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Sympathetic denervation of one white fat depot changes norepinephrine content and turnover in intact white and brown fat depots

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Abstract

It is well established that the sympathetic nervous system regulates adipocyte metabolism and recently it has been reported that sensory afferents from white fat overlap anatomically with sympathetic efferents to white fat. The studies described here characterize the response of intact fat pads to selective sympathectomy (local 6-hydroxydopamine injections) of inguinal (ING) or epididymal (EPI) fat in male NIH Swiss mice and provide in vivo evidence for communication between individual white and brown fat depots. The contralateral ING pad, both EPI pads, perirenal and mesenteric pads were significantly enlarged four weeks after denervating one ING pad, but only intrascapular brown fat (IBAT) increased when both ING pads were denervated. Denervation of one or both EPI pad had no effect on fat depot weights. In an additional experiment, NE turnover was inhibited in ING, retroperitoneal, mesenteric and IBAT two days after denervation of both EPI or of both ING pads. NE content was reduced to 10-30% of control values in all fat depots. There was no relation between early changes in NE turnover and fat pad weight 4 weeks after denervation, even though the reduction in NE content of intact fat pads was maintained. These data demonstrate that there is communication among individual fat pads, presumably through central integration of activity of sensory afferent and sympathetic efferent fibers,that changes sympathetic drive to white adipose tissue in a unified manner. In specific situations, removal of sympathetic efferents to one pad induces a compensatory enlargement of other intact depots.

Keywords

6-hydroxydopamine; fat pad weight; mice

INTRODUCTION

The sympathetic nervous system (SNS) clearly plays an important role in regulating the size of white fat depots. Adrenergic receptors in white adipose tissue are critical for the

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DISCLOSURE STATEMENT

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regulation of adipocyte lipolysis [2]. In rodents and humans, activation of β-adrenoreceptors stimulates cyclic AMP and activates hormone sensitive lipase (HSL) [6]. By contrast, activation of α2-adrenoreceptors inhibits cAMP and lipolysis [17]. The lipolytic response to adrenergic agonists varies across fat depots and this has been attributed, in part, to differences in the levels of expression of the different receptor subtypes (see [18] for review). Central control of SNS-dependent lipolysis was illustrated by agonism of third ventricle melanocortin 4 receptors increasing norepinephrine turnover (NETO) in intrascapular brown adipose tissue (IBAT) and selected white fat depots [4] with a simultaneous increase in phosphorylation of both perilipin A and HSL [28].

Several investigators have described a role for SNS regulation of fat depot size that is independent of lipolysis. Cousin et al [7] found that surgical denervation of retroperitoneal fat in rats resulted in an expansion of the fat depot due to cell proliferation without a measurable change in adipocyte metabolism. Similarly, there was a substantial increase in the size of surgically denervated inguinal fat pads of Siberian hamsters that was due to cell proliferation rather than cell hypertrophy [33]. These observations clearly demonstrate that the SNS controls adipose tissue growth in addition to lipolysis. A third example of SNS activity modifying the size of white fat stores is associated with transformation of white fat to brown fat in response to chronic activation of β3-adrenoceptors [15-16]. Increased expression of mitochondrial uncoupling protein 1 (UCP1) has the potential to promote fatty acid oxidation and heat loss due to proton leak at the inner mitochondrial membrane [20] which could ultimately decrease fat mass.

Viral tract tracing studies have shown that sympathetic outflow to white fat is controlled by multiple sites in the brain [1], illustrating an organized network that integrates multiple types of information to regulate metabolism of white fat in a depot-specific manner. A potential role for information exchange between white fat depots was demonstrated when selective destruction of the sensory nerves in epididymal fat pads of Siberian hamsters caused enlargement of the non-manipulated retroperitoneal and inguinal fat [26]. Subsequently, anterograde transneuronal viral tract tracing revealed the presence of sensory afferents from white adipose tissue that overlap in the spinal cord, brain stem and hypothalamus with SNS outflow to white fat [29]. These sensory nerves showed increased firing rates when lipolysis was stimulated. Taken together these observations suggest that there is a neural mechanism in place to allow white fat to respond to environmental and energetic conditions by adjusting both lipid metabolism and the number of cells available for lipid storage in each depot. If sensory neurons from one fat depot impinge on SNS output to a different fat depot in the brain or spinal cord, then there also is the opportunity to integrate the metabolic responses of individual fat depots to produce an appropriate whole animal response.

In a previous study, we tested whether the reduction in fat mass of leptin-treated rats and mice was dependent upon activation of the sympathetic nerves supplying the fat pads. Selective sympathetic denervation of one epididymal or one retroperitoneal fat pad changed the size and leptin responsiveness of distant, non-manipulated fat pads [25]. These unexpected results provide additional in vivo evidence that not only is there central control of the SNS drive to a specific fat depot, but that information on changes in metabolism in one fat pad is transmitted to other fat pads likely through CNS exchange of information. The

objective of the studies described here was to further characterize the relation between loss of sympathetic innervation in one fat pad and sympathetic activity in other pads or depots and also to determine whether the influence of one fat pad on other fat depots was uniform or whether it varied between depots. This was achieved by selectively destroying sympathetic nerve terminals in individual fat pads with local injections of 6-hydroxydopamine (6OHDA) [31] and then measuring norepinephrine content or turnover in denervated and intact pads. The results provide new in vivo evidence for indirect communication between individual white fat pads via the CNS.

MATERIALS AND METHODS

The studies described here used 5 week old male NIH Swiss mice (Harlan Laboratories Inc, Indianapolis, IN) housed individually in shoe-box cages with free access to chow (Purina Mouse Chow 5001, Purina Mills, MO) and water. The room was maintained at 28^{o}C and 55% humidity. Lights were on for 12 hours each day from 7 a.m. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Georgia.

Experiment 1: Time course of norepinephrine depletion in sympathetically denervated inguinal fat pads

Fifty mice were divided into five groups. Each mouse was anesthetized (100 mg/Kg ketamine, 10 mg/Kg xylazine, i.p.) and a small midline incision was made in the abdominal skin. One subcutaneous inguinal (ING) pad was exposed and kept moist with saline during injections. Denervated pads were injected 12 times with 2 μl of 9 mg/ml 6OHDA (Sigma, St. Louis, MO) in phosphate buffered saline containing 1% ascorbic acid. Similar injections of PBS 1% ascorbic acid were made in one ING pad of Sham mice. The 6OHDA solution was kept on ice and replaced every two or three hours. Injections were given using a Hamilton syringe with a 30-gauge needle. The needle was held in place for 45 seconds after each injection to minimize reflux from the site of injection. After the pads were injected skin incisions were closed with sterile suture. The mice were injected subcutaneously with analgesic (2 mg/kg ketoprofen) immediately after surgery and again the next day.

