Photoperiodic regulation of gene expression in brown and white adipose tissue of Siberian hamsters (*Phodopus sungorus*)

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1Center for Behavioral Neuroscience and 2Department of Biology, Georgia State University, Atlanta, Georgia 30303; 3Division of Gastroenterology and Hepatology, Department of Medicine, Medical University of South Carolina, Charleston, South Carolina 29425; and 4Pennington Biomedical Research Center, Baton Rouge, Louisiana 70808

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Demas, Gregory E., Robert R. Bowers, Timothy J. Bartness, and Thomas W. Gettys. Photoperiodic regulation of gene expression in brown and white adipose tissue of Siberian hamsters (*Phodopus sungorus*). *Am J Physiol Regulatory Integrative Comp Physiol* 282: R114–R121, 2002.—Siberian hamsters exhibit seasonal fluctuations in white adipose tissue (WAT) mass, with peaks in long “summerlike” days (LDs) and nadirs in short “winterlike” days (SDs). These responses can be mimicked in the laboratory after transfer from LDs to SDs. The purpose of the present study was to test whether changes in WAT and brown adipose tissue (BAT) gene expression that are mediated by the sympathetic nervous system in other obesity models are also associated with seasonal adiposity changes in Siberian hamsters. SDs decreased WAT mass and leptin mRNA, increased WAT β3-adrenoceptor mRNA, and induced retroperitoneal WAT uncoupling protein-1 mRNA (the latter measured by RT-PCR, others measured by ribonuclease protection assay) while increasing BAT uncoupling protein-1 and peroxisome proliferator-activated receptor-γ coactivator-1 mrnas. These effects were not due to SD-induced gonadal regression and largely occurred before the usual SD-induced decreases in food intake. Thus the SD-induced decreased adiposity of Siberian hamsters may be due to a coordinated suite of WAT and BAT gene transcription changes ultimately increasing lipid mobilization and utilization.

THE REGULATION OF ENERGY BALANCE in mammals consists of a complex network of feedback systems involving hormonal and neural control of energy input and energy output. Caloric imbalance leads to rapid changes in adipocyte metabolism, with the relative energy balance (i.e., energy intake vs. energy expenditure) determining whether triglyceride is mobilized or deposited. A chronic, positive energy balance promotes obesity, a condition not readily reversed in most animals, especially humans. There are, however, animal species that naturally pass between obese and lean states on an annual basis. In some species, seasonal obesity and leanness are regulated by the environment through changes in the photoperiod (day length) (for review see Ref. 5). By duplicating the changes in the photoperiod in the laboratory, these seasonal alterations in adiposity can be conveniently studied. We have focused on one such species, Siberian hamsters (*Phodopus sungorus*), which are naturally obese when housed in long “summerlike” days (LDs). Siberian hamsters decrease their body fat when exposed to short, “winterlike” days (SDs) (34). This SD-induced decrease in body mass is almost exclusively reflected as a decrease in body fat and is most rapid and nearly fully accomplished during the first 5 wk of SD exposure. At this time food intake is not significantly decreased (34, 39), suggesting that an increase in energy expenditure primarily underlies these changes in seasonal adiposity. This period, however, is subsequently followed by a naturally occurring phase from 8 to 18 wk of SD exposure that is characterized by decreases in food intake of ~30% and the attainment of their annual body fat nadir (34).

The sympathetic nervous system (SNS) recently has been identified as a significant factor involved in regulating Siberian hamster seasonal body fat cycles (12, 38, 39) and also most likely plays a role in modulating adiposity in other photoperiodic and nonphotoperiodic species (for review see Ref. 7). One way by which SNS activity can affect seasonal body fat changes is through increases in brown adipose tissue (BAT) thermogenesis and the consequent increase in energy expenditure via its well-established sympathetic innervation (36). The exact contribution of the sympathetically mediated increases in BAT thermogenesis to the SD-induced decreases in Siberian hamster body fat is not fully understood, however. Another mechanism by which SNS

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activity can affect seasonal body fat changes is through the stimulation of white adipose tissue (WAT) lipid mobilization (lipolysis) via its sympathetic innervation (for review see Ref. 3), which was identified recently in this species (1, 31, 38). This sympathetic innervation of WAT appears functionally important in that SDs increase the sympathetic drive (i.e., norepinephrine turnover) on WAT in proportion to the loss of lipid from the WAT pads (38). Moreover, surgical or chemical denervation of Siberian hamster WAT largely, but not totally, blocks SD-induced decreases in lipid mobilization (12, 39).

