Differential sympathetic drive to adipose tissues after food deprivation, cold exposure or glucoprivation

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Brito NA, Brito MN, Bartness TJ. Differential sympathetic drive to adipose tissues after food deprivation, cold exposure or glucoprivation. Am J Physiol Regul Integr Comp Physiol 294: R1445–R1452, 2008. First published March 5, 2008; doi:10.1152/ajpregu.00068.2008.—Surplus energy is principally stored in white adipose tissue (WAT) as triacylglycerol and mobilized via lipolysis through norepinephrine (NE) released from sympathetic nervous system terminals innervating WAT. We demonstrated that central melanocortin receptor agonism provokes differential sympathetic drives across WAT pads and interscapular brown adipose tissue (IBAT). Here we tested for differential WAT and IBAT sympathetic drive to known lipolytic stimuli (glucoprivation [2-deoxy-D-glucose (2-DG)], cold exposure (5°C), food deprivation (16 h), or both cold exposure and food deprivation) by measuring NE turnover (NETO). Only inguinal WAT NETO significantly increased across all stimuli. Dorsal subcutaneous WAT NETO only increased with glucoprivation. Retroperitoneal WAT NETO increased with glucoprivation, cold and cold + food deprivation, but not by food deprivation. Epididymal WAT NETO was unaffected by glucoprivation but increased with cold, cold + food deprivation or food deprivation, but to a small significant degree. IBAT NETO was unaffected by glucoprivation or food deprivation, but increased with cold and cold + food deprivation. Plasma glycerol was decreased with food deprivation and increased with 2-DG administration or cold exposure. Plasma glycerol was increased with food deprivation, cold, and their combination but not with 2-DG, whereas plasma free fatty acids increased with food deprivation, cold + food deprivation, and 2-DG. These data show differential sympathetic drive to WAT and BAT for four different lipolytic stimuli, exemplifying the fat pad-specific pattern of WAT sympathetic drive across lipid-mobilizing conditions and emphasizing the need to analyze multiple adipose depots for measures of NETO and likely most measures.

norepinephrine turnover; glycerol; free fatty acids; Siberian hamsters; 2-deoxy-D-glucose

The vast majority of lipid is stored by mammals in white adipose tissue (WAT) as triacylglycerol, with additional, but relatively nonsubstantial energy stored as glycogen in liver and muscle. When circulating or stored energy reserves do not meet energy requirements, glycogen is typically mobilized first, and, if these stores are not sufficient, then the triacylglycerol in WAT adipocytes is mobilized via lipolysis to generate free fatty acids (FFA) and glycerol (for review, see Ref. 48). Through our studies of the reversal of a naturally occurring seasonal obesity in Siberian hamsters (Phodopus sungorus; for review see Ref. 7 and 12), we discovered that WAT lipid mobilization is not uniform, that is, the obesity (body fat of ~50%) induced by long “summer-like” days is reversed by exposure to short “winter-like” days in the laboratory, and lipid is mobilized first and to the greatest degree by the more internally located WAT pads [e.g., epididymal WAT (EWAT) and retroperitoneal WAT (RWAT)] compared with the more externally located subcutaneous WAT [i.e., dorsosubcutaneous WAT (DWAT) and inguinal WAT (IWAT); e.g., Refs. 4, 8, and 14]. The underlying mechanism for these increases in lipid mobilization was not increases or decreases in hormones that change seasonally and that also directly or indirectly affect lipolysis (e.g., gonadal steroids, prolactin, insulin, thyroid hormones; for review, see Ref. 7). In addition, this photoperiod-induced naturally occurring reversal from the obese to the lean state was not due to increases in circulating concentrations of adrenal medullary epinephrine (Epi), a well-known inducer of lipolysis, because adrenal demedullation (and thus the removal of the sole source of circulating EPI) did not block short day-induced increases in lipid mobilization (25). This led to tests for the role of the sympathetic nervous system (SNS) innervation of WAT as the means by which lipid mobilization (lipolysis) occurs. This was a neuroanatomical possibility because we reported direct (tract tracing) innervation of WAT by the postganglionic sympathetic nerves innervating WAT using traditional (nontransneuronal) fluorescent tract tracers (72) and subsequently by defining the origins of the SNS outflow to WAT using a retrograde viral transneuronal tract tracer, the pseudorabies virus (PRV; see Refs. 2 and 13). Denervation of the sympathetic innervation of WAT, either surgically or more selectively with a SNS neurotoxin (guanethidine), blocked short day-triggered increases in lipid mobilization (25, 73). Subsequently, others have demonstrated the importance of the SNS innervation of WAT for lipolysis in humans (for review, see Ref. 26) and laboratory rats and mice (for review, see Refs. 5, 9, and 10) for a variety of lipolytic stimuli.