For three of the groups, one ING pad in each mouse was injected with 6OHDA (ING SDn) and in the two remaining groups one ING pad was injected with vehicle (ING Sham). One group of ING SDn mice and one group of ING Sham mice was killed 24 hours after the injections, one group of ING SDn mice was killed two weeks after injection and the remaining ING Sham and ING SDn groups were killed 4 weeks after injection. ING pads were dissected and NE content determined by HPLC, as described below.

Experiment 2: Sympathetic denervation of inguinal fat pads

Thirty mice were divided into three weight-matched groups: Sham (ING Sham), single denervation (ING SDn) in which one ING pad was denervated or ING DDn in which both of the bilateral ING pads were denervated. Injection of 6OHDA in ING SDn or PBS in sham mice was alternated between sides to control for potential lateral differences between pads.

Body weights of the mice were recorded twice each week for 4 weeks. Twenty-eight days after denervation the mice were killed and ING, epididymal (EPI), retroperitoneal (RP), perirenal (PR) and mesenteric (MES) white fat and intrascapular brown fat (IBAT) were rapidly dissected, weighed and snap frozen in liquid nitrogen for determination of norepinephrine (NE) content by HPLC. A small piece of each fat pad (~50 mg) was fixed in osmium tetroxide for determination of fat cell size and number by Coulter Counter and Channelizer, as described previously [21]. Briefly, the tissue was left in osmium tetroxide for one week, transferred to isotonic saline for 24 hours and then placed in 8 M urea for four days. The dissociated cells were filtered through sequential 250 um and 10 um nylon filters. Cells collected on the 10 um filter were diluted in 0.154 M saline, 0.1% Triton 100. Cell number and size distribution were determined using a Multisizer III Coulter Counter (Beckman Coulter Inc., Fullerton, CA).

Experiment 3: Sympathetic denervation of epididymal fat pads

This experiment was the same as Experiment 2 except that intraperitoneal, EPI pads of the mice were injected with 6OHDA. The treatment groups were EPI Sham, EPI SDn and EPI DDn. Only 10 injections per pad were used because EPI pads were smaller than ING pads. Cell size distribution and number were determined only in EPI and MES fat pads because there was little effect of EPI denervation on fat pad weight.

Experiment 4: Norepinephrine turnover in fat pads from mice in which inguinal or epididymal fat was sympathetically denervated

Baseline body weights of 105 mice were recorded three times over a 7 day period before the mice were divided into three weight-matched groups: Sham, ING DDn and EPI DDn. Fat pad denervation was achieved as described above. In the sham groups half of the mice had vehicle injected into EPI pads and half had vehicle injected into ING pads.

Two days after the injections the mice in each treatment group were subdivided into three weight matched subgroups. One of the subgroups (10 mice per treatment) was killed between 9.00 and 11.00 a.m. (time 0 hr) and the mice in a second subgroup from each treatment were injected i.p. with 300 mg/kg alpha methyl-*p*-tyrosine (αMPT: Sigma Chemical Company). Two hours after the first injection these mice received a second injection of 150 mg/kg of αMPT. Exactly 4 hours after the first injection the mice were killed (time 4 hr). The mice were decapitated and fat pads were rapidly dissected, weighed and snap frozen in liquid nitrogen for measurement of NE content. NE turnover (NETO) was calculated as described by others [5, 27].

NETO (K) was calculated as $K = k[\mathrm{NE}]_0$

$[\mathrm{NE}]_0$ = tissue NE concentration at time 0hr

k = the rate constant of NE efflux.

For each fat depot $k = (\lg \text{mean}[\mathrm{NE}]_0 - \lg \text{mean}[\mathrm{NE}\}_4)/(0.434 \times 4)$

$[\mathrm{NE}]_4$ is tissue NE concentration at time 4 hr.

Samples of ING and EPI fat collected at Time 0 were used to measure HSL (Cell Signaling Technologies 4107), UCP-1 (Abcam ab 23841) and MTCO-1 (Abcam ab14705), a subunit of cytochrome c and a marker of mitochondrial mass, by Western blot as described previously [19].

The remaining mice from each treatment group were killed four weeks after denervation, the fat pads were weighed and tissue NE content determined. Although Experiment 2 demonstrated that denervation of only one ING pad had a bigger effect than denervation of both ING pads on the intact fat pads in a mouse, we decided to make the analysis as simple as possible by denervating both pads of either the EPI or ING depots which required that only one sample for each fat depot had to be analyzed from each of the 105 mice. As shown in the results, there were significant changes in fat depot NE turnover, but no significant changes in fat depot weight, which was consistent with the results from Experiment 2.

Measurement of Tissue Norepinephrine

Tissue NE was measured by reverse–phase HPLC as described previously [25]. Briefly, frozen fat (15 – 250 mg) was homogenized in 0.2 M perchloric acid with 0.3 ug/ml ascorbic acid containing 25 ng/ml of dihydroxybenzylamine (internal standard). Samples were centrifuged and the supernatant was filtered through a 0.2 um nylon filter (Whatman Inc., Clifton, NJ). Catecholamines were assayed using an ESA (Bedford, MA) HPLC system with electrochemical detection (Culochem II). The mobile phase was 0.1 M sodium phosphate monobasic, 0.1 mM disodium EDTA, 0.3 mM 1-octanesulfonic acid, and 4% acetonitrile, pH of 3.1 and the column was a Phenomenex (150 × 4.6 mm) SYNERGI, 4 u, Max RP-80A column (ESA Inc., Chelmsford, MA).

Statistical Analysis

Body weight and adipocyte size distribution were compared between groups using repeated measures ANOVA. In Experiments 2 and 3 fat pad weight, tissue NE content and fat pad cell number were compared using a one-way ANOVA, comparing pads that were either ipsilateral or contralateral to the injected pad in sham or denervated mice. It was not possible to do a two-way ANOVA because the design was not balanced (DDn mice only had ipsilateral pads). Differences between specific groups were identified by post-hoc Duncan's Multiple Range tests. Differences in tissue NE content on Day 2 compared with Week 4 in Experiment 4 were determined by two-tailed unpaired t-test. All statistical analysis was completed using Statistica Software (Stat Soft, Tulsa OK). Differences were considered significant at $P < 0.05$.

RESULTS

Experiment 1

Denervation of one ING pad caused a significant decrease in tissue NE content of both the 6OHDA-injected and ING pad contralateral to the injection 24 hours and 4 weeks after injection (Figure 1A). At 24 hours, the NE content of the injected Sham pad was lower than that of the contralateral, non-injected pad, but 6OHDA caused a further reduction in NE content of both the injected and contralateral pads (6OHDA: $P<0.0001$, Injected vs.

contralateral: P<0.03, Interaction: P<0.01). At 4 weeks, NE was significantly lower in both the injected and contralateral ING pads of unilaterally 6OHDA-injected mice compared with the shams (Figure 1A: 6OHDA: P<0.002, Injected vs. contralateral: NS, Interaction: NS). The amount of NE in the denervated pads increased from ~40% of Sham levels at 24 hours to ~60% of Sham levels at 4 weeks. The increase in NE content of all of the ING fat pads over time was due to enlargement of the pad rather than an increase in NE concentration (ng/mg tissue: data not shown).

