Sensory or sympathetic white adipose tissue denervation differentially affects depot growth and cellularity

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Mammals primarily store energy as lipid in white adipose tissue (WAT), with the size of the WAT pads expanding and contracting according to fluctuations in energy intake and expenditure. When circulating or stored carbohydrates do not meet energetic needs, lipids stored as triacylglycerols in WAT adipocytes are catabolized through the process of lipolysis (37). Specifically and briefly, catecholamines [i.e., norepinephrine (NE) and epinephrine (EPI)] bind to membrane-bound β-adrenoceptors on fat cells, triggering the lipolytic cascade that ultimately results in the breakdown of triacylglycerols into free fatty acids and glycerol (37), an effect opposed in some species by membrane-bound α-adrenoceptors (32). Traditionally, the source of these catecholamines was believed to be primarily from the adrenal medulla, especially EPI because it robustly stimulates lipolysis in isolated white adipocytes (e.g., Ref. 50). The inability of adrenal demedullation (and thus the removal of circulating EPI and NE) to effectively block lipolysis under several physiological conditions (e.g., see Refs. 18, 39, 47) sheds doubt on this belief. In our studies of the reversible seasonal obesity of Siberian hamsters, we have focused on an alternate means of stimulating lipid mobilization: the sympathetic nervous system (SNS) innervation of WAT (52), as others did before us studying more common species (for review, see Refs. 6, 7). Fluorescent retrograde and anterograde tract tracers have defined the postganglionic sympathetic innervation of WAT (53), and the use of viral transneuronal tract tracers has revealed the central nervous system origins of the SNS outflow to WAT (3, 10, 43). Finally, the functional significance of the SNS innervation of WAT for lipid mobilization in Siberian hamsters (18, 53) and other species (8, 11, 12, 14) derives from the blockade of lipid mobilization by surgical denervation of WAT, but not by adrenal demedullation alone (18, 47). Thus the involvement of the SNS innervation of WAT in lipid mobilization seems undeniable. Although parasympathetic innervation of WAT has been suggested (29), there are critical interpretational problems with these findings (4). In addition, there is a complete lack of labeling for a well-established parasympathetic innervation marker (vesicular acetylcholine transporter) across all WAT pads in laboratory rats, mice, and Siberian hamsters (Giordano, Song, Bartness, and Cinti, unpublished observations).

A less appreciated, but more recent role of the SNS innervation of WAT is its control of fat cell number (FCN) through the sympathetically mediated inhibition of fat cell proliferation. Specifically, NE, the principal sympathetic postganglionic neurotransmitter, inhibits the natural proliferation of precursor fat cells in vitro (27). Conversely, in Siberian hamsters (10, 53) as well as in laboratory rats (15), surgical denervation of WAT triggers increases in FCN.

WAT also has sensory innervation, at least for laboratory rats, as demonstrated both by anterograde tract tracing (23) and histologically at the level of the WAT pad (25). In terms of the latter evidence, WAT contains immunoreactivity (ir) for nerve terminals of the sensory-associated neuropeptides substance P and calcitonin gene-related peptide (CGRP) (25). Presently, the function of this sensory innervation is virtually unknown, but it has been hypothesized to convey information to the brain about body fat levels and/or to participate in a feedback loop regulating the degree of SNS-mediated WAT lipolysis (6).

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Collectively, it is clear that WAT possesses several innervations. The SNS innervation of WAT is indisputable, and two of its roles are as the principal initiator of lipolysis and as a powerful factor modulating fat cell proliferation. The sensory innervation of WAT is also without doubt, at least in laboratory rats, but its function is unclear at this time. Therefore, we tested for the presence of these innervations histologically at the level of the fat pad and tested their function in the control of WAT growth and adipose tissue cellularity. It seemed particularly important to understand the existence and function of the sensory innervation of WAT in Siberian hamsters given that the majority of the recent work on WAT innervation has been done in this species (for review, see Refs. 6, 7). Therefore, we first used an immunohistochemical approach to demonstrate the sensory and SNS innervations of WAT pads by staining for CGRP, a sensory-nerve associated neuropeptide (45), and tyrosine hydroxylase (TH). TH is the rate-limiting enzyme for catecholamine synthesis and thus a marker for SNS innervation (25). To begin to understand the functional significance of WAT sensory innervation, we tested a selective chemical sensory denervation procedure using local injections of capsaicin, the pungent portion of chili peppers that selectively destroys unmyelinated sensory nerves (1). This local approach was inspired by its confined application to kill gastrointestinal sensory nerves (41). We tested the specificity of the sensory denervation by comparing the effects of capsaicin treatment with that of surgical denervation on TH-ir and CGRP-ir.