Despite this and other information regarding the sympathetic innervation of WAT and its role in lipid mobilization in Siberian hamsters, the SNS-mediated changes in WAT at the protein and gene level are virtually unknown. In the more frequently studied laboratory rat and mouse obesity models, several sympathetically mediated WAT and BAT factors have been identified but have largely been unexplored within the context of seasonal adjustments of adiposity. Specifically, increases in BAT thermogenic activity primarily appear to be due to the functions of one of a family of uncoupling proteins, UCP-1 (23), which is predominantly expressed by BAT adipocytes (23). The effects of SDs on Siberian hamster BAT UCP-1 gene expression have been examined but only after extended SD exposure (20), well past the rapid decline in body mass, and peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1), a critical coactivator of UCP-1 (29), has not been measured. Another important sympathetically related factor in the control of adiposity is the β3-adrenergic receptor (β3-AR), the primary postsynaptic SNS adrenoceptor subtype in rodent WAT that plays a critical role in WAT lipolysis (32). Siberian hamster β3-AR has only been studied in primary culture of brown adipocytes (19), but not in vitro or in vivo in Siberian hamster WAT to our knowledge. Finally, leptin, the protein product of WAT (24) and BAT (10) adipocytes postulated to inform the brain of body fat levels (for review see Ref. 6), also appears to regulate gene expression in WAT and BAT through the SNS (17, 33), in part via coupling to the β3-AR (13, 14). Leptin content is decreased in WAT from the closely related Djungarian hamster exposed to SDs (27), as is leptin gene expression in SD-exposed Siberian hamsters (20). SD-induced changes in leptin gene expression, however, have not been examined in the context of these other sympathetically mediated factors that are involved in thermogenesis and/or lipolysis (e.g., UCP-1, PGC-1, and β3-AR). Therefore, the purpose of the present study was to test whether changes in WAT and BAT gene expression, thought to be mediated sympathetically in other obesity models, are associated with the SD-induced decreases in adiposity of Siberian hamsters. This was accomplished by examining the naturally occurring changes in gene expression of leptin, UCP-1, PGC-1, and β3-AR in WAT and BAT from Siberian hamsters transferred from LDs to SDs.

Materials and Methods

Experimental design. SD-housed hamsters were killed after 5 wk of SD exposure, when the decrease in body fat is most rapid and independent of food intake (34), and after 10 wk of SD exposure, when the decline in body fat becomes asymptotic and is associated with a significant decrease in food intake (34). Because any observed SD-induced changes in gene expression could be secondary to SD-induced gonadal regression and the concomitant decreases in serum testosterone concentrations (2) [e.g., leptin (37)], additional LD-housed hamsters were castrated to match the naturally occurring SD “functional castration.”

Animals and housing conditions. Eighty adult (>60 days of age) Siberian hamsters (P. sungorus) were obtained from our laboratory breeding colony. This colony was originally derived from stock supplied by Dr. Bruce Goldman (University of Connecticut) in 1988 and interbred with wild-trapped hamsters in 1990 from Dr. Katherine Wynne-Edwards (Queens University). Hamsters were weaned at 21 days of age and housed with same-sex siblings. Two weeks before the initiation of the experiments, animals were housed individually in polypropylene cages (27.8 × 7.5 × 13.0 cm) in colony rooms under a 16:8-h light-dark photoperiod (lights on at 0300 Eastern Standard Time). Temperature was kept constant at 20°C, and relative humidity was maintained at 50 ± 5%. Food (Purina Rat Chow) and tap water were available ad libitum throughout the experiment. All experimental procedures were approved by the Georgia State University Institutional Animal Care and Use Committee in accordance with Public Health Service and US Department of Agriculture guidelines.