Experiment 2

Sympathetic denervation of one or both ING pads had no effect on the body weight of the mice (Figure 1B), but there was a 35% increase in the weight of dissected white fat from ING SDn mice (P<0.03: Figure 1C). This increase in white fat mass was due to enlargement of the non-injected (contralateral) ING pad (P<0.01), both EPI pads, the PR (P<0.04) and the MES depot (P<0.02, Figure 2A). There was no change in the weight of the injected ING pads. For the ING DDn mice, there was no significant change in the weight of any specific white fat pad. IBAT was significantly larger in ING DDn than ING Sham mice (P<0.02: Figure 2A).

Sympathetic denervation reduced NE content of injected ING pads in both ING SDn and ING DDn mice compared with ING fat from sham mice. The NE content of the contralateral ING pad in ING SDn mice was lower than in the contralateral pad from ING Sham mice (P<0.001). NE content of IBAT from ING SDn mice was lower than for the other two treatment groups (P<0.001, Figure 2B). Decreases in NE content of the MES depot from ING SDN and ING DDN mice and of the contralateral RP pad of ING SDn mice did not reach statistical significance (P<0.06). The NE content of IBAT and of MES fat was substantially higher than in other white fat depots, whereas that of PR fat was highly variable possibly due to the presence of brown fat and blood in this depot (data not shown).

Adipocyte number increased in the 6OHDA-injected ING pads from both ING SDn and ING DDn mice and was increased in the EPI pad contralateral to the ING SDn pad compared with EPI pads from ING sham mice (Table 1). There were significantly more cells in the small size ranges (Figure 3: 30-50um: Treatment: P<0.005, Size: P<0.0001, Interaction: P<0.001) in 6OHDA-injected pads, whereas there was an increase in the number of larger cells in the EPI pad contralateral to the 6OHDA injected ING pad (Figure 3: Treatment: P<0.03, Size: P<0.0001, Interaction: P<0.003). There also was an increase in the number of larger cells in the MES pad from ING SDn mice compared with ING Sham and ING DDn mice (Figure 3: Treatment: P<0.08, Size: P<0.0001, Interaction: P<0.0008).

Experiment 3

Denervation of one or both EPI pads had no significant effect on the weights of any fat pad or the total weight of dissected fat (data not shown). At the end of the study, the NE content of injected EPI pads and RP pads ipsilateral to denervated EPI pads in both EPI SDn and DDn mice (P<0.03, Figure 4). IBAT NE content was lower in EPI DDn mice than either EPI sham or EPI SDn mice (P<0.02: Figure 4). There were no significant differences in EPI pad cell number or cell size distribution.

Experiment 4

Although EPI and ING Sham groups were expected to be identical, fat pad weights and NETO (Figure 5) were different between the groups. Therefore, the groups were analyzed separately, with only 5 or 6 mice per group, and were used as the controls for their respective DDn treatment groups. Denervation of both ING fat depots significantly inhibited NETO not only in the denervated pads (Figure 5A), but also in other fat pads that had not been injected. Two days after 6OHDA injection NETO was significantly inhibited in RP and IBAT fat depots from ING DDn mice compared with ING Sham mice (Figure 5B and D). By contrast, NETO was below detectable levels in EPI fat depots and this was not changed by denervation of either the EPI or ING pads (data not shown). There was, however, a significant inhibition of NETO in RP, ING and IBAT depots in EPI DDn mice (Figure 5A, B and D). NETO in ING pads from ING sham mice was lower than that in ING fat from EPI Sham mice (Figure 5A), suggesting that vehicle injections had disrupted sympathetic activity. Consistent with this disruption there also was a decrease in NETO in RP and MES fat, but an increase in IBAT NETO in ING Sham mice compared with EPI Sham mice.

All of the changes in NETO for a specific depot were associated with a decrease in NE content of the tissue at time 0 hr, rather than a change in the rate constant (*k*) (data not shown) although *k* changed between depots. NE content of all fat depots was decreased 2 days after denervation of either the ING or the EPI depots (Table 2). This decline was fully reversed in ING fat 4 weeks after denervation, but only partially reversed in EPI, MES, RP and IBAT (Table 2). The change in NE turnover did not correlate with any significant changes in fat depot weight 4 weeks after denervation. There was no specific effect of either EPI or ING DDn on the weight of any fat depots (data not shown).

Injection of either vehicle or 6OHDA into EPI pads inhibited expression of HSL and UCP1protein compared with EPI pads from ING sham or ING DDn mice (Figure 6: $P<0.01$). MTCO1 also was decreased in these EPI DDn pads compared with EPI pads from ING DDn mice, suggesting a reduced mitochondrial mass. By contrast, injection of ING pads had no effect on MTCO1 or HSL in ING DDN mice, but there was a reduction in ING UCP-1 protein expression in both the sham injected and denervated ING pads from these mice (Figure 6: $P<0.002$).

DISCUSSION

The results from these studies clearly show that destruction of the sympathetic nerves to one fat pad or both pads in one bilateral depot reduced the NE content and inhibited NETO in other white fat pads and of IBAT, representing altered sympathetic trafficking. This implies that there is indirect communication between individual fat pads within a bilateral depot and between different fat depots and is consistent with recent reports of sensory afferents from white fat overlapping anatomically with the sympathetic drive to white fat [29]. A change in NE content was the most consistent response observed in intact fat depots because changes in fat pad size varied depending upon which fat depot was denervated (ING or EPI) and whether one or both pads within a bilateral depot were denervated (ING SDn or ING DDn). For example, at the end of Experiment 4 there was no effect of either EPI or ING DDn on

the weights of the intact fat depots, but NE content and NETO were decreased in intact RP and IBAT from denervated compared with sham mice.

Experiments 2 and 3 demonstrated that there are different consequences of denervating EPI versus ING fat. For example, denervation of one ING fat pad increased the size of intact contralateral ING fat pads, both EPI pads and the MES fat in ING SDn mice and decreased the NE content of the contralateral ING pad and of IBAT. By contrast, denervation of one EPI fat pad had no effect on the size of any intact fat pad and decreased NE content of the ipsilateral RP fat pad. In Experiment 4 we found very low levels of NETO in EPI fat and this may explain why denervation of EPI pads did not have any effect on the size of other fat depots. These depot-specific effects of fat pad denervation are not unique. There are reports of different degrees of sympathetic innervation between fat depots with substantially fewer noradrenergic nerves in EPI than RP fat [12]. Miller and Faust [23] reported a decrease in the number of measureable ING fat cells, possibly due to lipolysis reducing the size of the cells below detectable levels, but an increase in the number of EPI fat cells in rats that were cold exposed for 24 weeks, providing a very clear demonstration that chronic activation of the SNS produces different effects in ING and EPI fat. In addition, there are differences in rates of proliferation [32] and fat cell size between subcutaneous and omental fat [30] and subcutaneous fat is more responsive to the lipolytic effects of testosterone [10], whereas omental fat is more responsive to lipolytic adrenergic agonists [30]. Therefore, the many cellular and metabolic differences between fat depots could account for the differential effects of denervation of ING versus EPI fat on intact fat depots.