Second, we tested the effects of sensory vs. surgical denervation on the control of WAT growth and cellularity. Here, we took advantage of the unilateral innervation of WAT in which one WAT pad was either surgically denervated or injected with capsaicin and its contralateral mate received sham denervation or vehicle injections. We did these denervations to two WAT pads that show different propensities for fat cell proliferation and fat pad growth: epididymal WAT (EWAT) and inguinal WAT (IWAT) (e.g., see Refs. 19, 20). Cellularity measurements [FCN, fat cell size (FCS)] were made 12 wk later, a time that we have previously used to test the effects of chemical and surgical SNS denervation on WAT cellularity and function (18, 53).

**MATERIALS AND METHODS**

**Animals and housing conditions.** Eighty 3-mo-old adult male Siberian hamsters were obtained from our breeding colony. The genealogy of the hamster colony was described recently (44). Hamsters were weaned at 21 days of age and housed with same-sex siblings in groups of 8–12 in polyvinyl cages (48 × 27 × 15 cm) in a long summer-like day (16:8-h light-dark cycle, with lights on at 0300) until used in the present experiments. Temperature was kept constant at 20°C, and relative humidity was maintained at 50 ± 5% throughout the experiments. Animals had ad libitum access to Purina rodent diet (no. 5001) and tap water. All experimental procedures were approved by the Georgia State University Institutional Animal Care and Use Committee in accordance with National Institutes of Health and U.S. Department of Agriculture guidelines. Two weeks before the initiation of the experiments, animals were housed individually in polypropylene cages (27.8 × 17.5 × 13.0 cm) containing corn cob bedding and cotton nestlets, and body mass was monitored weekly for 2 wk. Hamsters were then divided into eight groups matched for body mass and percent body mass change during the previous 2 wk (n = 10 hamsters/group).

**Experimental design.** Animals received either unilateral IWAT or EWAT surgical denervation or unilateral IWAT or EWAT chemical sensory denervation by the use of capsaicin (both denervations are as described below). IWAT and EWAT pads are unilaterally innervated, at least at the level of their postganglionic innervations (for review, see Ref. 6). Therefore, we took advantage of a within-animal experimental design, manipulating one WAT pad with its contralateral mate as a within-animal control. Because there are no significant differences in the effects of left or right WAT pad sympathetic (53) or sensory denervation (pilot experiments) on growth, cellularity, or neural immunohistochemical responses, the left IWAT or left EWAT pads received one of the denervation procedures, with their contralateral counterpart receiving the control manipulations.

Body mass was measured weekly to the nearest 0.01 g. Twelve weeks later, WAT pads from one-half of the animals were processed for histology analyses of TH-ir and CGRP-ir as described below. In the remaining half of the animals, a sample of the manipulated IWAT or EWAT for each type of denervation was processed for cellular analysis, with the remainder used for HPLC analysis of NE content.