Hamsters (n = 40) were selected randomly and assigned to one of two photoperiodic conditions. At week 0, half of the animals (n = 20) were transferred from the LDs to an SD (8:16-h light-dark) photoperiod, while the remaining animals (n = 20) continued to be housed in LDs. After 5 wk, half of the animals from each photoperiodic condition were selected randomly, removed from the colony, and killed via an overdose of pentobarbital sodium. Paired testes, epididymal WAT (EWAT), inguinal WAT (IWAT), and retroperitoneal WAT (RWAT) were removed, weighed to the nearest 0.1 mg, and stored in RNAlater (Ambion, Austin, TX) for subsequent statistical analyses. The two time points (e.g., 5 and 10 wk) were chosen for several reasons. First, SD-housed hamsters display significant reductions in food intake after 10 wk, but not 5 wk, of SDs. Second, the reduction of body fat by SDs occurs at its greatest rate during the first 5 wk of SD exposure, whereas the nadir in body fat typically occurs at ≥10 wk of SD exposure (34). Therefore, these time points allow us to determine changes in gene expression at times when changes in body fat are at their most dynamic and static, respectively.

To control for the effects of reduced serum testosterone concentrations that could potentially underlie any SD-induced change in gene expression, as discussed above, additional hamsters (n = 40) were selected randomly and assigned to one of two conditions. Half of the animals (n = 20) were castrated; the remaining hamsters were subjected to sham castration. After 1 wk, half of the animals from each
group were selected randomly, weighed, and killed via an overdose of pentobarbital sodium, and adipose tissues were harvested, weighed, and stored as described above. The remaining animals \((n = 10)\) were kept in the colony rooms and then killed 5 wk after their respective surgeries. Hamsters were killed at these two time points \((e.g., 1 \text{ and } 5 \text{ wk after castration})\), because these time points allowed us to capture any potential changes in gene expression in adipose tissue during the height and nadir of castration-induced changes in body fat. These time points roughly map onto comparable time points of SD-induced reductions in body fat \((e.g., 5 \text{ and } 10 \text{ wk})\).

**Materials.** T1 ribonuclease and TRIzol reagent were obtained from Life Technologies (Gaithersburg, MD). T7 and SP6 RNA polymerases (RNAPs), Taq polymerase, Moloney’s murine leukemia virus reverse transcriptase, and the pGEM-3Z cloning vector were purchased from Promega (Madison, WI). The pCRII cloning vector was purchased from Invitrogen (Carlsbad, CA). Oligonucleotide primers corresponding to UCP-1, PGC-1, and \(\beta_3\)-AR genes were prepared by the DNA core facility at the Medical University of South Carolina. The primers used to amplify the leptin gene fragment were prepared by Sigma Genosys (St. Louis, MO). \(^{[32P]}\)CTP was purchased from Dupont-New England Nuclear Radiochemicals (Boston, MA).

**Isolation of RNA from adipose tissue depots.** After dissection, all adipose tissues were homogenized in TRIzol reagent using an Ultraturrax Tissumizer (Tekmar, Cincinnati, OH) according to the protocol of the manufacturer. Thereafter, RNA was isolated from the homogenized samples following the protocol of the manufacturer, which is a modification of the guanidinium thiocyanate-phenol-chloroform one-step extraction method of Chomczynski and Sacchi \((9)\).