Although the SNS innervation of WAT has been suggested as long as ~110 years ago (27), its function only has recently been appreciated (see above); however, the SNS innervation of brown adipose tissue (BAT) has long been established unequivocally with the advent of histofluorescence (e.g., Ref. 68) and readily accepted as provoking the major mechanism underlying thermogenic and morphological changes in this tissue (for review, see Ref. 18). BAT plays a role in heat generation through a unique property of its mitochondria involving uncoupling protein-1 (UCP-1) (47). The primary stimulator of UCP-1 synthesis and activation, as with WAT lipolysis, is norepinephrine (NE) released from the postganglionic nerve endings of the SNS innervation of this tissue (e.g., Ref. 44).
BAT contributes to thermogenesis under a variety of conditions, including non-shivering-induced thermogenesis (e.g., Ref. 34), and is a primary site for diet-induced thermogenesis (e.g., Ref. 59).

We have been testing the differential sympathetic drives to WAT vs. BAT, as well as differential sympathetic drives across the various WAT depots. For example, as noted above, short-day exposure increases NE turnover (NETO, a neurochemical measure of sympathetic drive) to a greater degree in EWAT than IWAT (72). BAT NETO was not measured in that study, but others have shown short day-induced increases in interscapular BAT (IBAT) NETO in this species (40). In addition, central stimulation of melanocortin 3/4 receptors by the agonist melanotan II (MTII) differentially increases the sympathetic drive to Siberian hamster WAT, increasing NETO in IWAT, RWAT, and DWAT, but not in EWAT, as well as increasing IBAT NETO (16). Thus, for short days or melanocortin receptor agonism, both WAT and BAT sympathetic drives increase. In a preliminary study, we identified some neurons in the hypothalamic paraventricular nucleus and A5 of the brain stem that were part of the circuits to both IWAT and IBAT (62), suggesting a possible neuroanatomical basis for the dual stimulation of both types of adipose tissues. The sympathetic stimulation of both WAT and BAT also occurs in laboratory rats, where cold exposure increases IBAT, EWAT, and RWAT NETO (28).

There are physiological conditions, however, where the sympathetic drives to both WAT and BAT do not occur. For example, food deprivation increases the sympathetic drive (NETO) to EWAT and RWAT (~3-fold) but decreases IBAT NETO (42), which intuitively makes sense given the need to mobilize lipid stores but not to increase energy expenditure at a time when energy is in demand. Not only are the exact mechanisms unknown as to how differential sympathetic trafficking occurs across WAT, BAT, and other peripheral tissues (for review, see Ref. 43), but the conditions under which this occurs also are not fully explored, especially across the various WAT depots. For example, a common nonphysiological, but often insightful, means of triggering metabolic emergency responses is glucoprivation induced by the nonmetabolizable glucose analog 2-deoxy-o-glucose (2-DG), resulting in increases in adrenal medullary catecholamines (for review, see Ref. 56) as well as increases in WAT sympathetic nerve activity [measured electrophysiologically (49)]. Changes in sympathetic drive across WAT pads in response to glucoprivation have not been formally tested, although we have preliminary data suggesting increases to some, but not all, WAT pads (11). It is important to understand mechanisms such as differential sympathetic drives to WAT and other energy balance-related tissues (BAT) because, in terms of the former, the distribution of body fat is critical for its deleterious health effects (e.g., Ref. 65), with the severity of these secondary health consequences of obesity substantially reduced through decreases in visceral lipid stores (e.g., Ref. 54). BAT is a primary means of thermogenesis in rodents and some other species (for review, see Ref. 18) and perhaps is more important in humans than is generally believed given recent evidence of its abundance in adult humans (e.g., Ref. 46).

