum. Serum samples were stored at −80°C until assayed.

**Statistical Analysis.**

**Experiment 1.** Initial and terminal tissue and body masses, cumulative food intake, the percent change in body and graft masses, and FCN for grafts and intact WAT were analyzed by two-factor, univariate ANOVA (general linear model, version 11.5; SPSS, Chicago, IL), with the factors “surgery” (EWATx, EWATx + graft, sham) and “location” (dorsal, ventral), followed by post hoc testing using Duncan’s new multiple range tests when appropriate. If the interaction of “location × surgery” was significant, then separate one-way ANOVAs for each location were done next, followed by Duncan’s new multiple range post hoc tests to determine differences among surgery types on each location. Because there were suggestive but not statistically significant differences in IWAT mass between the two sham-operated control groups (P = 0.064, data not shown) and because IWAT mass was used in the calculation of total dissected WAT (combined mass of dissected IWAT, EWAT, RWAT, and grafts), IWAT mass and total dissected WAT (with and without grafts included) in EWATx + dorsal sham, EWATx + dorsal grafts, EWATx + ventral sham, and EWATx + ventral grafts animals were expressed and analyzed as percentages of the mean masses of their appropriate sham-operated control group. Paired t-tests were used to compare the initial vs. the final graft masses. Weekly body mass and food intake were analyzed by two-factor ANOVA for repeated measures with the ANOVA design of surgery × location × time (for body mass from surgery to week 12, 3 × 2 × 13; for food intake week 1 to week 12, 3 × 2 × 12). The proportion of WAT cells that were between 30 and 240 µm diameter were analyzed in 10-µm increments by two-factor ANOVA for repeated measures with the ANOVA design of surgery × location × cell size (3 × 2 × 21). For all repeated-measure ANOVAs, if the main effects of week or cell size were significant, post hoc two-factor ANOVA was done next, followed by Duncan’s new multiple range tests to determine differences between treatments for each week or size range. If the interaction of “location × surgery” was significant for the two-factor ANOVA, separate post hoc one-way ANOVAs were done next, followed by Duncan’s new multiple range tests to determine differences between treatments for each location.

**Experiment 2.** For all tests, data were analyzed separately for animals receiving EWAT vs. IWAT grafts because EWAT and IWAT transplants differ in size (Table 1). Initial and terminal tissue and body masses, cumulative food intake, percent change in body and tissue masses, and circulating leptin concentrations were analyzed by one-way ANOVA, with the factor being “donor type” (none, sibling, nonsibling). Paired t-tests were used to compare the initial vs. the final graft masses. Weekly body mass and food intake were analyzed with one-way ANOVA for repeated measures with the ANOVA “donor type” × “time” (for body mass, 3 × 13; for food intake, 3 × 12), with post hoc testing using one-way ANOVA to determine differences between treatments for each week. Two-tailed Pearson’s product moment correlations were used to determine covariation among variables.

For all statistical tests in both experiments, differences among means or correlations were considered significant at P < 0.05; in most cases, exact probabilities and test values were omitted for simplification and clarity of the presentation of the results.

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<th>Table 1. Initial and final graft masses for experiments 1 and 2</th>
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Values are means ± SE. EWAT, epididymal white adipose tissue; IWAT, inguinal white adipose tissue. *P < 0.05 initial vs. final graft mass.
RESULTS

Experiment 1: Does the Effect of Autologous WAT Transplantation Vary Depending on the Location of Graft Placement?

EWAT lipectomy deficits, graft mass, and graft cellularity. The mass of EWAT removed by lipectomy did not differ among lipectomized groups nor did the mass of EWAT grafts replaced as autologous dorsal vs. ventral grafts (Table 1). The mass of all grafts was significantly decreased by ~35% at week 12 (P < 0.05; Table 1), and both the absolute and percent decreases in mass were the same regardless of their placement (dorsally or ventrally; Table 1). The total FCN in the dorsal and ventral graphs was also similar (Fig. 2A). There were, however, differences in the FCS distributions between the grafts. Thus there were significantly more smaller adipocytes for the ventral grafts (30–40 μm diameter) and significantly more larger adipocytes for the dorsal grafts (60–100 μm diameter) (P < 0.05; Fig. 2, B and C).

Weekly food intake and body mass. Food intake did not significantly vary among the groups across the experiment with the exception of an inhibition of food intake during the first week, an effect likely due to surgery-associated stress. At that time, food intake was significantly decreased by the EWATx dorsal graft hamsters compared with the other two ventral graft groups (sham EWATx + ventral sham and sham EWATx + ventral sham; P < 0.05; Fig. 3A). The initial inhibition of food intake was short lived, however, with the remaining weekly intakes not differing among the groups (Fig. 3A). In addition, cumulative food intake was not different among the groups across the experiment (Fig. 3B). Weekly body mass also did not vary significantly among the groups across the 12 wk (data not shown).