**Ribonuclease protection assay.** Reverse transcription followed by the polymerase chain reaction (RT-PCR) was used to generate cDNA from the total RNA isolated from EWAT or BAT. Oligomers based on the mouse UCP-1 and PGC-1 genes were used as PCR primers to amplify fragments of these genes from BAT cDNA. The UCP-1 primers \((5’-\text{GATC}-\text{CAAGGTGAAGGCCAGG} \text{ and } 3’-\text{GGTGAACAGTTCTGTGTTGG})\) encompass nucleotides 357–746 of *Mus musculus* UCP-1, and the *P. sungorus* PCR product displays 90% identity to this gene. The PGC-1 primers \((5’-\text{AAGGATTGTCGGAC} \text{ TAAGGGTATGGCAGCCTT} \text{ and } 3’-\text{ATAGGTACGGGATATG-GTGATCGGGAAC})\) correspond to nucleotides 1535–1840 of the murine PGC-1 gene, and the hamster PCR product displays 95% identity to this gene. The \(\beta_3\)-AR and leptin gene fragments were amplified using cDNA derived from EWAT as a template. The \(\beta_3\)-AR primers \((5’-\text{CTCTGGCTTGGAGCGCTAC} \text{ and } 3’-\text{GGCATGGTGAGGGAAGGAAC})\) amplify nucleotides 948–1146 of the *M. musculus* gene, and the *P. sungorus* PCR product displays 91% identity. To design primers to amplify a portion of the *P. sungorus* leptin gene, published sequences from diverse vertebrate leptin genes were analyzed for highly conserved regions (http://www.nlm.nih.gov). The primers \((5’-\text{GACACCAAAACCCCTCATCA} \text{ and } 3’-\text{CARRGCCACCCCTCNGT})\), where *R = A + G и \(N = A + G + C + T*\) correspond to nucleotides 113–1267 of the murine gene, and the *P. sungorus* PCR product displays 92% identity. TA cloning was utilized to clone these gene fragments into the pCRII vector (Invitrogen). Thereafter, the fragments were subcloned into the pGEM-3z vector, which contains SP6 and T7 RNAP promoters flanking the multiple cloning site. In vitro transcription reactions, in which plasmids linearized on either side of the insert were used as templates, utilized these promoters to prepare runoff cRNA transcripts. The cRNA transcribed by one RNAP is the sense strand, whereas the cRNA produced by the other RNAP was radiolabeled with \(^{[32P]}\)CTP and used as a riboprobe in ribonuclease protection assays essentially as described previously. Briefly, 2 \(\times 10^4\) cpm of \(^{32P}\) labeled riboprobe were coprecipitated with 0.5–5.0 \(\mu\)g of adipose tissue RNA. An 18S rRNA riboprobe was included to ensure that equal amounts of RNA from each sample were used. After ethanol precipitation and resuspension in hybridization buffer, RNA was denatured at 80°C and hybridized at 55°C overnight. Unhybridized RNA was digested by addition of 300 U of T1 ribonuclease to each sample and incubation at 37°C for 1 h, and the reaction was stopped with 5 mM EDTA. After precipitation, protected dsRNA was resuspended in dye and electrophoretically resolved on 6% denaturing polyacrylamide gels containing 8 M urea. The gels were exposed to film, and the autoradiograms were scanned with a densitometer. Known amounts of sense strand standards were included in each assay to allow quantification of protected mRNA. Because the densitometric area of the autoradiographic bands is a linear function of the amount of sense strand added, it is possible to generate a standard curve to quantify the message protected in each lane.

**Semiquantitative RT-PCR.** Because of the limited amount of RNA obtained from the retroperitoneal depot, the RNA from five hamsters was pooled to yield two samples for each photoperiodic condition and time point. The RNA \((1 \mu\)g) was chromatographed to assess quality and confirm that each pooled sample contained the same amount of RNA. Then 400 ng of each sample were reverse transcribed into cDNA using oligo(dT) as a primer. The UCP-1 primers described above were used to amplify hamster UCP-1. To quantify the UCP-1 mRNA in experimental samples by PCR, 100 pCi of \(^{[32P]}\)CTP were added to each PCR, and the following PCR conditions were used: 1 cycle of 95°C for 5 min; 32 cycles of 95°C for 45 s, 56°C for 30 s, and 72°C for 45 s; and 1 cycle of 72°C for 5 min. Pilot experiments confirmed that after 32 cycles the PCR amplicon was still being amplified exponentially. After the PCR products were electrophoretically resolved on a 2% agarose gel, the amplicons were cut from the gel and purified using the QIAquick gel extraction kit (Qiagen, Valencia, CA). The amount of radiolabeled CTP incorporated was determined by scintillation counting.