Therefore, the purpose of the present work was to test the influence of several energy challenges on sympathetic drives across WAT and BAT. Three acute energetic challenges were tested: food deprivation, cold exposure, and glucoprivation. In addition, to create an even greater acute energetic challenge, food deprivation was combined with cold exposure. These acute energy challenges were predicted to produce dissimilar sympathetic drives (NETO), with cold exposure increasing both WAT and BAT drives and food deprivation and glucoprivation increasing WAT but decreasing BAT, with a more difficult to predict outcome occurring with the combination of food deprivation (increase in WAT, not BAT) and cold (increase in WAT and BAT). This was accomplished by testing Siberian hamsters that were food deprived, cold exposed, food deprived and cold exposed, or injected peripherally with 2-DG and measuring NETO as a neurochemical measure of sympathetic drive across the major WAT pads (IWAT, DWAT, EWAT, RWAT) and the major BAT pad (IBAT). In addition, where applicable, food intake was measured, and, in all experiments, fat pad masses and plasma concentrations of glucose, glycerol, FFAs, leptin, Epi, and NE were assessed.

**METHODS**

**Animals**

Two hundred and fifty-six male Siberian hamsters (P. sungorus, 3–4 mo old) were obtained from our breeding colony. The hamsters were single-housed in plastic cages (23 x 26 x 30 cm) and maintained under a long-day photoperiod (16:8-h light-dark period, lights on at 0300) at 22 ± 1°C. Food (Rodent Chow no. 5001; Purina, St. Louis, MO) and tap water were available ad libitum throughout the experiment. Body mass was monitored for 2 wk at which time they were divided into experimental groups matched for absolute body mass and percent body mass change. Housing and all procedures were approved by the Georgia State University Institutional Animal Care and Use Committee and were in accordance with the Public Health Service and United States Department of Agriculture guidelines.

**Experiment 1: Cold Exposure, Food Deprivation, or Cold Exposure Combined with Food Deprivation**

One hundred and sixty hamsters were used. On the day of the test, the animals were provided with clean cages (food and water availability detailed below) and were weighed, and food/bedding was removed from their pouches. One-half the animals was transferred to a modified chromatography refrigerator equipped with a muffin fan to ensure air exchanges equivalent to that of the vivarium. The cold test lasted for 16 h and began at 2000. In addition, one-half of the animals in each temperature condition was food deprived for the 16 h, whereas the remainders were given a new provision of food at the start of the cold or ambient conditions. At this time, one-half of the animals in each temperature/feeding group was killed to obtain baseline tissue NE content measures for subsequent between-animal calculation of NETO, as we have done previously (16, 72). The other one-half of the hamsters was injected intraperitoneal with AMPT (250 mg AMPT/kg; 25 mg/ml) at this time, and a supplemental dose of AMPT (125
mg/kg body mass, at a concentration of 12.5 mg/ml) was administered to these animals 2 h after the injection to assure the maintenance of tyrosine hydroxylase inhibition. These animals were then killed at the end of the 16-h test (i.e., 4 h after the initial AMPT injection) by rapid decapitation. IWAT, RWAT, EWAT, DWAT, and IBAT were rapidly harvested, weighed, frozen in liquid nitrogen, and then stored at −80°C until assayed for catecholamine content to determine NETO.

The NE tissue content was measured using reverse-phase HPLC with electrochemical detection, following our modification (72) of the method of Mefford (41). Briefly, tissue was thawed and homogenized in a solution containing dihydroxybenzylamine (DHBA, internal standard) in 0.2 M perchloric acid (PCA) with 1 mg/ml ascorbic acid (AA). The amounts of tissue processed and DHBA added were varied to obtain NE values within the range of the standards (~250 mg of WAT was used with 50 ng of DHBA added; ~150 mg of IBAT was used with 100 ng of DHBA added). Following centrifugation for 15 min at 7,500 g at 5°C, catecholamines were extracted from the homogenate in an inert atmosphere with alumina and were eluted into the PCA/AA HPLC system with electrochemical detection (Coouloch III). The mobile phase was Cat-A-Phase II, and the column was a HR-80 reverse-phase column. NETO was calculated in IWAT, EWAT, RWAT, DWAT, and IBAT by subtracting the NE content (ng NE/tissue) from the 0-h group from the 4-h group according to the method of Brodie et al. (17). Specifically and briefly, calculations were made according to the following formula: 

