White adipose tissue sensory nerve denervation mimics lipectomy-induced compensatory increases in adiposity

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Shi, Haifei, and Timothy J. Bartness. White adipose tissue sensory nerve denervation mimics lipectomy-induced compensatory increases in adiposity. Am J Physiol Regul Integr Comp Physiol 289: R514–R520, 2005.—The sensory innervation of white adipose tissue (WAT) is indicated by the labeling of sensory bipolar neurons in the dorsal root ganglion after retrograde dye placement into WAT. In addition, immunoreactivity (ir) for sensory-associated neuropeptides such as calcitonin gene-related peptide (CGRP) and substance P in WAT pads also supports the notion of WAT sensory innervation. The function of this sensory innervation is unknown but could involve conveying the degree of adiposity to the brain. In tests of total body fat regulation, partial surgical lipectomy triggers compensatory increases in the mass of nonexcised WAT, ultimately resulting in restoration of total body fat levels in Siberian hamsters and other animals. The signal that triggers this compensation is unknown but could involve disruption of WAT sensory innervation that accompanies lipectomy. Therefore, a local and selective sensory denervation was accomplished by microinjecting the sensory nerve neurotoxin capsaicin bilaterally into epididymal WAT (EWAT) of Siberian hamsters, whereas controls received vehicle injections. Additional hamsters had bilateral EWAT lipectomy (EWATx) or sham lipectomy. As seen previously, EWATx resulted in significantly increased retroperitoneal WAT (RWAT) and inguinal WAT (IWAT) masses. Capsaicin treatment significantly decreased CGRP- but not tyrosine hydroxylase-ir, attesting to the diminished and selective sensory innervation. Capsaicin-treated hamsters also had increased RWAT and, to a lesser degree, IWAT mass largely mimicking the WAT mass increases seen after lipectomy. Collectively, these data suggest the possibility that information related to peripheral lipid stores may be conveyed to the brain via the sensory innervation of WAT.

capsaicin; calcitonin gene-related peptide; obesity; hamster

THE NOTION THAT TOTAL BODY FAT is regulated was initially articulated by Kennedy (16) as the major tenant of his lipostatic hypothesis. Since then, the regulation of body fat has been studied using a variety of primarily indirect treatments to alter total body fat levels. For example, fasting or overfeeding decreases or increases body fat, respectively, and body fat is restored to prestarch or overfeeding levels with refeeding or with termination of overfeeding (e.g., 5, 12). In addition to changing body fat levels, both fasting and overfeeding affect more than just white adipose tissue (WAT) mass; for example, fasting and tube feeding both affect thermogenesis (e.g., 18, 30), and fasting inhibits reproductive function (e.g., 31). A more direct approach to the manipulation of total body fat is the removal of selective WAT pads—partial lipectomy, hereafter referred to as lipectomy. Lipectomy immediately affects only body fat and then triggers changes in physiology such as decreased sympathetic drive to brown adipose tissue (33), alterations in the growth and cellularity of the nonexcised WAT pads (e.g., 19, 21) and behavior such as increases in food hoarding (36) but not food intake (e.g., 19, 21, 22, 36). Fasting, by contrast, immediately affects physiology and behavior and then triggers decreases in body fat (for a review, see Ref. 29). Thus lipectomy directly reduces body fat levels and thereby directly challenges the hypothesized total body fat regulatory system (for a review, see Ref. 25).

The response to lipectomy is pervasive across species occurring in laboratory rats and mice, as well as in species that undergo seasonal or circannual fluctuations in body fat content, such as ground squirrels and Syrian hamsters and Siberian hamsters (Phodopus sungorus), our species of study here (for a review, see Ref. 25). These compensatory increases in WAT pad mass ultimately result in restoration of total body fat levels and occur without changes in food intake (for a review, see Ref. 25). Although much still remains to be known regarding the mechanisms underlying the compensatory responses to the lipid deficit that ultimately replenish total body fat levels, here, we focus on determining the signal that initiates the compensatory responses.