WAT mass and cellularity. IWAT and RWAT masses from EWATx + dorsal graft animals were significantly larger than their respective control groups (P < 0.05; Fig. 4A). Specifically, IWAT and RWAT masses from the EWATx + dorsal graft hamsters increased ~100% compared with their sham-operated counterparts. By contrast, IWAT and RWAT masses from EWATx + ventral graft hamsters were not different from their lipectomized and sham-operated controls (Fig. 4A). EWAT mass was significantly reduced by EWATx, indicating little or no regrowth of this pad (Fig. 4A). Total dissected WAT mass was significantly reduced by EWATx, indicating little or no regrowth of this pad (Fig. 4A). Total dissected WAT...
Although placement site of the EWAT graft did not affect the distribution of FCS for RWAT adipocytes, when both graft locations were considered together, EWATx + graft consistently and significantly shifted the FCS distribution toward larger adipocytes compared with the EWATx + sham animals, beginning with 90- to 100-μm and 100- to 230-μm-diameter adipocytes (P < 0.05; Fig. 6) except for 170- to 190-μm and 200- to 210-μm adipocytes (Fig. 6C).

Experiment 2: Does the Source of the Donor Graft [i.e., WAT Pad (IWAT vs. EWAT), Sibling vs. Nonsibling] Affect Compensatory Body Fat Responses?

Initial and final transplant mass. Because we transplanted the entire fat pads removed from the donors to the recipients and because EWAT mass is approximately one-half the mass of IWAT, EWAT grafts were smaller than IWAT grafts at the beginning and end of the study (P < 0.05; Table 1). For both EWAT and IWAT transplants, the initial and final graft masses did not differ between groups receiving transplants from a sibling vs. a nonsibling. Both types of WAT pads, whether from siblings or nonsiblings, significantly decreased in mass by ~25–50% across the 12-wk experiment (P < 0.05; Table 1). Overall, the larger the size of the initial graft, the greater the final decrease in graft mass (R = 0.356, P = 0.05).

Weekly food intake and body mass. In general, food intake was not different across the experiments among the EWAT or IWAT groups except for decreased food intake, which was likely attributable to surgery-associated effects at week 1 by sibling EWAT recipients vs. nonsibling EWAT animals, by both groups compared with their sham-operated controls, and by the nonsibling IWAT hamsters compared with their Sham counterparts (P < 0.05; data not shown). These were transient decreases in food intake because cumulative food intakes were not different among the groups (data not shown).

Weekly body mass did not differ significantly among EWAT and IWAT groups, and cumulative body mass did not gain (data not shown).

WAT mass. IWAT mass of EWAT-grafted animals was significantly greater (~55%) than that from sham-treated controls (P < 0.05; Fig. 7A) regardless of whether the donor was a sibling. By contrast, RWAT mass was only significantly greater (~55%) than controls for sibling EWAT-grafted...

Fig. 5. Fat cell number for each WAT type in experiment 1. Values are means ± SE. *P < 0.05 vs. all groups on that location.
sters (P < 0.05; Fig. 7A). EWAT mass was not significantly different among EWAT graft groups (Fig. 7A). Total dissected WAT was significantly greater in both groups that received EWAT grafts than in the sham-operated animals (P < 0.05; Fig. 7A), and this was not solely due to the graft mass because these significant differences persisted after the graft masses were subtracted from the total dissected WAT (P < 0.05; Fig. 7A).

Unlike the stimulation of fat pad growth by EWAT grafts, IWAT grafts did not stimulate WAT growth compared with sham controls (Fig. 7B), although the pattern of nonsignificant increases was similar to that of EWAT grafted hamsters. Total dissected WAT, however, was significantly greater in both grafted groups (P < 0.05; Fig. 7B), but this difference was solely due to the contribution of the IWAT graft with the statistical significance disappearing with subtraction of graft mass from the total dissected WAT (Fig. 7B).

Circulating leptin concentrations and correlations with WAT mass. Mean serum leptin concentrations ranged from 11.75 to 15.36 ng/ml (range 3.45–38.60 ng/ml) and did not differ among treatments for either EWAT or IWAT grafts (Fig. 8A). Serum leptin was significantly positively correlated with body mass at week 12 and with IWAT mass or with RWAT mass (R = 0.452, 0.494, and 0.452, respectively, P < 0.05; Fig. 8, B and C, RWAT data not shown).