\[
\text{NETO} = \text{[NE]}_0 \times \left( \ln \left( \frac{\text{[NE]}_0}{\text{[NE]}} \right) \right) - \left(0.434 \times 4\right) 
\]

and 

\[
K = k[\text{NE}]_0, \text{ where } k \text{ is the constant rate of NE efflux, [NE]}_0 \text{ is the initial NE concentration, } [\text{NE}]_4 \text{ is the final NE concentration, and } K = \text{NETO.}
\]

Using this procedure, we find NE decay after AMPT to be linear for all WAT pads, an example of which was shown (see above). The blood was centrifuged for 20 min at 3,000 g at 3,000 g, 5°C. Plasma was removed and stored at −80°C until assayed. We measured plasma glucose (Asceision Elite blood glucose strips; Bayer, Mishawaka, IN), FFA (NEFA C kit; Wako Chemicals, Richmond, VA), and glycerol, the latter based on the Wieland method (48) adapted to fluorometric analysis in microplates according to the method of Laurell and Tibbling (39). Plasma leptin was measured by enzyme-linked immunosorbent assay (ELISA) with a commercial kit (mouse leptin ELISA kit, Linco Research, St. Charles, MO). Plasma Epi and NE also were processed with a commercial kit (plasma catecholamine analysis kit; ESA Biosciences, Chelmsford, MA), but assayed by HPLC (see above).

\[
\frac{[\text{NE}]_0}{[\text{NE}]_4} \quad \text{lg}\frac{[\text{NE}]_0}{[\text{NE}]_4} = -\frac{\text{lg}[\text{NE}]_0 - \text{lg}[\text{NE}]_4}{(0.434 \times 4)} \quad \text{and} \quad K = k[\text{NE}]_0,
\]

\[
\text{where } k \text{ is the constant rate of NE efflux, } [\text{NE}]_0 \text{ is the initial NE concentration, } [\text{NE}]_4 \text{ is the final NE concentration, and } K = \text{NETO.}
\]

\[
\text{Using this procedure, we find NE decay after AMPT to be linear for all WAT pads, an example of which was shown previously (16), except sometimes not for EWAT (as we have previously noted; e.g., 72.}
\]

Plasma hormones and metabolites. Because of the likelihood that AMPT administration would alter several physiological systems that might impact concentrations of hormones and circulating metabolic fuels, we conducted a parallel experiment to the NETO test where all procedures were done exactly as described in experiment 1 above. Four hours after the first AMPT injection (2 h after the second AMPT injection), animals were rapidly killed by decapitation, and the tissues were harvested and stored as described in experiment 1 for subsequent assay of NE content.

PLASMA HORMONES AND METABOLITES. As with experiment 1, because of the likelihood that AMPT administration would alter several physiological systems that might impact concentrations of hormones and circulating metabolic fuels, a parallel experiment to the 2-DG NETO experiment was done except there was no AMPT administration. Blood collection, plasma hormone, and metabolite measures were done exactly as described in experiment 1 above.

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\text{Statistical Analysis}
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In experiment 1, NETO and circulating concentrations of glucose, glycerol, FFAs, leptin, Epi, and NE were statistically analyzed using a two-way ANOVA (temperature × feeding condition), whereas, in experiment 2, a one-way ANOVA was used (2-DG vs. saline) using Sigma Stat version 2.0 (Systat Software, San Jose, CA). For experiment 1, Duncan’s New Multiple-Range tests were used as post hoc tests when appropriate. Differences between the means for all tests 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