These depot-specific differences do not account for the different responses we observed in ING SDn versus ING DDn mice in Experiment 2. Denervation of one ING pad caused a significant increase in the weight of the intact, contralateral ING pad and of intact EPI, PR and MES fat, but denervating both ING pads did not increase the size of any fat depot. These results imply that one type of compensatory response is initiated when there is a relatively small apparent reduction in body fat caused by removal or denervation of one fat pad, but that a different response is initiated if there is a larger reduction in fat mass. Alternatively, it is possible that there has to be a response in the intact contralateral ING pad in order for the signal promoting enlargement to be transmitted to other intact pads and with double denervation there are no contralateral pads. This explanation seems unlikely given that sensory nerves are functional in both denervated and intact fat pads. The studies described here did not attempt to determine whether communication between fat depots was based on a neural network or on release of a circulating factor. If the response is mediated by a circulating factor, then the different effects of ING and EPI denervation could be explained by differences in production and/or secretion of the factor by the fat depots, similar to depot-specific differences in expression of leptin mRNA [14] [13] and adiponectin protein [11]. Selective denervation of white fat produces some of the same compensatory responses in intact depots as seen in lipectomized animals [22], suggesting that the animals are responding to the loss of, rather than the generation of a signal. If we assume that there is overlap in the mechanisms that compensate for real (lipectomy) or perceived (denervation) loss of fat, then it would be difficult to imagine the involvement of a circulating signal because the fat depot that is the source of the signal is no longer present in lipectomized animals.

It is likely that the communication between fat depots has a neural basis. Others have reported that the neural supply to white fat regulates both lipolysis [17] and proliferation of adipocytes [3, 8]. Surgical denervation of ING fat depots in hamsters doubles the size of the depot in 11 weeks due to adipocyte hyperplasia [33] whereas in rats surgical denervation of RP fat results in a significant enlargement of the fat depot within a week due to both hyperplasia and hypertrophy of the adipocytes [7]. Shi and Bartness [26] demonstrated that specific sensory denervation of ING white fat in hamsters by local administration of capsaicin caused an enlargement of adipocytes, but no increase in fat cell number [26]. The hypertrophy of adipocytes with specific sensory denervation appeared to be independent of sympathetic tone because tyrosine hydroxylase immunoreactivity was unchanged in capsaicin-treated fat pads [26]. This leads to the conclusion that hyperplasia caused by surgical denervation may be due to loss of sympathetic fibers whereas hypertrophy results from destruction of sensory fibers. In Experiment 2 described here the increase in size of the denervated ING fat pad was associated with hyperplasia, whereas enlargement of intact pads in the same animal was associated with hypertrophy of adipocytes, suggesting reduced activity of sensory afferents.

As noted above, sympathetic denervation of a single fat pad caused hyperplasia in the denervated pad, but hypertrophy in the intact depots. Therefore, if communication between depots is mediated by a bidirectional neural network, it is possible that the loss of efferent signals to one depot results in a change in sensory output from that pad. This information is received and responded to at a central site of integration in the brain, which then inhibits efferent outflow to the intact pads. A reduction in sympathetic activity in these pads is indicated by reduced NE tissue content and reduced NETO. This decrease in efferent activity may then produce a compensatory decline in activity of sensory afferent fibers in the intact fat depots, as is implied by enlargement of fat cells in some of these pads. Because Experiment 4 clearly shows a decrease in NETO and of NE content of multiple intact fat depots irrespective of which fat pad is denervated, the compensatory decline in sympathetic drive to other white and brown fat depots appears to be non-specific. By contrast, because we found that only ING SDn caused a change in the size of the intact depots it appears that the regulation of sensory output, or of the relative activity of sympathetic and sensory fibers, is more complex and may be dependent upon additional signals beyond simply a reduction in sympathetic tone in one fat depot. Recently, Nguyen et al [24] reported that IL-4 activated alternative macrophages in adipose tissue release catecholamines. They report that the macrophage activation is required for a coordinated white and brown adipose tissue response to cold exposure. It is possible that changes in the NE content and NETO of denervated fat pads in studies described here resulted from loss of catecholaminergic input from both sympathetic nerves and macrophages, but this would explain the selective responses to denervation of ING versus EPI pads.

It is important to note that denervation of white fat also reduced NETO and NE content of IBAT, which may represent a reduction in thermogenic activity of the tissue. Inhibition of IBAT NETO has been found in hamsters 3 weeks after surgical removal of both EPI pads [27], suggesting that compensation for an apparent loss of energy stores includes an attempt to reduce energy expenditure. Future studies should examine whether the down-regulation

of NETO in IBAT translates into an effective means of increasing efficiency of energy utilization in an animal that is responding to a perceived loss of lipid stores.

We did not attempt to measure metabolism in either intact or denervated fat pads, but whether or not the size changed, there remains the possibility of changes in metabolic status or hormonal responsiveness of intact pads. We previously reported that sympathetic denervation of one EPI pad in rats or mice reduced the leptin-responsiveness of other, intact fat pads [25] implying that inhibition of NETO has metabolic consequences. In Experiment 4 described here basal levels of HSL and UCP-1 were measured in pads taken from EPI DDN and ING DDN mice and we found no association between expression of these proteins and NE turnover. The measures were made in basal conditions and we may have found differences if the animals had been in a stimulated state because the SNS drives phosphorylation of both HSL and perilipin A [4, 28] and increases UCP-1 expression in white fat [15-16].

The selective down-regulation of sympathetic innervation in this study was achieved by making multiple small injections of 6OHDA into specific fat pads. Others reported that local injection of guanethidine produced a permanent functional sympathectomy in white fat of Siberian hamsters [9], however, as discussed previously [25], we were unable to repeat these effects in mice or rats. Although 6OHDA produces a selective destruction of sympathetic innervation to a tissue by destroying adrenergic neurons, the effect is not permanent [31]. Experiments 1 and 4 show that the initial reduction in NE of 6OHDA injected white fat is more marked several days after injection than 4 weeks after surgery. These results are similar to those reported by Thureson-Klein et al [31] for whole animal sympathetic denervation in rats. Specifically, 6OHDA treatment reduced the NE content of IBAT to 10–35% of control values 2 days after injection, but these levels doubled over the next 6 days [31]. The studies described here show that, although the inhibition of NE synthesis produced by 6OHDA denervation was partially reversed over time, the effect of ING SDn on the size of intact fat depots and the reduction in NE content of intact pads in ING DDn and EPI DDn mice was maintained for at least 4 weeks after the denervation.