**Surgical denervation.** Animals were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg). The hair was removed from the incision area, and then the area was wiped with 95% ethanol-soaked gauze. For IWAT surgical denervation surgery, an incision was made at the dorsal hindlimb of the animal, lateral to the spinal column that continued rostrally and then ventrally to the ventral hindlimb. Care was taken with the depth of the incision to avoid the underlying blood vessels and musculature. Using a dissecting microscope, we separated the IWAT pad from the skin and abdominal wall, being careful not to damage the blood vessels entering or leaving the pads. For the EWAT denervation surgery, a single abdominal midline incision was made through which bilateral EWAT pads could be accessed. For both surgical denervations, a drop of 1% toluidine blue was applied to the fat pad to facilitate visualization of the nerves. The nerves were then freed from the surrounding tissue and vasculature. Small nerves were cut in 2 or more locations, and 3-mm segments were removed from nerves large enough to permit it to prevent possible, but unlikely, reconnection. For IWAT or EWAT sham surgeries, fat pads were gently lifted or pushed with tissue forceps to visualize the nerves without damaging either the nerves or the blood vessels. Throughout denervation and sham surgeries, fat pads were kept moist with 0.15 M NaCl-soaked gauze. After surgery, the denervated or sham pads were replaced back to their original locations. For EWAT surgeries, the musculature was closed with silk sutures and the skin was closed with wound clips, whereas for IWAT surgeries only the skin was wound clipped. Nitrofurazone powder was applied to the wound surface to decrease the risk of infection.

**Capsaicin sensory denervation.** A capsaicin (Sigma, St. Louis, MO) stock solution was made at a concentration of 200 μg/μl in 100% ethanol and then diluted to a final concentration of 20 μg/μl using 10% of stock solution, 80% of 0.15 M NaCl, and 10% of Tween 80 before injection. Hamsters were anesthetized with pentobarbital sodium as above, and access to the pads and wound closures occurred in exactly the same manner. Capsaicin or vehicle [10% ethanol, 10% Tween 80, and 80% 0.15 M NaCl] was injected unilaterally into either the left IWAT or EWAT pads, and the right pads received equiovolemic injections of the vehicle. We administered capsaicin and the vehicle to the pads using 20 microinjections (2 μl per injection) spread across the full extent of the pads as we have done previously for chemical sympathetic denervations (17, 18). Reflux of the solutions was reduced by holding the needle at each injection site for 60 s before removal.

**Tissue harvesting.** Twelve weeks after surgery, a time when our group previously observed surgical denervation-induced increased FCN (10, 53), animals were deeply anesthetized with intraperitoneally injected pentobarbital sodium (80 mg/kg). The animals were subdivided into two groups matched for their percent body mass change...
from the beginning of the experiment and by their 12-wk absolute body masses. For animals used in the WAT cellularity and NE content analyses, the IWAT or EWAT pads were bilaterally removed, chilled on wet ice, blotted dry, weighed, and then prepared for cellularity and NE content measurements as described below. Testes also were removed and weighed as an indicator of reproductive status because of the possibility of damage to the testicular blood supply during the EWAT pad manipulations, given the role of gonadal steroids in WAT growth and function (for review, see Ref. 49). For animals used in the immunohistochemical analyses, the hamsters were perfused transcardially with 100 ml of saline, followed by 150 ml of 4% paraformaldehyde in 0.1 M phosphate buffer solution (pH 7.4). Bilateral EWAT and IWAT pads were then dissected, weighed, and stored in 4% paraformaldehyde until they were processed for immunohistochemistry, as were their positive control tissues (brain for TH, skeletal muscle for CGRP).