**Experiment 1: Cold Exposure, Food Deprivation, or Cold Exposure Combined with Food Deprivation**

**Body mass and food intake.** As expected, the body mass loss of food-deprived + cold-exposed hamsters was greater than that of both ad libitum-fed and food-deprived hamsters at 22°C (P < 0.05; Table 1). The availability of food in the cold-exposed-only (5°C) hamsters appeared blocked any decrease in body mass associated with this single treatment (Table 1). Food intake was significantly increased in cold hamsters compared with their ambient counterparts (P < 0.05; Table 1). NETO. NETO is presented on a whole fat pad basis to reflect the overall sympathetic drive and physiological impact for each tissue. Food deprivation and/or cold exposure differentially increased NETO across the WAT depots. Specifically, food deprivation alone triggered significant increases in IWAT and EWAT NETO compared with the ad libitum-fed ambient (22°C) counterparts (P < 0.05, Fig. 1), but did not affect RWAT, DWAT, or IBAT NETO. Cold exposure triggered a more broad increase in NETO by IWAT, EWAT, RWAT, and IBAT by cold (5°C) hamsters compared with their ambient controls (P < 0.05; Fig. 1), but did not affect DWAT NETO. Thus, to these two energy-demanding stimuli and their combination, the only common increases in NETO were in IWAT and EWAT.
Plasma metabolites. As expected, plasma glucose concentration was significantly reduced by food deprivation in both cold and ambient hamsters and significantly increased in cold ad libitum-fed hamsters (P < 0.05; Table 2). Both plasma glycerol and FFA concentrations were significantly increased by food deprivation and cold + food deprivation (P < 0.05; Table 2), whereas plasma glycerol, but not FFAs, was increased by cold exposure alone (P < 0.05; Table 2). Superimposing cold exposure with food deprivation yielded significantly greater plasma glycerol concentrations than either treatment alone (P < 0.05; Table 2). Food deprivation did not affect plasma Epi and NE concentrations, but cold-exposed ad libitum-fed hamsters significantly decreased plasma Epi concentrations compared with all the other groups (P < 0.05; Table 2). Cold exposure or food deprivation alone did not affect plasma leptin concentration, but it was significantly decreased with the combined treatment (P < 0.05; Table 2).

**Experiment 2: Glucoprivation**

**NETO.** Glucoprivation induced by 2-DG also provoked dissimilar sympathetic drives, as evidenced by differential NETO across WAT pads and BAT depots. Specifically, both doses of 2-DG significantly increased IWAT, RWAT, and DWAT NETO (P < 0.05; Fig. 2); however, a dose-response increase in NETO only was seen in IWAT (P < 0.05; Fig. 2). 2-DG did not trigger increased NETO in EWAT or IBAT (Fig. 2).

**Plasma metabolites.** Plasma glucose, FFA, Epi, and NE plasma concentrations, but not glycerol, were significantly increased by the high dose of 2-DG (500 mg/kg) compared with the saline controls (P < 0.05; Table 3), whereas the low dose of 2-DG (250 mg/kg) only increased plasma NE concentrations (P < 0.05; Table 3).

**DISCUSSION**

The results of the present experiment demonstrate that there is differential sympathetic drive among WAT pads as well as between WAT and BAT, as assessed neurochemically by NETO, in response to four lipolytic stimuli [glucoprivation (2-DG), cold exposure, food deprivation, or the combination of cold exposure and food deprivation]. IWAT NETO was the only WAT depot exhibiting significantly increased sympathetic drive across all four of these stimuli compared with their respective controls. More specifically, common significant NETO increases to cold exposure and food deprivation only occurred in IWAT and EWAT. IBAT NETO was increased with cold and cold + food deprivation only. The most notable similarity between 2-DG treatment and the other lipolytic stimuli on NETO was the increase in IWAT NETO mentioned above, whereas the lack of an increase in EWAT NETO by 2-DG contrasts with that seen for all the other stimuli as does the 2-DG-induced increase in DWAT NETO. The changes in plasma concentrations of glucose followed expectations based on these treatments in laboratory rats and mice, with significantly decreased glucose concentrations occurring with food deprivation and increased concentrations after 2-DG or cold exposure (e.g., 61; however, c.f., Ref. 63). Although all four stimuli are known to increase lipid mobilization, especially when chronic (cold) or at maximal intensities (high 2-DG doses, long-term food deprivation or restriction; e.g., Refs. 21, 30, and 31), FFAs and glycerol, the products of triacylglycerol breakdown, were not always increased. That is, although plasma glycerol concentrations significantly increased with food deprivation, cold, and cold + food deprivation, they were not increased by 2-DG treatment. Plasma FFA concentrations increased significantly with food deprivation and the highest dose of 2-DG, but not by cold or cold + food deprivation. Some possible reasons for nonuniform increased plasma FFAs and glycerol responses to these known lipolytic stimuli are discussed below.