An obvious circulating factor that could trigger the lipectomy-induced increases in body fat is leptin, the largely adipose-derived cytokine thought of as a conveyor of body fat levels to the brain (for a review, see Ref. 37). It does not appear, however, that leptin is necessary for the lipectomy-induced stimulation of lipid accumulation by the nonexcised WAT pads because animals with nonfunctional leptin signaling (ob/ob and db/db mice) recover lipectomy-induced body fat losses despite not having leptin or leptin receptors (13). Alternatively, the trigger for lipectomy-induced body fat compensation could be neural. WAT has sensory innervation, as first demonstrated by tract tracing (10). Specifically, implantation of the retrograde trach tracer, true blue, into laboratory rat WAT pads labels bipolar sensory neurons in the dorsal root ganglia (10). In addition, WAT contains immunoreactivity (ir) for sensory-associated neuropeptides such as substance P and calcitonin gene-related peptide (CGRP; 11, 33). Although the exact function of the sensory innervation of WAT is unknown (for a review, see Refs. 2 and 3), the neuroanatomical evidence for its presence is strong.

Therefore, to begin to determine the functions of WAT sensory innervation, we tested the possibility that WAT sensory innervation informs the central nervous system of peripheral lipid stores. Specifically, we tested whether bilateral epididymal WAT (EWAT) sensory denervation triggers increases in the mass of intact WAT pads analogous to the increases in the mass of...
nonexcised WAT pads after EWAT lipectomy (EWATx) (e.g., Refs. 19, 22, 23). Intra-WAT pad microinjections of capsaicin, a sensory neurotoxin (14), were used to denervate EWAT locally and selectively, as we have described previously (33).

**MATERIALS AND METHODS**

**Animals and Housing Conditions**

Our Siberian hamster (Phodopus sungorus) colony was established in 1988, and its genealogy has been described recently (6). Hamsters were exposed to a long summer-like day (16:8-h light-dark cycle with lights on at 0300 EST). Room temperature at 20°C, and relative humidity was maintained at 50 ± 5% throughout the experiment. All animals had ad libitum access to Purina Rodent Diet (#5001) and tap water. All experimental procedures were approved by the Georgia State University Institutional Animal Care and Use Committee in accordance with Public Health Service and United States Department of Agriculture guidelines.

**Does Sensory Denervation Trigger Lipectomy-Like Increases in WAT Mass Similar to That Seen After Lipectomy?**

Experimental design. Sixty, 3-mo-old adult male Siberian hamsters were obtained from our breeding colony and divided into groups matched for body mass and percent body mass change after a 2-wk single-housing adaptation period, during which they were weighed once each week (n = 10 hamsters/group). Each group received one of the following treatments: bilateral EWAT sham lipectomy (Sham EWATx), bilateral EWATx, bilateral EWAT Sham denervation (Sham Den), bilateral surgical denervation [Surg Den; disrupting both sensory and sympathetic nervous system (SNS) innervation], bilateral EWAT treatment with vehicle, or capsaicin, a sensory neurotoxin (14) that disrupts sensory but not SNS innervation (33). Body mass and food intake were measured weekly to the nearest 0.01 g for 12 wk, a time when compensation for lipectomy-induced lipid deficits is complete (e.g., 19, 23). At the end of this experiment, the animals were killed and fat pads were harvested, weighed, and processed for histological verification of the denervation procedure (see below).

**General surgical procedures.** Hamsters were anesthetized with pentobarbital sodium (50 mg/kg ip) for all surgeries. The hair was removed from the incision area and then the area was wiped with 50% ethanol-soaked gauze. A single abdominal midline incision was made, through which bilateral EWAT pads could be accessed and care in the depth of the incision was taken to avoid the underlying blood vessels and musculature. Fat pads were kept moist with 0.15 M NaCl-soaked ethanol-soaked gauze. After the surgeries, the abdominal peritoneum was closed with sterile silk sutures, and the skin was closed with sterile wound clips. Nitrofurazone powder was applied to the incision site surface to decrease the incidence of infection.

**Surgical lipectomy.** For EWATx, pads were excised with care taken to avoid damage to the testicular blood supply, as described previously (19, 23, 33). Sham EWATx surgery consisted of fat pad manipulation, in which both left and right EWAT pads were lifted out of the peritoneal cavity intact and then replaced.

**Surgical denervation.** Surgical denervation was performed as we described previously (33). Briefly, a drop of 1% toluidine blue was applied to the EWAT pads to facilitate visualization of the nerves. The nerves were then freed from the surrounding tissue and vasculature to minimize disturbances in the blood flow and cut in two or more places. For Sham Den surgery, fat pads were gently lifted or pushed with tissue forceps to visualize the nerves without damaging either the nerves or the blood vessels. After surgery, the denervated or sham-denervated pads were replaced back to their original locations.