The biological significance of a change in sympathetic tone and responsiveness to metabolically active hormones following sympathetic denervation of selective fat pads remains to be determined. The reaction of an intact pad to destruction of efferent fibers in a distant fat pad suggests that sympathetic tone leads to the production of a metabolic signal from adipocytes that may be sensed by afferent fibers as a measure of the size of the energy store. A reduction in this signal within a discrete fat pad would be interpreted as a reduction in energy stores and would initiate a compensatory response in other fat depots within an animal.

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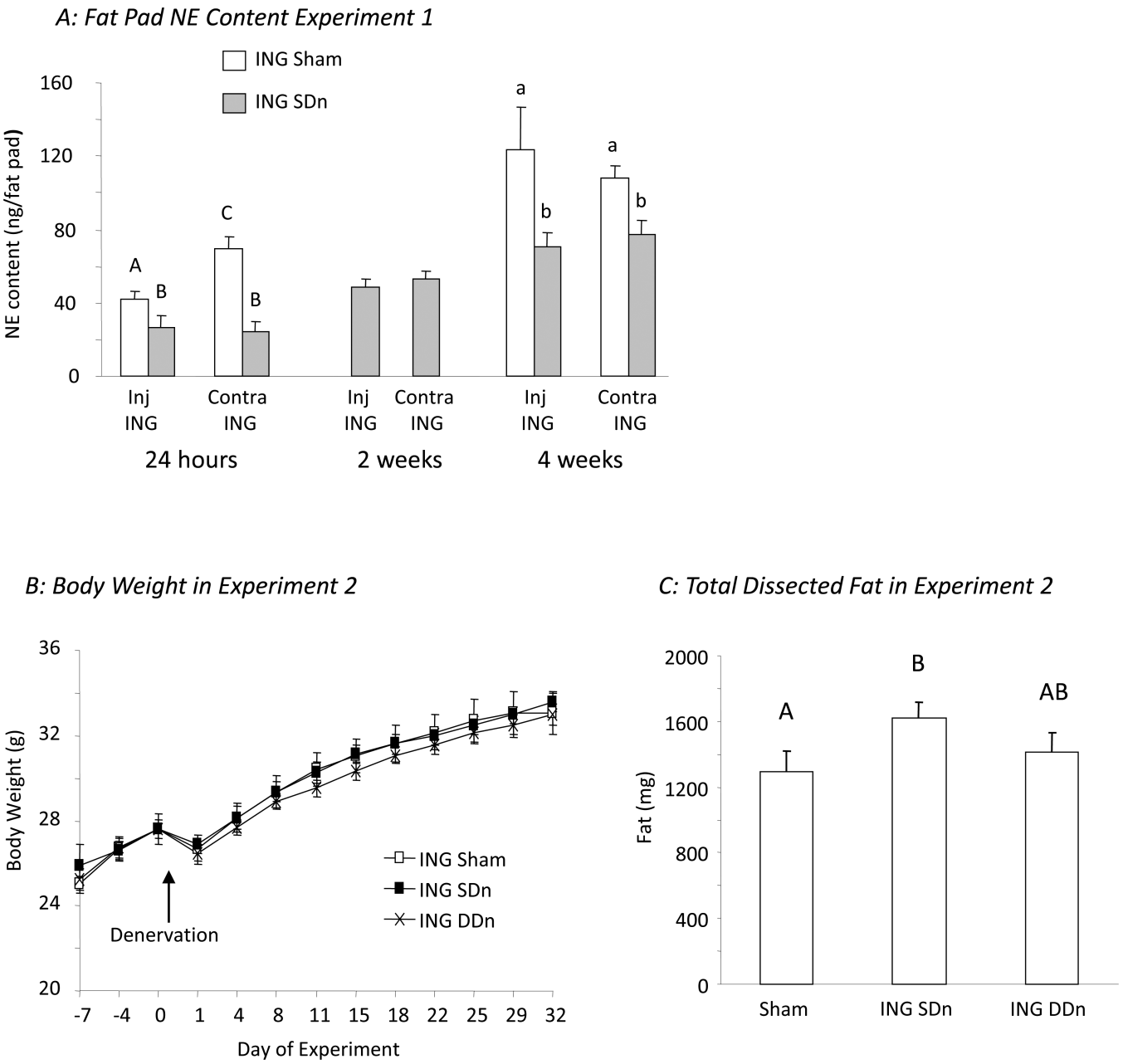

Figure 1.

Panel A is the average NE content of individual fat pads from mice in Experiment 1. Panel B is the average body weight of the groups of mice in Experiment 2. Panel C shows the total weight of the fat that was dissected at the end of the experiment (ING + EPI + RP + MES + PR). Values that do not share a common superscript are significantly different at $P<0.05$. Data are means ± sem for groups of 10 mice.