It should be noted that our control housing temperature was 22°C, which is close to the temperature for thermoneutrality for Siberian hamsters (23°C, see Refs. 37 and 67). This is potentially important for understanding some of the results of this experiment, because animals at thermoneutrality, by definition, do not have to expend additional energy to keep warm; therefore, sympathetic drive will be severely dampened to
BAT because there is no need for what Cannon and Nedergaard (18) have termed “thermoregulatory thermogenesis” and likely also for WAT because there would be no need for additional lipid fuels to be converted to heat by BAT. Therefore, the low NETO in the control animals for both experiments likely is due, at least in part, to this feature of the housing environment.

Acute cold exposure increased sympathetic drive to WAT and BAT, but nonuniformly with significantly increased IWAT, EWAT, RWAT, and IBAT NETO, but not DWAT NETO. One might expect that IWAT NETO (and hence lipid mobilization) would be dampened in the cold given the insulative function of WAT (e.g., 69). Because IWAT is the largest WAT store of lipid fuels (22), it may be that the need for lipid to fuel thermogenesis surpassed the need to maintain insulation. Cold exposure greatly increased IBAT NETO, consistent with such increases in sympathetic drive (e.g., 28 and 32) and sympathetic nerve electrophysiological activity in laboratory rats (50). As suggested above, such increases in BAT sympathetic drive should translate into increased thermogenesis to combat the low ambient temperature. Indeed, with identical treatments of cold exposure and cold exposure + food deprivation, Siberian hamster IBAT temperature is increased (C. H. Vaughan and T. J. Bartness, unpublished observations).

As with the other lipolytic stimuli, food deprivation triggered differential NETO among the WAT pads, as well as differences compared with IBAT. Specifically, IWAT NETO was most markedly increased by food deprivation, nearly tripling compared with ad libitum-fed controls at the same temperature, whereas a more modest but yet significant increase in the low basal EWAT NETO also occurred. These data are relatively comparable to the only other studies testing the effects of food deprivation on WAT NETO. Specifically, IWAT (but also RWAT) NETO was significantly increased by food deprivation in adult laboratory rats (42), and EWAT NETO was significantly increased by food deprivation in young laboratory rats (70). We saw no hint of an increase in RWAT NETO with food deprivation, so the differences between studies could be the result of several factors, at the very least, species differences. Finally, food deprivation did not alter IBAT NETO in the present study, nor did it affect IBAT NETO in laboratory rats (42), Syrian hamsters (64), or laboratory mice (32) that were food deprived, as would be predicted for a stimulus that completely eliminates energy intake; therefore, mechanisms to preserve, not increase, energy expenditure should be marshaled into service.

The addition of food deprivation to cold exposure results in a complex environmental challenge (the complete restriction of energy input at a time when energy expenditure is increased due to the fall in ambient temperature). Such a combined condition can easily be envisioned to occur in nature. WAT NETO was increased by either condition alone in the present experiment, as others have found as well (see above), but there was not an exaggeration in WAT NETO when these two stimuli were combined (although there was a suggestion of such for IWAT). This lack of exaggerated NETO with food deprivation superimposed on cold exposure could be due to a number of factors, the most parsimonious of which is that NETO is maximal with cold exposure alone (ceiling effect). Unlike WAT where, in principle, the combination of cold and food deprivation should separately and together promote increases in NETO, this combination of lipolytic stimuli poses a more complex challenge to BAT. That is, the acute cold stimulus should enhance its thermogenic activity via increases in sympathetic drive to BAT thermogenesis.
in the sympathetic drive to this tissue, but food deprivation at ambient temperature normally inhibits the heat production of BAT (for review, see Ref. 18). In the present study, the cold-induced increased IBAT NETO was not attenuated by the addition of food deprivation, suggesting that, under these conditions, the response to cold was dominant. Although no one else has measured NETO in adipose tissues with this combination, when these two stimuli are combined, the cold exposure-induced increases in heart NETO are markedly attenuated (71), as are urinary catecholamines (71). Because SNS outflow appears very tissue specific to a variety of stimuli (for review, see Ref. 43), the attenuation of sympathetic activity of the heart and, more generally, NETO (urinary catecholamines) by superimposed food deprivation might not reflect the sympathetic drives to WAT and/or BAT. In addition, it may be that, with the impressive degree of naturally occurring obesity in our hamsters, 16 h of food deprivation were not much of a metabolic challenge and therefore the response to cold was not curtailed.