Immunohistochemistry. Immunohistochemistry was performed on the WAT according to the method of Giordano et al. (25). Briefly, the WAT pads were washed with 0.015 M PBS (pH 7.4) and then dehydrated with a series of increasing concentrations of ethanol. Specifically, tissues were incubated into 75% ethanol (60 min), 95% ethanol (75 min × 2), and 100% ethanol (60 min × 3). After dehydration, fat pads were incubated in xylene (60 min × 2). Tissues were then infiltrated with paraplast embedding media, which is a refined compound of purified paraffin (Sigma), at 60°C overnight and then embedded with fresh paraplast. We then sliced each pad across its extent at 4 μm using a rotary microtome (American Optical Instrument, Buffalo, NY). Immediately after they were sliced, sections were placed in a tissue floating water bath (37°C) and then mounted on glass slides. Slides were left on a slide-warming table (37°C) to air dry overnight. Slides were grouped into levels of approximately <200 μm, and two slides from each level were used for localization of TH-ir or CGRP-ir. Sections were deparaffinized with xylene and hydrated with a series of decreasing concentrations of ethanol and distilled water immediately before immunohistochemistry was started. Sections were first incubated in methanol with 0.3% H2O2 for 30 min at room temperature to block endogenous peroxidase activities. After they were washed twice with 0.015 M PBS, sections were preincubated to minimize nonspecific immunoreactivity with 1:10 normal serum in 0.15 M PBS for 20 min. Sections were then incubated with the primary polyclonal antibodies (1:300) mouse anti-TH (Chemicon International, Temecula, CA) or (1:500) mouse anti-CGRP (Chemicon International) in a humid chamber for 48 h at 4°C. 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 no immunoreactivity. After incubation with the primary antibody, the slides were rinsed with 0.015 M PBS three times and then incubated with 1:200 IgG biotinylated anti-mouse (Jackson Immunoresearch Laboratories, West Grove, PA) in 0.15 M PBS for 30 min at room temperature. Next, the slides were washed three times with 0.015 M PBS and then incubated in a 1:100 solution of the avidin-biotin horseradish peroxidase complex (Vectastain ABC elite kit; Vector Laboratories, Burlingame, CA) for 1 h at room temperature. The horseradish peroxidase was made visible by incubation with 0.075% 3,3′-diaminobenzidine tetrahydrochloride and 0.02% H2O2 in 0.05 M Tris buffer (pH 7.6) for 3 min. The slides were then washed with tap water and counterstained with hematoxylin (Vector Laboratories). After dehydration with a series of increasing concentrations of ethanol and xylene, the slides were covered with Histomount (National Diagnostics, Atlanta, GA). The 3,3′-diaminobenzidine tetrahydrochloride chromagen product was visualized by light microscopy.

Quantitative microscopy. Surgical and chemical denervations were verified by quantifying TH-ir and CGRP-ir. Five parenchymal fields were evaluated from WAT sections at each of 200-μm intervals. The numbers of individual TH-ir or CGRP-ir staining sites were counted visually.

WAT cellularity analyses. WAT samples for cellularity analyses were finely minced and fixed in 37°C osmium tetroxide for 7 days, according to our modified method (33) of Hirsch and Gallian (26). After cell washing, the samples were counted using a Coulter counter (Multisizer 3 Coulter counter, Beckman Coulter) to obtain FCN, average FCS, and FCS distributions.

HPLC analysis of WAT NE content. NE content was measured to verify the degree of the sympathetic denervation and to test the selectivity of the chemical sensory denervation. NE content was measured by reverse-phase HPLC with electrochemical detection following our modifications (10) of the method of Mefford (35).

Briefly, samples were thawed on wet ice and finely minced; ~100 mg of WAT were added into polypropylene microcentrifuge tubes containing 790 μl of 0.2 M perchloric acid with 1 μg/ml ascorbic acid and 10 μl of 1,000 ng/ml dihydroxybenzylamine (internal standard). Each sample was sonicated and homogenized on wet ice (30 s × 3). After centrifugation for 10 min at 7,500 g, 600 μl of the homogenate were added to microcentrifuge tubes containing 150 μg of alumina. Cat-echolamines were adsorbed by adding 1 ml of 0.5 M Tris buffer (pH 8.6) followed by vortexing for 30 s. Alumina was washed three times with HPLC grade water (ESA, Bedford, MA), and 200 μl of the perchloric acid-ascorbic acid mixture were added; this was followed by vortexing (30 s) to elute the catecholamines. Supernatant (180 μl) was removed and frozen at −80°C until assay (~30 days later) using the HPLC system with electrochemical detection (Cuoiochem II; ESA) at guard cell +55 mV, cell 1 +10 mV, and cell 2 −30 mV. The mobile phase used was Cat-A-Phase II, and the column was a C-18 reverse-phase column. Standard solutions containing known concentrations of catecholamines and dihydroxybenzylamine were run on the HPLC before and after sets of unknowns.