**Chemical sensory denervation.** The WAT pads were sensory denervated using our previously described procedure (33). Briefly, the EWAT pads for hamsters in the Capsaicin group were injected with 20 microinjections (2 μl per injection) of 20 μg/μl of capsaicin (Sigma Chemical, St. Louis, MO) dissolved in 10% of ethanol, 10% of Tween 80 and 80% of 0.9% NaCl, whereas the sham pads (Vehicle) received the same volume and numbers of injections of the vehicle. The dose of capsaicin was based on the lowest effective dose that significantly decreased CGRP-ir in pilot studies and used previously to demonstrate the presence of capsaicin-sensitive nerves in WAT (33). Reflux of the solutions was reduced by holding the needle at each injection site for 60 s before removing the microsyringe. The capsaicin and vehicle injections were placed so as to cover the full extent of the pads.

**Tissue harvesting.** Twelve weeks after surgery, animals were deeply anesthetized with pentobarbital sodium (80 mg/kg ip) and perfused transcardially with 100 ml of saline followed by 150 ml of 4% paraformaldehyde in 0.1 M phosphate buffer solution (pH = 7.4). EWAT, inguinal WAT (IWAT), retroperitoneal WAT (RWAT), and dorsal subcutaneous WAT (DWAT) were harvested and weighed. Testes also were removed and weighed as an indicator of reproductive status. The treated EWAT pads were stored in 4% paraformaldehyde until processing for immunohistochemical quantification (see Immunohistochemistry and quantitative microscopy) of tyrosine hydroxylase (TH) and CGRP-ir to verify chemical sensory or surgical denervations. Positive control tissues (ventral medulla for TH and skeletal muscle for CGRP) also were run.

**Immunohistochemistry and quantitative microscopy.** Immunohistochemistry for TH- and CGRP-ir was performed on WAT using the avidin-biotin complex peroxidase method, according to the method of Giordano et al. (11). Primary polyclonal antibodies [1:300] mouse anti-TH (Chemicon International, Temecula, CA) or [1:500] mouse anti-CGRP (Chemicon International) were used, and antibody specificity was demonstrated by incubating sections without the primary antibody and by preadsorption of the primary antibody with its antigen; each procedure resulted in neither TH-ir nor CGRP-ir. The presence of TH-ir or CGRP-ir using the above specific primary antibodies indicated the presence of sympathetic or sensory nerves, respectively, and was considered as a positive staining.

Surgical and chemical denervations were verified by quantifying TH-ir and CGRP-ir using our previously described methodology (33) with the researcher blind to the treatment of the pad. Briefly, WAT pads were continuously sectioned and grouped at 200-μm intervals. Two slides were randomly chosen from each interval and used for immunohistochemistry to localize the TH-ir and CGRP-ir. For each slice on each of these slides, TH-ir and CGRP-ir were quantified from five parenchymal fields. This was accomplished by first viewing each slide to determine how many areas showed positive staining for any given section. If more than five areas with positive staining were found, the first five encountered were used. If less than five areas with positive staining were found, then another slide was chosen or, if this were not possible (many slides from denervated WAT had one or two areas of positive staining), then any areas with positive staining on the two slides were chosen. The average numbers of TH-ir and CGRP-ir were compared between groups.

**Statistical analyses.** Weekly body mass and food intake were analyzed using repeated-measures ANOVA [SigmaStat 2.0, San Rafael, CA; treatment × time (6 × 12)]. Two-way ANOVA (SigmaStat 2.0) was used to analyze terminal measures of tissue mass [treatment × peptide (6 × 2)]. Differences between means were considered statistically significant if P < 0.05. Exact probabilities and test values were omitted for simplicity and clarity of the presentation of the results.

**RESULTS**

**Does Sensory Denervation Trigger Lipectomy-Like Increases in WAT Mass Similar to That Seen After Lipectomy?**

Three of the ten Capsaicin animals, 3 of the 10 Surg Den animals and 2 of the 10 EWATx animals either died or...
appeared unhealthy during the immediate postsurgical recovery period and therefore were euthanized and excluded from study yielding final group numbers of $n_{/H11005}7$, $n_{/H11005}7$ and $n_{/H11005}8$, respectively. All control animals survived ($n_{/H11005}10$ per group), and all surviving animals from all groups were healthy, as indicated by their body mass increase and food intakes (see below).