DISCUSSION

Lipectomized animals across a variety of species show compensatory increases in the growth of nonexcised WAT pads in response to the surgically induced lipid deficit (for review, see Ref. 26). The compensatory increase in nonexcised WAT growth by EWATx Siberian hamsters is exaggerated when the surgically removed EWAT is transplanted to another location within the same animal (i.e., autologous grafts) even though no net initial decrease in total body fat occurs (19). The results of the present study show that this exaggerated response to lipectomy in autologous graft-bearing Siberian hamsters only occurred when the grafts were in direct contact with intact WAT. Thus autologous grafts on the dorsum in contact with IWAT triggered increased masses of nonexcised WAT pads that were significantly greater than those triggered by lipectomy alone, whereas autologous grafts on the ventrum did not do so. In addition, the WAT cellularity of the nonexcised WAT was differentially affected by the autologous grafts such that

![Graph A](image1.png)

**Fig. 6.** Percent total RWAT cells per 10-μm-diameter size class for hamsters with dorsal graft treatments in experiment 1. Values are means ± SE. A: percent RWAT cells of 30–100 μm diameter. B: percent RWAT cells of 100–170 μm diameter. C: percent RWAT cells of 170–240+ μm diameter. *P < 0.05 between bracketed groups.

![Graph B](image2.png)

**Fig. 7.** WAT pad mass expressed as percent of sham transplant values for experiment 2. Values are means ± SE. A: IWAT, EWAT, RWAT, and total dissected WAT (sum of masses of all pads), including and excluding graft mass for animals with EWAT grafts. B: IWAT, EWAT, RWAT, and total dissected WAT, including and excluding graft mass for animals with IWAT grafts. *P < 0.05 vs. SHAM. Total dissected WAT (no grafts), sum of masses of all pads excluding grafts.
The mechanism through which autologously transplanted EWAT triggers these exaggerated compensatory increases in nonexcised WAT mass after lipectomy (present study and Ref. 19) is unknown. The exaggerated growth of nonexcised WAT seems consistent with the notion that the presence of the grafts goes unrecognized by the central nervous system. One way that the brain may receive information as to the presence or size of WAT pads may be via the sensory innervation of WAT (11, 27, 30), and the grafted WAT may not get reinnervated by the sensory nerves. Indeed, we have failed to see calcitonin gene-related peptide immunoreactivity, indicative of sensory nerves (12, 30), in autologous grafted pads (Lacy and Bartness, unpublished results). It also could be that the failure to respond to autologously transplanted WAT may be due to the absence of a circulating signal (e.g., leptin) conveying the presence or size of WAT to the central nervous system. An intact leptin signaling system, however, is not necessary for the lipectomy-induced compensatory responses of nonexcised WAT pads because ob/ob and db/db mice show normal compensatory WAT responses to lipectomy (5, 14).

In the present experiment, if the autologous grafts were transplanted so as to have direct contact with the intact WAT (IWAT) in lipectomized hamsters, then the nonexcised WAT pads increased their growth beyond that of lipectomy alone. By contrast, their ventrally transplanted graft counterparts that had no direct contact with intact WAT did not do so. One possible underlying mechanism for this effect could involve a circulating factor because not only was there an increase in the mass of WAT that was in contact with the graft (IWAT) but there was an increase in the mass of the distally located WAT that was not in contact with the graft (RWAT). It is not clear, however, whether this hypothesized contact-induced WAT growth factor originates from the graft, from the intact pad, or from both; speculation as to possible candidates is difficult because this is a hitherto undiscovered phenomenon in any species. Alternatively, a circulating factor may not be involved, despite the response of the distal RWAT pad. That is, a locally produced (paracrine) factor from the graft and/or intact IWAT could trigger the exaggerated responses of WAT in contact with the graft, as well as those distal and not in contact with the graft, via changes in the sympathetic drive to these WAT pads. Thus the signal generated from the WAT-graft contact could reach the brain via the sensory innervation of the intact WAT (11, 30), that, in turn, could decrease activity of the centrally located sympathetic outflow circuits to WAT (1). Decreased sympathetic drive to the WAT would result in decreased lipid mobilization (i.e., increased lipid accumulation) (2, 4, 7) and/or increased adipocyte proliferation (3, 6, 30, 32). Indeed, our recent work (29) suggests that lipectomy triggers a decrease in the sympathetic drive to WAT, as measured by norepinephrine turnover. This decrease in sympathetic drive would, in turn, promote decreases in basal lipolysis, thereby enhancing lipid accumulation and/or increasing fat cell proliferation in the nonexcised WAT pads. These hypothesized decreases in the sympathetic drive to, and responses of, nonexcised WAT pads in lipectomized hamsters bearing autologous grafts remain to be tested.