Statistical analyses. Weekly body mass measurements were analyzed by repeated-measures ANOVA with surgery type (surgical denervation or chemical denervation) and WAT pad (IWAT or EWAT) as between-group factors and time as a within-group factor (NCSS 11.5, Chicago, IL). FCS distribution was analyzed by repeated-measures ANOVA with surgery type and EWAT pad as between-group factors. The interactions of the terminal measures of IWAT and EWAT mass, FCN, FCS, NE content, or numbers of TH-ir or CGRP-ir with surgery type and treated pad were analyzed by an SAS program (SAS Institute, Cary, NC). The percent differences between sham controls and treated WAT pads within an animal were compared (SAS Institute). Student-Newman-Keuls tests were used as post hoc tests (SAS Institute). 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 results.

RESULTS

All animals were allowed to recover for 12 wk after surgery. Two of the ten hamsters receiving unilateral IWAT surgical denervation, and three of the ten hamsters receiving unilateral EWAT surgical denervation died during the recovery period. All capsaicin-treated animals survived for 12 wk after their treatments.

Body mass. Body mass was not significantly different among the animals that received unilateral IWAT or EWAT surgical denervation or unilateral IWAT or EWAT capsaicin treatment (data not shown).

Tissue masses. IWAT and EWAT masses were significantly increased by surgical denervation compared with their contralateral sham-operated control pads (P < 0.05; Fig. 1, A and B). EWAT mass in IWAT-denervated hamsters and IWAT mass in EWAT-denervated hamsters were not affected by unilateral IWAT or EWAT surgical denervation, respectively, nor were retroperitoneal WAT (RWAT) and dorsosubcutane-
ous WAT masses (Fig. 1, A and B). Chemical sensory denervation did not affect any WAT pad mass (Fig. 1, C and D).

The mass of testes was not significantly affected by any of the WAT denervations, including EWAT denervation, that had the potential to damage the testes: left and right masses for unilateral IWAT surgical denervation were $0.423 \pm 0.025$ and $0.436 \pm 0.025$ g, left and right masses for unilateral IWAT capsaicin injection were $0.473 \pm 0.092$ g, EWAT were $0.470 \pm 0.042$ g, RWAT were $0.095 \pm 0.014$ g, and DWAT were $1.001 \pm 0.070$ g. For unilateral left IWAT capsaicin denervation (C), right IWAT were $0.839 \pm 0.092$ g, EWAT were $0.470 \pm 0.042$ g, RWAT were $0.095 \pm 0.014$ g, and DWAT were $1.001 \pm 0.070$ g. For unilateral left EWAT capsaicin denervation (C), right IWAT were $1.237 \pm 0.165$ g, EWAT were $0.415 \pm 0.045$ g, RWAT were $0.085 \pm 0.011$ g, and DWAT were $0.784 \pm 0.088$ g. For unilateral left EWAT capsaicin denervation (D), right IWAT were $0.893 \pm 0.114$ g, EWAT were $0.534 \pm 0.059$ g, RWAT were $0.081 \pm 0.013$ g, and DWAT were $0.677 \pm 0.063$ g.

TH-ir and CGRP-ir were found in IWAT (Figs. 2A, 3A, and 4) and EWAT (Figs. 2B, 3B, and Fig. 4). TH- and CGRP-ir were varicose-like and were found adjacent to blood vessels as well as in the parenchymal areas between adipocytes, with more TH-ir or CGRP-ir associated with the vasculature. The density and staining patterns of TH-ir and CGRP-ir were similar in IWAT and EWAT. Because these animals were fed ad libitum, the ability to detect nerves for either substance in the parenchyma was diminished due to the close packing of the adipocytes, whereas the presence of TH-ir and CGRP-ir adjacent to blood vessels is more readily seen (Ref. 13 and unpublished observations). Some stained fibers in the parenchymal area were not obviously associated with any arterioles or capillaries, however (Figs. 2 and 3).

Capsaicin injections significantly decreased CGRP-ir by $\sim 40\%$ in IWAT and EWAT compared with the vehicle injections in EWAT ($P < 0.05$; Fig. 4A) and significantly increased
TH-ir by ~40% in capsaicin-treated IWAT (P < 0.05; Fig. 4B); however, TH-ir was not significantly changed with capsaicin-treated EWAT. Surgical denervation decreased CGRP-ir fibers to a significantly greater extent than capsaicin treatment, with an >80% decrease in IWAT and EWAT CGRP-ir (P < 0.05; Fig. 4A). Surgical denervation also significantly decreased >70% of TH-ir fibers in IWAT and >90% of TH-ir fibers in EWAT compared with the sham-operated controls (P < 0.05; Fig. 4B).