**Body mass and food intake.** Body mass was not significantly affected by any treatment (Figs. 1A, 2A, and 3A). EWATx animals decreased food intake for the first 2 wk after surgery compared with that for Sham EWATx-treated animals ($P < 0.05$; Fig. 1B). Food intake was not significantly different between the Capsaicin and Vehicle groups (Fig. 2B). Food intake was significantly decreased in Surg Den hamsters the first week after surgical denervation compared with Sham Den animals (Fig. 3B). Food intakes for the remaining weeks, as well as cumulative food intake, however, were not different among the groups (Figs. 1B, 2B, and 3B).

**WAT and testes masses.** RWAT and IWAT masses of both EWATx and Capsaicin groups were significantly greater than their respective controls ($P < 0.05$; Fig. 4). DWAT masses did not differ among the groups (Fig. 5, top). EWAT mass of the Surg Den group showed the expected increase in mass compared with that of the Sham Den controls ($P < 0.05$; Fig. 5, bottom). Not surprisingly, the remnant EWAT mass of EWATx hamsters was significantly smaller than that of all other groups ($P < 0.05$; Fig. 5, bottom). None of the treatments affected total dissectible WAT mass (total dissectible WAT [IWAT+DWAT+EWAT+RWAT] means ± SE in grams: Sham: EWATx: 4.18 ± 0.26; EWATx: 4.07 ± 0.40; Sham surgical denervation: 4.17 ± 0.23; EWAT surgical denervation: 4.45 ± 0.31; EWAT vehicle: 4.03 ± 0.34; EWAT capsaicin: 4.78 ± 0.55), a result highly suggestive of equivalent total body fat levels.

Testes mass was significantly decreased in EWATx hamsters compared with their Sham EWATx controls ($P < 0.05$, 0.63 ± 0.07 vs. 0.91 ± 0.12 g). Testes mass was not different after bilateral capsaicin treatment [0.92 ± 0.05 g (Capsaicin) vs. 0.93 ± 0.05 g (Vehicle)] or surgical denervation [0.87 ± 0.05 g (Surg Den) vs. 0.97 ± 0.12 g (Sham Den)].

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**Fig. 1.** Means ± SE weekly body mass (A), weekly food intake and cumulative food intake (B) for hamsters that underwent bilateral epididymal white adipose tissue removal (EWATx) or Sham lipectomy (Sham EWATx). *$P < 0.05$ EWATx vs. Sham EWATx.

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**Fig. 2.** Means ± SE weekly body mass (A), weekly food intake and cumulative food intake (B) for hamsters that underwent bilateral epididymal white adipose tissue capsicain treatment (Capsaicin) or vehicle treatment (Vehicle).
EWAT histology. Consistent with our previous study (33), CGRP-ir \( (P < 0.05; \text{Fig. 6A}) \), but not TH-ir, was significantly decreased in the Capsaicin compared with Vehicle WAT documenting disruption of sensory, but not sympathetic innervation of EWAT. Similar to our previous study (33), surgical denervation of EWAT (Surg Den) significantly decreased both CGRP-ir and TH-ir \( (P < 0.05; \text{Fig. 6B}) \), suggesting disruption of both sensory and sympathetic innervations.

**DISCUSSION**

The results of the present study suggest that local microinjections of the sensory nerve neurotoxin capsaicin into EWAT triggers increases in RWAT and IWAT mass analogous to that which accompanies EWAT lipectomy. This capsaicin-induced increased mass of the noninjected WAT pads appears to be due to at least a partial sensory denervation because capsaicin-treated EWAT pads had significantly decreased CGRP-ir, but not TH-ir, indicating the selectivity of the destruction of sensory nerves, as we have seen previously (33). EWAT capsaicin treatment did not affect EWAT pad mass, confirming previous observations (33). By contrast, here and in our previous work (33), EWAT mass significantly increased after surgical denervation, an effect likely due to the removal of sympathetic inhibition of fat cell proliferation and decreased basal lipolysis (6, 7, 38). The simplest interpretation of the capsaicin-induced increases in the masses of the noninjected WAT pads suggests that the sensory innervation of WAT conveys information to the brain related to body fat levels and when the information is diminished or absent, the animal responds as if the WAT pads were removed as is seen with lipectomy. This condition, in turn, marshals the compensatory increases in the mass of the sensory intact WAT pads, similar to that which follows lipectomy, through as yet imprecisely understood mechanisms that include reduced sympathetic drive on brown adipose tissue (decreases in norepinephrine turnover;
Ref. 32). This decrease in sympathetic drive to brown fat likely leads to decreases in energy expended as heat, with the resulting energy savings used to replenish the body fat deficit. These results suggest that one possible function of the sensory innervation of WAT is to inform the brain of the size of body fat stores based on information derived from individual fat depots. Neither the significantly increased WAT masses of the noninjected fat pads after capsaicin treatment nor the significantly increased WAT masses of the nonexcised fat pads after lipectomy were associated with a measurable increase in food intake, as we have seen for lipectomy previously (e.g., Refs. 19, 24, 32).