Although the intact pad in contact with the graft (IWAT) had increased FCN and the fat pad distal to and not in contact with the graft (RWAT) had increased FCS, this does not necessarily mean that proliferation and lipid accumulation, respectively,
were the only or even primary responses of these depots. That is, it has been hypothesized (10) and tested and confirmed by us in a number of animal models (15) that adipocytes first fill to capacity and then, through as yet unidentified mechanisms, trigger a proliferative response. Thus the accumulation of lipid may have occurred for both pads, but the lipid filling in IWAT resulted in a more rapid achievement of maximum FCS that, in turn, triggered the likely proliferative response. By contrast, the maximum FCS may not have been achieved by RWAT at the time of death and thus no proliferative response occurred.

The compensatory increase in mass of the nonexcised WAT pads after EWATx without replacement in experiment 1 was somewhat less than that seen in our previous studies with similarly treated hamsters (20, 21, 23–25). Specifically, the IWAT mass of EWATx hamsters was nonstatistically significantly increased (~30%) with considerable variability across animals, whereas previously the compensatory increase of IWAT was ~40–50% with less variability across animals (20, 21, 23–25). One reason for this muted compensatory response to lipectomy in the present study may be differences in surgical procedures between this work and our previous studies. That is, less EWAT mass was removed in experiment 1 of the present study compared with our previous studies (20, 21, 23–25) because of a more aggressive tendency to remove all EWAT in the earlier work, whereas a more conservative approach was taken by the surgeon here. Regardless of the differences between the previous studies and the present study, this compensatory response to lipectomy was clearly exaggerated in animals with dorsal grafts and was statistically significantly greater than EWATx alone.

Transplantation of EWAT but not IWAT in nonlipectomized hamsters (i.e., fat additions) significantly increased native IWAT mass regardless of the relatedness of the donor and also increased RWAT mass when the donor was a sibling, with a suggestive but not statistically significant increased mass with nonsibling donors. The individual increased WAT masses resulted in significantly increased total dissected WAT mass, even when graft mass was taken into account, an effect seen previously by us (19) in our study of EWAT transplants between siblings. Thus we extended the results here to show that, regardless of the relatedness of the donor (brother or nonbrother), the response to EWAT addition is an increase in body fat. This is in sharp contrast to our recent study with laboratory mice (28) in which WAT additions (EWAT grafts) triggered decreases in native fat pad masses, ultimately resulting in no change in total body fat. The response by these mice is the predicted result if total body fat is regulated and if the added WAT is incorporated into the sensing of total body fat. One possible difference between these two studies is the species (Siberian hamsters vs. laboratory mice). Both species, however, respond similarly to fat subtractions (lipectomy) (14, 20, 21, 23–25), making such an explanation plausible only if fat additions promote species-specific effects.

As noted above, EWAT but not IWAT graft additions to nonlipectomized hamsters increased native WAT pad growth (IWAT and RWAT, but not EWAT). The underlying mechanism for this fat pad-specific ability of EWAT to increase WAT growth in all but its native counterpart pad as well as the inability of IWAT to significantly affect any WAT pad (although there was a suggestive, but nonsignificant similar pattern of increases) is unknown. Unfortunately, we did not measure the cellularity responses of the grafts or of the native pads in experiment 2, making it more difficult to speculate as to the possible mechanisms underlying this phenomenon on the basis of known factors that stimulate adipocyte hypertrophy or hyperplasia. The ability of the EWAT grafts to trigger increases in some but not all pads should facilitate subsequent investigations of factors that stimulate adipocyte proliferation and/or lipid accumulation, allowing for within-animal comparisons among the native WAT pads.

Collectively, the present data show a remarkable stimulation of body fat by either the addition of body fat to lipectomized or nonlipectomized hamsters. A common feature of these increases in adiposity is the contact of the grafted WAT pad with native/intact WAT pads. The mechanism underlying this interesting phenomenon is unknown at present, but the finding appears to have important implications for understanding the growth of WAT. Specifically, although several factors, both circulating and paracrine, have been suggested as stimulators of FCN and/or proliferation (for review, see Ref. 16), the hypercellularity associated with obesity is poorly understood at best, despite it being a hallmark of this disease. WAT grafts, therefore, afford a useful and relatively simple in vivo means of stimulating WAT growth including increases in FCN and/or proliferation with the goal of investigating underlying circulating or paracrine factors involved in this process. Finally, the ability of WAT grafts to stimulate WAT growth of pads with which it makes contact as well as distant pads with which it has no contact has implications for the use of WAT in reconstructive and cosmetic surgery in humans (for review see, Ref. 9). That is, if humans respond to WAT grafts in a manner similar to Siberian hamsters, then a potentially adverse consequence of such a surgery might be increases in adiposity, perhaps in visceral fat, where health risks could increase as a consequence.

ACKNOWLEDGMENTS

The authors thank the Georgia State University Animal Care staff for efforts in maintaining the animals.

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GRANTS

This research was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant R01 DK-35254 to T. J. Bartness.

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