**HPLC analysis of WAT NE content.** WAT IWAT and EWAT NE contents were significantly decreased by surgical denervation (~60% and ~80%, respectively; P < 0.05, Fig. 5). Capsaicin-induced sensory denervation did not affect NE content in either IWAT or EWAT pads (Fig. 5).

**WAT cellularity.** The significant surgical denervation-induced increased IWAT mass (see above) was reflected as a significantly increased total FCN or average FCS (Fig. 6B). FCS distribution analysis indicated, however, that surgically denervated EWAT had significantly increased medium-sized cells (i.e., 75–100 and 100–125 μm diameters; P < 0.05; Fig. 6B). In contrast to the surgical denervation, capsaicin-induced sensory denervation did not affect total FCN or the distribution of FCS for either IWAT or EWAT (Fig. 7, A and B). There was, however, a capsaicin-induced significantly increased average FCS in IWAT (P < 0.05) but not in EWAT (Fig. 7A).

**DISCUSSION**

Although the first reports of the innervation of WAT occurred over 100 years ago (21; for review, see Ref. 6), the exact nature of this innervation, as well as delineation of all its functions, is still being revealed. To date, the most compelling neuroanatomic and functional case can be made for the SNS innervation of WAT, with undeniable neuroanatomic evidence for WAT sensory innervation (Refs. 23, 25, and the present investigation) with little knowledge of its function. Here, we...
surgical denervation in laboratory rats (15) and Siberian hamsters (10). In addition, we found that the surgical denervation-induced increased IWAT mass was exclusively reflected as increased total FCN, confirming previous studies (10, 17, 18, 53). More specifically, the increased IWAT FCN was associated with significantly increased small adipocytes, suggestive of increased fat cell proliferation. Surgically denervated EWAT, however, only exhibited a suggestive, nonsignificant increase in total FCN, but the number of medium-sized fat cells (75–100 and 100–125 μm diameters) was significantly increased. By contrast, with surgical denervation, capsaicin sensory denervation did not increase WAT pad mass, but significantly increased average FCS to a small extent in IWAT, but not in EWAT. This sensory denervation-induced increased FCS implicates a role for sensory nerves in inhibiting lipid mobilization and/or stimulating lipid uptake/lipogenesis, likely working through interactions with WAT SNS innervation.

Two functions of the sympathetic innervation of WAT are recognized: 1) the principal initiator of lipid mobilization and 2) an inhibitor of WAT growth via inhibition of FCN (Refs. 10, 15, 53, and the present study). This latter function is not well understood, but clearly functionally important both for a more complete understanding of WAT growth and for understanding the pathology of adiposity because increased fat cell proliferation and the subsequent hypercellularity are hallmark signs of obesity (22). In the present study, despite the incomplete nature of the surgical denervation, a result our group (17, 18, 53) as well as others (15) had previously found for surgical or chemical WAT denervations, impressively increased fat pad mass and FCS (~2-fold) still occurred. This suggests the potential for even greater increased growth and FCN if a complete SNS denervation is achieved, unless what we observed after incomplete denervations represents a ceiling on the magnitude of these changes. Nonetheless, collectively, these data emphasize the important role of the SNS in WAT growth

![Fig. 4. Staining sites as a percentage of control (sham surgical denervation or vehicle capsaicin sensory denervation) in WAT. Results are means ± SE. CGRP-ir (A) and TH-ir (B) were measured after unilateral IWAT or EWAT capsaicin sensory denervation (cap) or surgical denervation (den). *P < 0.05 compared with controls (i.e., 100%). P < 0.05 for different letters. Results for TH-ir staining sites are as follows: for IWAT sham denervation, 19.78 ± 3.48; for IWAT vehicle capsaicin denervation, 17.80 ± 2.30; for EWAT sham denervation, 17.00 ± 0.51; for EWAT vehicle capsaicin denervation, 20.90 ± 2.50. Results for CGRP-ir staining sites are as follows: for IWAT sham denervation, 17.80 ± 2.04; for EWAT vehicle capsaicin denervation, 11.90 ± 6.42; for EWAT sham denervation, 17.00 ± 3.08; for EWAT vehicle capsaicin denervation, 18.20 ± 2.04.](http://ajpregu.physiology.org/)