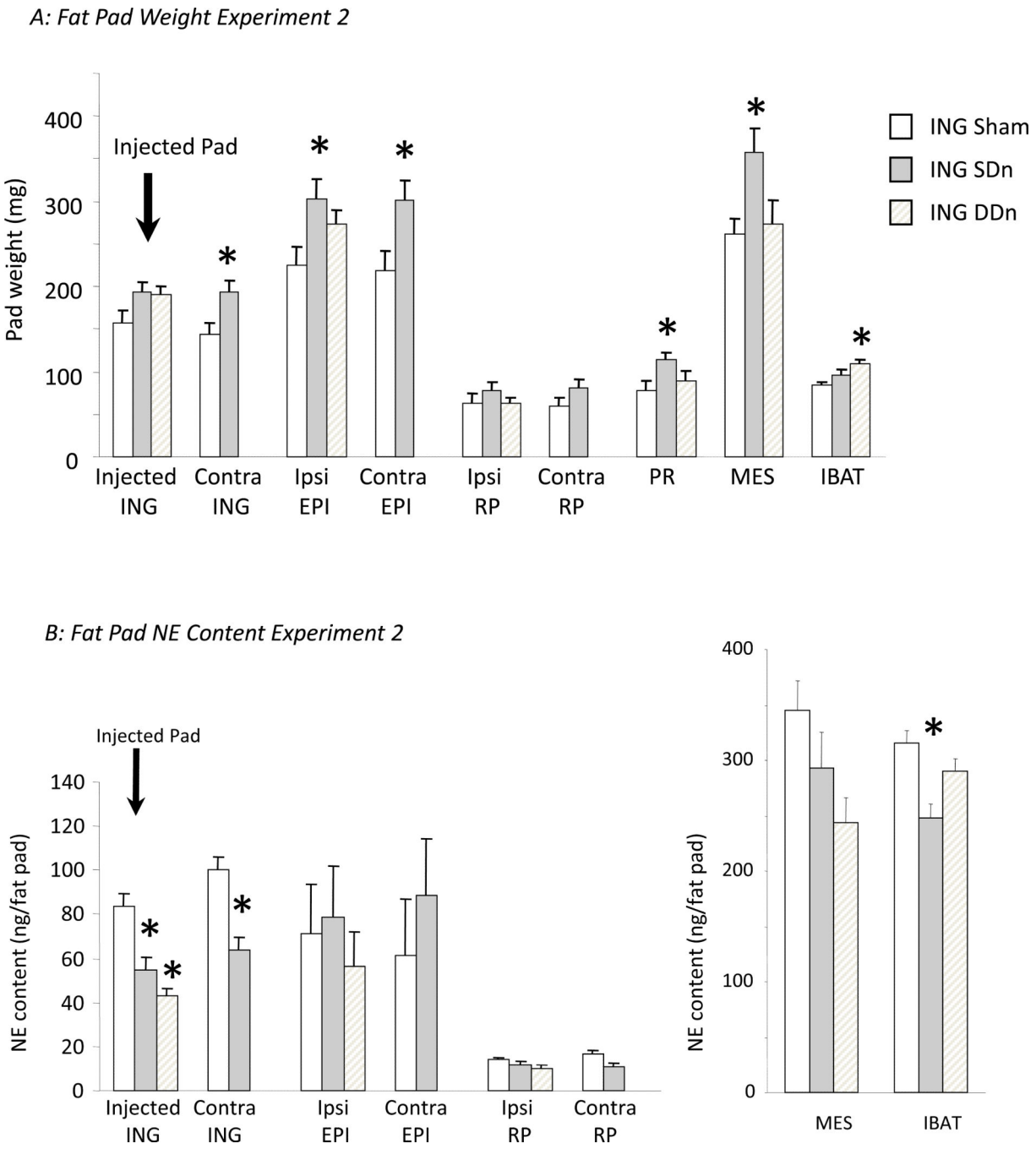

Figure 2.

Panel A is the average weight of individual fat pads from mice in Experiment 2 An asterisk indicates a pad that is significantly larger than the equivalent pad from ING Sham mice. Data are means + sem for groups of 10 mice. Panel B is the NE Content of the pads at the end of Experiment 2. An asterisk indicates a significantly ($P<0.05$) lower NE content than in the equivalent pad from ING Sham mice. Ipsi is a pad that is on the same side of the mouse as the denervated (injected) ING pad and Contra is a pad on the opposite side of the denervated ING pad.

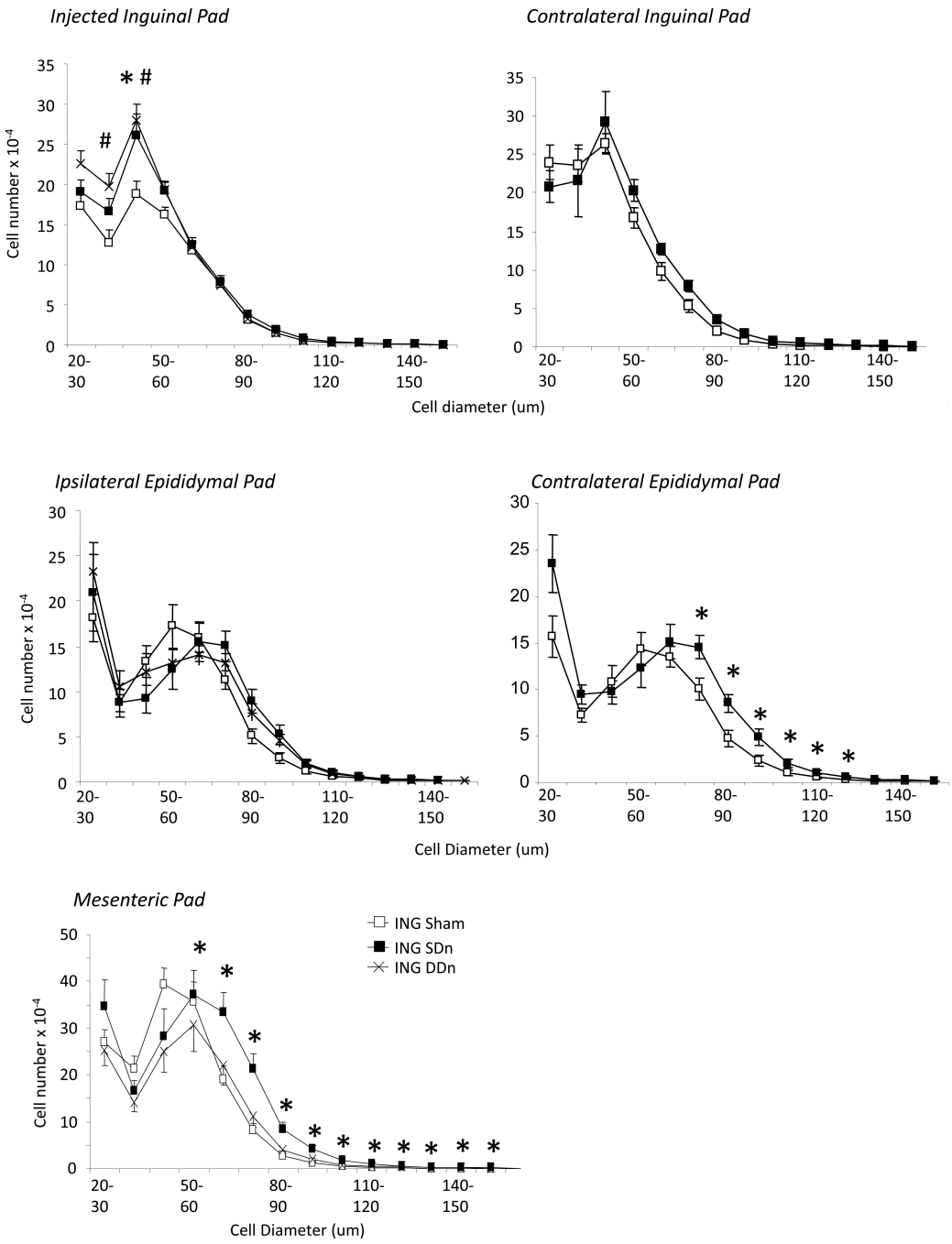

Figure 3.

Cell size distribution in fat pads from mice in Experiment 2. An asterisk indicates a significant ($P<0.05$) difference between ING Sham and ING SDn groups and # indicates a significant difference between ING Sham and ING DDN mice. Data are means ± sem for groups of 10 mice.