Surgical denervation, in which both the sympathetic and sensory innervations of WAT are at least partially destroyed (present study; Ref. 33), did not trigger increased RWAT or IWAT mass. Because surgical denervation significantly decreased CGRP-ir to the same or greater extent as capsaicin treatment (present study and Ref. 33) and then if the signal for triggering lipectomy- (e.g., 19, 21, 23, 32) and capsaicin-induced increases in intact WAT growth is sensory nerve disruption, one would expect surgical denervation-induced increases in intact pad growth. This lack of growth by the nonsurgically denervated WAT pads seems to be explained by another effect of WAT surgical denervation—the near doubling of the denervated WAT pad mass [~50% increase after surgical denervation of IWAT or RWAT (6, 7, 38), ~30% increase for EWAT (present study and Ref. 33)] largely reflected as increases in fat cell number (e.g., Refs. 6, 33, 38). These sympathetic denervation-induced increases in fat cell number (6–8, 33, 38) have been attributed to removal of sympathetic inhibition of fat cell proliferation (15). Sensory

Fig. 5. Means ± SE fat pad mass dorsosubcutaneous white adipose tissue (DWAT; top) and epididymal white adipose tissue (EWAT; bottom) of hamsters that underwent EWAT capsaicin, surgical denervation, or lipectomy treatment. Dissimilar letters are significantly different from one another (P < 0.05).

Fig. 6. Means ± SE numbers of calcitonin gene-related peptide (CGRP)- and tyrosine hydroxylase (TH)-immunoreactive (ir) sites of the vehicle-treated epididymal white adipose tissue (EWAT; Vehicle) and capsaicin-treated EWAT (Capsaicin) (A) and sham-denervated EWAT (Sham Den) and surgically denervated EWAT (Surg Den) (B). *P < 0.05 Vehicle vs. Capsaicin or Sham Den vs. Surg Den.
denervation, however, does not increase WAT mass or fat cell number (33). In the present study, the smaller increase in WAT mass after EWAT surgical denervation compared with that after IWAT or RWAT surgical denervation (6, 33, 38) seems to be due to the lesser propensity of the former pad for growth by adipocyte proliferation compared with the latter pads (e.g., Ref. 9). Thus the surgical denervation-induced increased accumulation of lipid by EWAT in the present study likely inhibited lipid accumulation by nonsurgically denervated WAT pads.

If the sensory innervation of WAT conveys body fat information to the brain, then what is the nature of the lipid-associated signal? Quite simply, this is not known, but some possibilities exist (for a review, see Refs. 2 and 3). One possibility would be that a factor associated with lipid storage or mobilization may roughly correlate with body fat levels and, in its absence, would trigger responses leading to compensation by other lipid depots. For example, lipolysis results in the catabolism of triacylglycerols into free fatty acids and glycerol (27), and WAT sensory nerves could respond to changes in either or both, thereby providing the brain with some information as to the status of lipid storage in WAT depots (2). To our knowledge, the response of WAT sensory nerves to glycerol, free fatty acids, or for that matter, to any substance, has not been tested. Precedent exists, however, for sensory nerves responding to such signals because some gastrointestinal sensory nerves are activated by fatty acids (17, 26). Although the role of leptin in total body fat regulation has been questioned (see Introduction), leptin appears capable of activating WAT sensory nerves (28). That is, direct injections of leptin into the EWAT of laboratory rats increases sensory nerve firing rates (28), suggesting that leptin may act as a paracrine rather than a circulating factor to convey adiposity information to the brain via a neural sensory conduit.

Finally, although we found that, testes mass was significantly decreased in EWATx hamsters compared with their Sham EWATx controls, testes of this diminished size are still fully functional with regard to serum testosterone concentrations (20). This is important because testosterone affects body and lipid mass in this species (1, 4, 34); however, unlike laboratory rats and mice, decreases in serum testosterone are unknown, and the current study is a beginning toward a deeper understanding of its role in body fat regulation. The role of leptin in total body fat regulation has been questioned (20). This is important because testosterone affects body fat in hamsters.

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REFERENCES