![Fig. 5. WAT pad norepinephrine (NE) content as a percentage of control (sham surgical denervation or vehicle capsaicin sensory denervation) after unilateral IWAT or EWAT capsaicin sensory denervation or surgical denervation. Results are means ± SE. *P < 0.05 compared with controls (i.e., 100%). P < 0.05 for different letters. NE results are as follows: for IWAT sham denervation, 19.78 ± 3.48 μg/g tissue; for IWAT vehicle capsaicin denervation, 17.80 ± 2.30 μg/g tissue; for EWAT sham denervation, 17.00 ± 0.51 μg/g tissue; for EWAT vehicle capsaicin denervation, 20.90 ± 2.50 μg/g tissue.](http://ajpregu.physiology.org/)
and cellularity. In the present study, as well as in all the previous studies, bona fide adipocyte proliferation was not measured; rather, only increased FCN was measured. Increased FCN, although indicative of fat cell proliferation, also could result from denervation-induced increased lipid filling via the inhibition of lipolysis that, in turn, would increase the probability of the adipocytes exceeding the Coulter counter threshold size for detection (20 μm) and thereby increase FCN (5). We have preliminary data, however, showing that SNS denervation triggers increased fat cell proliferation. Specifically, local surgically denervated WAT pads, but not their contralateral sham surgery counterparts, had significantly decreased WAT NE content associated with increased fat cells labeled with bromodeoxyuridine (a marker for cell division; Ref. 42) that also were labeled with an antibody for a white adipocyte-specific membrane protein (i.e., AD3) (51). The presence of these denervation-induced, double-labeled cells attests to their denervation as bona fide proliferating cells and identifies their ultimate mature cell type as that of adipocytes (M. T. Foster and T. J. Bartness, unpublished observations). Collectively, these findings suggest that SNS denervation triggers fat cell proliferation in vivo, supporting the ability of NE to inhibit in vitro proliferation of adipocyte precursor cells (27). The mechanisms underlying the inhibition of fat cell proliferation by NE, or release from this inhibition with denervation, are not known beyond the activation of adipocyte membrane-bound β-adrenoceptors (27) found in adipocyte precursor cells (30).

The α-adrenoceptors, the other class of adipocyte adrenoceptors, also could be involved in the sympathetic control of WAT cellularity. The best-documented function of the α-adrenoceptors is that they oppose lipid mobilization (antilipolysis) through activation of the α2-adrenoceptor subtype found on fat cell membranes of many species, including humans and Syrian hamsters (for review, see Ref. 31). Siberian hamsters also appear to possess these α2-adrenoceptors, but they have a limited antilipolytic effect (2). Nevertheless, α2-adrenoceptor activation has been suggested to trigger fat cell proliferation in vitro in laboratory rat and human adipocyte precursor populations (9, 48). This could be important in the present study because compensatory increases in one arm of the SNS can occur when the other is disabled. For example, chemical sympathetic denervation causes increases in adrenal medullary EPI secretion (46). If such an effect occurred here, albeit due to surgical sympathetic denervation of one WAT pad, then, if EPI release was increased, activation of WAT α2-adrenoceptors would be likely because EPI is a high-affinity agonist for this adrenoceptor subtype (for review, see Ref. 30). Activation of α2-adrenoceptors by EPI increases lysophosphatidic acid release (48), which, in turn, triggers fat cell proliferation, at least in vitro (48). Thus this scenario could underlie some of denervation-induced increases in this response. The continued presence and function of the sympathetic innervation of the non-denerverated WAT pads would tend to antagonize any α2-

![Fig. 6. A: total fat cell number (FCN; inset) and fat cell distributions for sham (sham) or surgically denervated (den) IWAT (top) and EWAT (bottom). Results are means ± SE. B: total FCN (inset) and fat cell distributions for vehicle-treated (vehicle) or capsaicin-treated (cap) IWAT (top) and EWAT (bottom). *P < 0.05 sham vs. den.](http://ajpregu.physiology.org/doi/10.1152/ajpregu.00681.2004)
adrenoeceptor stimulation by the increased EPI in these pads and thus oppose any tendency for fat cell proliferation there.