There also was a differential sympathetic drive across the WAT pads and the IBAT pad in response to glucoprivation. Specifically, 2-DG markedly increased IWAT, RWAT, and DWAT NETO, with IWAT showing the only dose-response increases, whereas EWAT and IBAT NETO was unchanged, as we have seen previously in a pilot study (11). Despite the well-known 2-DG-induced sympathetic activation (for review, see Ref. 57), we are unaware of any studies testing for changes in NETO in adipose tissues except our pilot study (11); however, intracerebroventricularly administered 2-DG in laboratory rats decreases the electrophysiological activity of IBAT sympathetic nerves (35). The lack of a 2-DG effect on IBAT NETO (present study) and decrease in IBAT sympathetic nerve activity (35) makes sense in that the emergency metabolic crisis imposed by 2-DG-induced glucoprivation promotes decreases in energy expenditure (58) and, at the other end of the energy balance equation, triggers increases in food intake in many species (for review, see Ref. 6), but for some reason not in Siberian or other hamster species (for review, see Ref. 6).

One would expect that these lipolytic stimuli would promote increases in circulating concentrations of glycerol and FFAs, the products of lipolysis, based on responses of isolated white adipocytes to lipolytic stimuli [e.g., food deprivation (1)], especially given the increases in WAT NETO by these stimuli we found. Indeed, plasma glycerol concentrations were generally increased with food deprivation, cold exposure, and cold + food deprivation, but not with glucoprivation. Increases in plasma FFA concentrations were much more sporadic, being increased with food deprivation and glucoprivation only. The lack of cold-induced increase in FFAs could be due to increased oxidation of this metabolic fuel in muscle to spare glucose (48). Indeed, in terms of the latter, plasma glucose levels were increased by cold exposure, perhaps somewhat reflecting this and/or increased glycolysis, with the latter also suggested by the significant cold-induced increase in circulating Epi concentrations. The addition of food deprivation to cold exposure pushed the plasma glycerol concentrations even higher than either lipolytic stimulus alone and significantly increased plasma FFA concentrations, an effect not seen with cold exposure alone. The lack of cold-induced increased plasma FFA concentration is not surprising because this was an acute cold exposure, and Siberian hamsters tolerate extreme cold −35°C very well, maintaining body temperature for several hours (33). Indeed, cold-exposed (5°C) Siberian hamsters show only a modest, at best, increase in FFAs and then only early at that temperature (5−90 min) with subsequent waning concentrations (34). The ability to tolerate cold so well, even when housed under the more inhibitory influence of a long summer-like photoperiod, may, in part, be due to their ability to rapidly stimulate BAT mitochondrial content, doubling it by 24 h in the cold (55) with a possible subsequent increase in thermogenesis, as well as their naturally extreme obesity, as discussed above. The increase in plasma glucose, FFAs, Epi, and NE plasma concentrations after 2-DG treatment were as expected, despite the lack of a feeding response to 2-DG in hamster species, including Siberian hamsters (for review, see Ref. 6). Plasma glycerol concentrations were not increased by 2-DG treatment, however. Laboratory rats clearly show 2-DG-triggered increases in both FFAs and glycerol (e.g., 51). It is possible that plasma glycerol concentrations were elevated, but we missed this with our 4-h post-2-DG injection sampling. It also is possible that the released glycerol in the present experiment was transported to liver, where it served as a gluconeogenic precursor. Thus, the inability to utilize glucose due to the occupation of glycolytic enzymes trying to metabolize the nonmetabolizable 2-DG may have triggered liver gluconeogenesis using glycerol and other substrates to produce glucose.