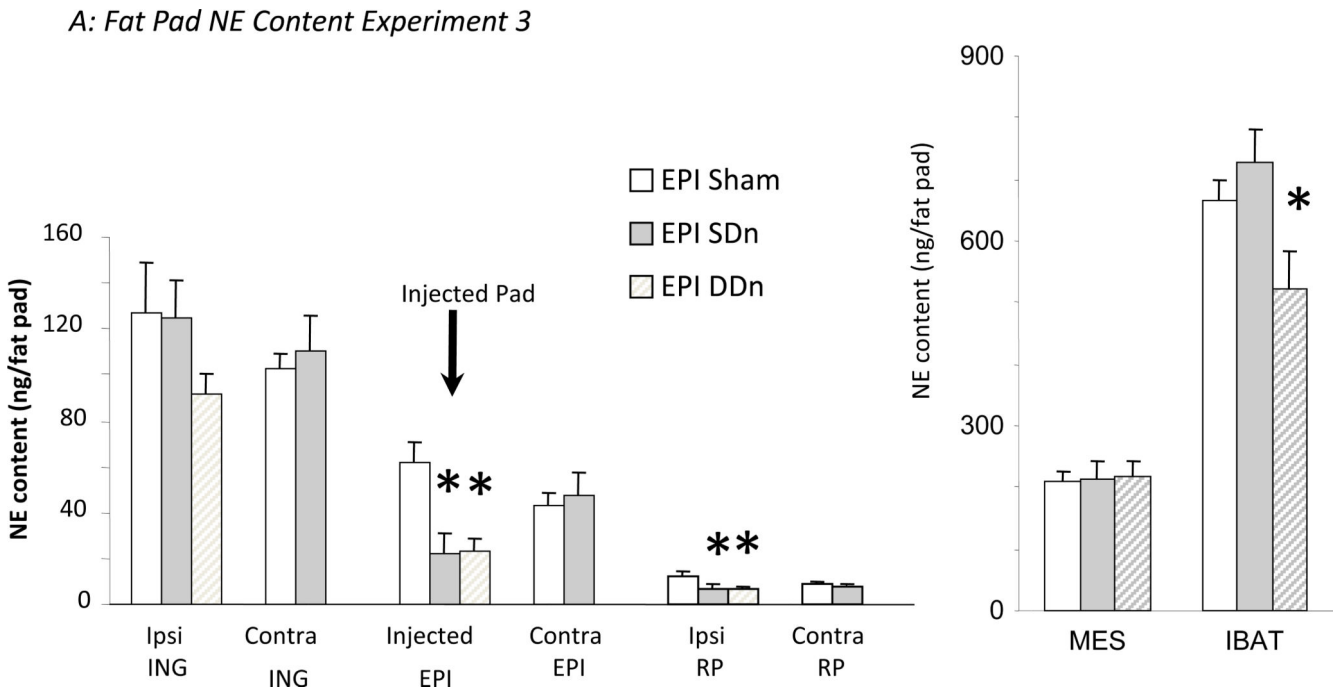

Figure 4.

NE content of fat pads for mice in Experiment 3. An asterisk indicates a significant difference from EPI Sham ($P<0.05$). Data are means + sem for groups of 10 mice.

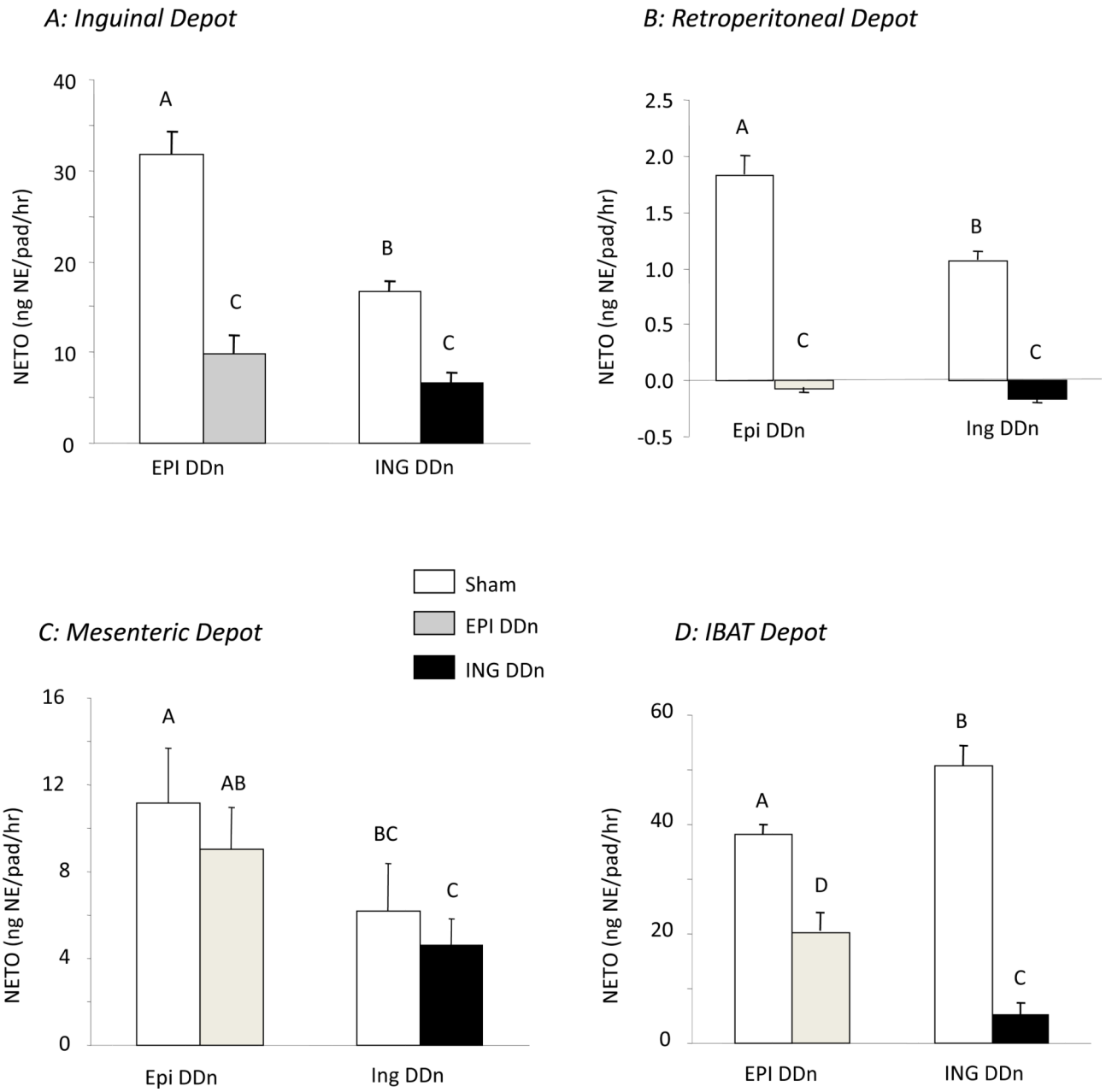

Figure 5.

Norepinephrine turnover (NETO) in fat depots from mice in Experiment 4 measured 2 days after denervation of both epididymal (EPI DDn) or both inguinal (ING DDn) pads. Values within a specific axis that do not share a common superscript are significantly different at $P<0.05$. Data are means + sem for 5 to 10 observations.