The lack of an increase in FCN after selective sensory denervation suggests that the severing of sensory nerves that accompanies surgical denervation of WAT, as evidenced here by the decreased CGRP-ir after axotomy, does not play a significant role in the surgical denervation-induced increase in FCN. Sensory denervation of WAT did have some effect on cellularity, however, in that capsaicin injections increased the average FCS, but only in IWAT. Increases in FCS suggest increased lipogenesis or lipid uptake and/or decreased lipolysis. The lack of increased FCS in capsaicin-treated EWAT is not explicable at this time, but nonuniform responses across fat pads appear to be the exception, not the rule, for many cellularity responses (for reviews, see Refs. 5, 28). For example, there appears to be a programmed propagation of WAT pads to alter FCS or FCN after manipulations that affect body fat generally. Thus rat EWAT and mesenteric WAT grow the most by increases in FCS rather than in FCN, whereas IWAT and RWAT show the opposite growth responses (20).

To our knowledge, the role of sensory innervation in any of the aforementioned processes is unknown. There is a report, however, that neonatal global capsaicin treatment promotes lipid mobilization, resulting in decreased body fat (16, 36), a finding opposite to the suggested increase in average FCS in IWAT in the present study. The discrepancy between the effects of capsaicin on body fat between these studies and the present one could be attributable to a number of factors, including species differences, the postinjection interval before assay, and global vs. local application of capsaicin.

Finally, of technical note here is the adaptation of the local application of capsaicin (40) to WAT as a means of selective sensory denervation. Our group (24) previously showed that global capsaicin treatment in neonatal rats decreases CGRP-ir, as well as that of substance P, another sensory nerve-associated peptide (45). Here, we show that a similar decrease in the magnitude of CGRP-ir can be accomplished with local administration of capsaicin and, moreover, that it is selective in that TH-ir was not decreased. The elimination of CGRP-ir after local capsaicin treatment was far from complete, accounting for an ~40% decrease in staining, but robust physiological responses can result from a far less than perfect WAT sensory denervation. That is, when WAT pads are removed from many animals (for review, see Ref. 34), the nonexcised fat pads enlarge in an apparent compensation for the lipectomized WAT pads. A possible signal for this compensatory response is the lowering of the sensory innervation of the fat pads that accompanies lipectomy. Indeed, bilateral EWAT capsaicin treatment identical to that done here, although equally incomplete as also assessed by CGRP-ir, triggers lipectomy-like enlargement of the noninjected fat pads, even though the EWAT pads were removed from many animals (52). Moreover, the present study shows sensory innervation of WAT to include Siberian hamsters and support, via immunohistochemical staining, the sympathetic innervation of WAT shown by tract tracing experiments in this species (52). Moreover, the present study provides, for the first time, tests of the role of WAT sensory innervation in its growth and cellularity, finding that the capsaicin-induced decreased CGRP-ir was not associated with changes in EWAT or IWAT growth, but did affect cellularity in a fat pad-specific manner by significantly increasing FCS in IWAT. The increased surgical denervation-induced WAT growth (IWAT and EWAT) and increased FCN (IWAT) of the present study support the notion that the sympathetic innervation of WAT inhibits fat pad growth and/or FCN (fat cell proliferation). Because sensory denervation did not produce similar effects, these results suggest that severing of sensory nerves associated with surgical denervation, as suggested here by the surgical denervation-induced decreased CGRP-ir, does not contribute to these responses. The increased FCS in capsaicin-treated WAT suggests a possible influence on lipid
accumulation in normal conditions, but much remains to be done to test this notion. Finally, a novel technique for the local application of capsaicin to produce selective sensory denervation of WAT was demonstrated.

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