Finally, how are the differential patterns of sympathetic drive across WAT and BAT pads produced? This is not known at this time, and, in a broader sense, the trafficking of sympathetic outflow to peripheral tissues remains one of the great mysteries of regulatory biology. We (e.g., 2 and 3) and others (e.g., 20, 38, and 52) have determined the origins of the sympathetic outflows from brain to WAT and BAT. In brief, the circuits to both types of adipose tissue contain both common and uncommon structures across the neural axis. Regarding only WAT, there is an apparent viscerotopic separation of parts of the sympathetic innervation of WAT at the level of the sympathetic chain (72) and spinal cord and higher levels of the neuroaxis (C. K. Song and T. J. Bartness, unpublished observations; see Ref. 38) that could account for some of the fat pad-specific NETO differences. We have postulated (11) that it seems unlikely that the large number of neurons participating in the sympathetic outflow from the CNS to WAT (and BAT), as revealed by the PRV technique (see above), is all activated in response to each of the various lipolytic stimuli (e.g., food deprivation, cold, exercise); rather, we believe subsets are turned on with some shared neurons as we have seen in a preliminary study where individual neurons in the hypothalamic paraventricular nucleus are components of the SNS outflow to WAT and BAT (62).

In conclusion, these data demonstrate, for the first time, differential sympathetic drives to WAT and BAT, as measured neurochemically with NETO, for four lipolytic stimuli (cold, food deprivation, glucoprivation, and cold + food deprivation), with only IWAT NETO significantly increased across all stimuli. We previously found differential sympathetic drives to WAT when MTH1 was injected in the third ventricle (16), and we would predict that such results are likely to occur for other lipolytic and anti-lipolytic stimuli (e.g., exercise and refeeding, respectively). The most difficult task ahead is to determine which sets of the neurons comprising the sympathetic outflows
to WAT and BAT are activated by these and other stimuli, but use of the immediate-early gene product c-fos, a proven marker of cell activation (53), in animals with the SNS outflow to WAT or BAT labeled via PRV is not possible because PRV stimulates c-fos expression (53, 66). An alternative approach is to conduct the PRV labeling of SNS outflow in one set of animals and, in a parallel set, apply the stimulus and immunohistochemically labeled c-fos protein, as has been done for cold exposure and BAT SNS outflow (20). Although one might be confident of overlap in specific nuclei with this approach, it would not, of course, be possible to be absolutely certain that specific cells or subnuclei were activated as well as part of the SNS outflow circuit. Regardless, the overall result shown here is that different stimuli, in general, engender differential sympathetic drive across WAT depots and BAT and emphasizes the necessity of sampling of multiple adipose tissue pads when making measurements related to the SNS control of WAT and BAT, and likely other adipose tissue measures as well.

**Perspectives and Significance**

The results of these studies, as well as our previous study testing the effects of central melanocortin receptor agonism on sympathetic drive (NETO) across WAT depots and IBAT, are clear examples that run counter to the often-prevailing view that originated with Walter Cannon in the late 1920s [(19) that the sympathetic outflow to peripheral tissues is “all-or-nothing” and uniform (for review, see Ref. 43)]. This early line of thinking was further propagated by the interpretation of electrophysiological recordings from the sympathetic nerves innervating IBAT often resulting in extrapolation of decreases or increases in the sympathetic nerve activity of this tissue to other tissues, including WAT (e.g., 15). As we have noted previously (10), tapping into the SNS system by any means at any point in the innervation of any tissue, including adipose tissue, is not analogous to tapping into any electric outlet in any point in the innervation of any tissue, including adipose tissue. However, we do see at many outlets of the sympathetic outflow circuit. Regardless, the overall result shown here would not, of course, be absolutely certain that we are confident of overlap in specific nuclei with this approach, it would not be possible to be absolutely certain that specific cells or subnuclei were activated as well as part of the SNS outflow circuit. Regardless, the overall result shown here is that different stimuli, in general, engender differential sympathetic drive across WAT depots and BAT and emphasizes the necessity of sampling of multiple adipose tissue pads when making measurements related to the SNS control of WAT and BAT, and likely other adipose tissue measures as well.

**GRANTS**

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