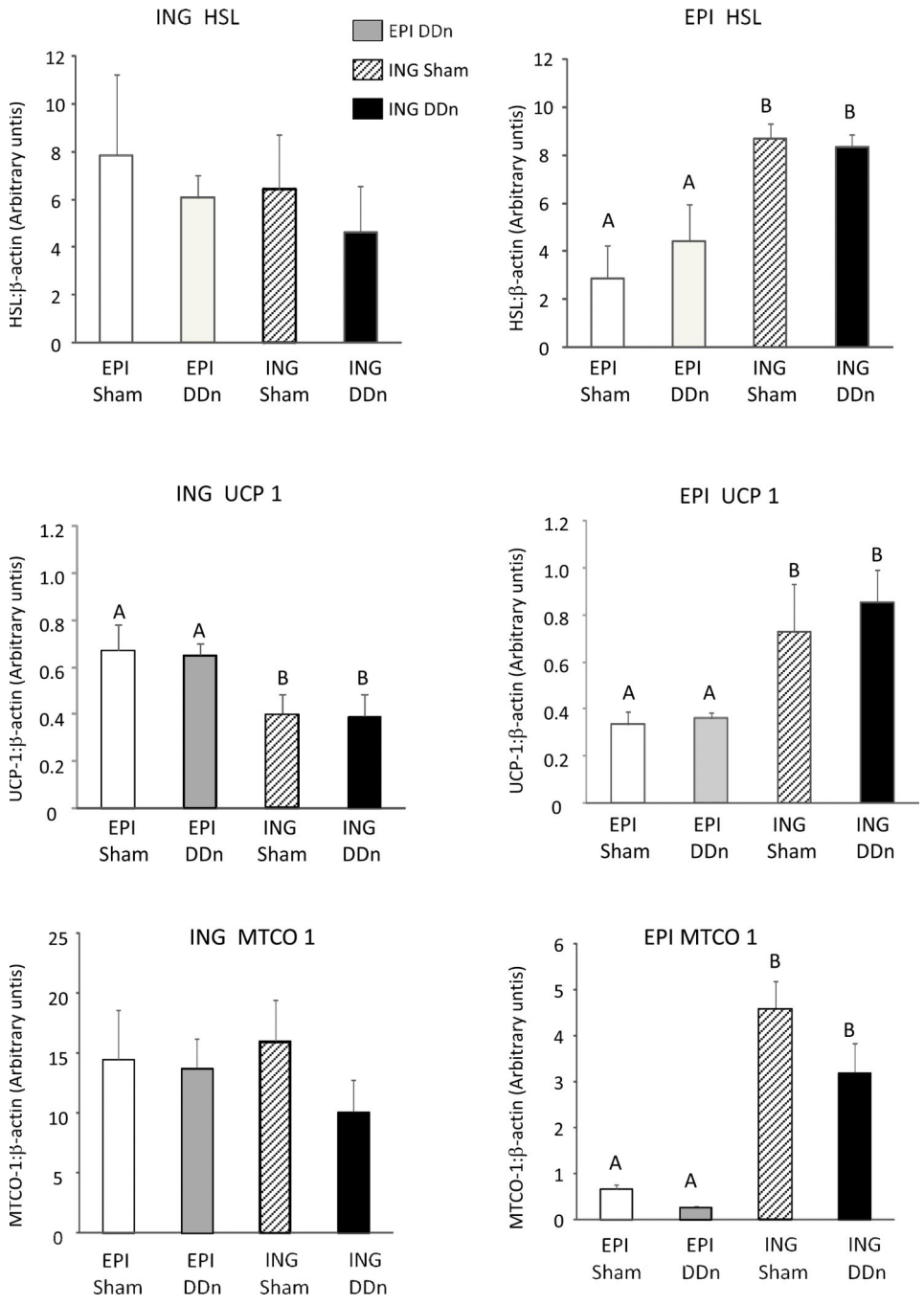

Figure 6.

Expression of HSL, UCP-1 and MTCO 1 protein in EPI and ING fat from mice killed at Time 0 two days after denervation of both epididymal (EPI DDn) or both inguinal (ING DDn) pads in Experiment 4. Data are means + sem for 5 to 10 mice. Values on a specific axis that do not share a common superscript are significantly different at $P<0.05$.

Table 1

Cell number ($\times 10^{-4}$) in different white fat pads from mice in Experiment 2

Fat pad	ING Sham	ING SDn	ING DDn
Injected ING	0.90 ± 0.04	1.09 ± 0.07*	1.15 ± 0.04*
Contra ING	1.10 ± 0.07	1.19 ± 0.11	
Ipsi EPI	0.95 ± 0.07	1.00 ± 0.12	1.03 ± 0.09
Contra EPI	0.81 ± 0.06	1.03 ± 0.07*	
Ipsi RP	0.35 ± 0.02	0.27 ± 0.03	0.27 ± 0.01
Contra RP	0.31 + 0.04	0.30 + 0.02	
MES	1.56 ± 0.10	1.88 ± 0.18*	1.37 ± 0.18

Data are means ± sem for groups of 10 mice. An asterisk indicates a significant difference between the cell numbers for pads from denervated mice compared with the sham control ($P<0.05$).

Table 2

NE content (ng/fat depot) of fat depots from mice killed 2 days and 4 weeks after EPI or ING denervation in Experiment 4

	EPI Depot		ING Depot		RP Depot		MES Depot		IBAT	
	Day 2	***Week 4***	***Day 2***	***Week 4***	***Day 2***	***Week 4***	***Day 2***	***Week 4***	***Day 2***	***Week 4***
EPI Sham	24 ± 4^{A}	84 ± 37^{a}	157 ± 12^{A}	$215 \pm 16^{a*}$	12 ± 1^{A}	$16 \pm 1^{a*}$	65 ± 15^{A}	79 ± 10^{ab}	223 ± 9^{A}	200 ± 26^{a}
EPI DDn	13 ± 4^{B}	30 ± 9^{b}	40 ± 8^{B}	$115 \pm 9^{a*}$	2 ± 1^{B}	$7 \pm 1^{b*}$	16 ± 3^{B}	$68 \pm 8^{b*}$	33 ± 7^{B}	$167 \pm 7^{b*}$
ING Sham	30 ± 9^{A}	77 ± 25^{a}	122 ± 10^{C}	144 ± 25^{a}	11 ± 1^{A}	$15 \pm 1^{a*}$	43 ± 15^{A}	$97 \pm 13^{a*}$	244 ± 17^{A}	229 ± 10^{a}
ING DDn	7 ± 1^{B}	$25 \pm 7^{b*}$	29 ± 5^{B}	$86 \pm 11^{a*}$	1 ± 1^{B}	$7 \pm 1^{b*}$	10 ± 1^{B}	$54 \pm 7^{b*}$	28 ± 5^{B}	$163 \pm 11^{b*}$

Data are means ± sem for groups of 4 to 12 mice killed either 2 days or 4 weeks after pads were denervated with 6OHDA. In DDn groups there were 12 mice on day 2 and 10 mice on Week 4. In sham groups there were 6 mice on Day 2 and 4 or 6 on Week 4. Values within a column that do not share the same superscript are significantly different at $P<0.01$, determined by one-way analysis of variance and post-hoc Duncans Multiple Range test. An asterisk indicates a significant difference ($P<0.0001$) between values at Day 2 and Week 4.