**Data analyses.** The estimated concentrations of tissue mRNA for leptin, UCP-1, PGC-1, and \(\beta_3\)-AR were obtained by reverse calibration from standard curves as described above. Comparisons were made using separate 2 (photoperiod or surgery) \(\times 2\) (week) between-groups analyses of variance (SigmaStat, Jandel, San Rafael, CA). Post hoc differences between means were conducted using Tukey's honestly significant difference tests as appropriate and were considered statistically significant at \(P < 0.05\). Exact probabilities and test values have been omitted for simplification and clarity of the presentation of the results.

**RESULTS**

**Body and adipose tissue masses.** Animals maintained in SDs for 5 or 10 wk (SD-5 and SD-10, respectively) weighed significantly less than LD animals \((P < 0.05; \text{Table } 1)\). SD-5 and SD-10 hamsters also had significantly reduced paired testes mass compared with LD animals \((P < 0.05)\), and EWAT, IWAT, RWAT, and BAT pad masses were significantly smaller in SD-5 and SD-10 than in LD animals \((P < 0.05; \text{Table } 1)\). IWAT and BAT pads were significantly smaller in SD-10 than in SD-5 animals \((P < 0.05)\); there was a
small, nonsignificant trend toward smaller RWAT pad masses in SD-10 than in SD-5 animals (Table 1). There were no significant differences in body, paired testes, or EWAT pad mass between SD-5 and SD-10 hamsters (Table 1).

Body masses were significantly smaller in the castration control than in the sham-operated animals at 1 and 5 wk after surgery (P < 0.05) but were not different within each group at these two times (Table 2). At 1 wk after surgery, EWAT and IBAT pad masses were significantly smaller in castrated than in sham-operated animals (P < 0.05); IWAT and RWAT were not significantly different between castrated and sham-operated animals at 1 wk after surgery (Table 2). There were no significant differences between any of the WAT or IBAT pad masses between castrated and sham-operated hamsters at 5 wk after surgery.

**Gene expression in IBAT.** Because SDs are associated with increased thermogenic capacity of BAT (18, 35), we hypothesized that PGC-1 and UCP-1 expression would be upregulated in SD-housed Siberian hamsters. Our results demonstrate that UCP-1 and PGC-1 mRNA were significantly higher in SD-10 hamsters than in LD-10 hamsters (P < 0.05; Figs. 1 and 2). IBAT UCP-1 also was significantly elevated in SD-10 animals compared with LD-10 animals (P < 0.05; Fig. 1), although there was no difference in PGC-1 expression between SD-10 and LD-10 animals in this tissue (Fig. 2). In addition, β3-AR mRNA expression was significantly higher in IBAT from SD-10 hamsters than LD-10 animals (P < 0.05; Fig. 3); β3-AR mRNA expression in IBAT did not differ between LD-5 and SD-5 animals. There was no difference in IBAT leptin mRNA expression among any of the experimental groups (Fig. 4) or between castrated and sham-operated hamsters at 1 or 5 wk after surgery (Table 3).

**DISCUSSION**

The results of the present study demonstrate that SD exposure promotes marked changes in several genes involved in the regulation of energy balance in Siberian hamsters. The naturally occurring SD-induced decreased body and WAT pad masses were generally associated with decreased WAT leptin gene ex-

### Table 1. Body and adipose tissue masses in hamsters housed in LDs or SDs for 5 or 10 weeks

<table>
<thead>
<tr>
<th></th>
<th>Body (g)</th>
<th>EWAT (g)</th>
<th>IWAT (g)</th>
<th>RWAT (g)</th>
<th>IBAT (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD-5</td>
<td>42.52 ± 1.57</td>
<td>0.846 ± 0.046</td>
<td>1.065 ± 0.059</td>
<td>0.826 ± 0.072</td>
<td>0.219 ± 0.015</td>
</tr>
<tr>
<td>SD-5</td>
<td>36.08 ± 1.72*</td>
<td>0.874 ± 0.008†</td>
<td>0.530 ± 0.082†</td>
<td>0.153 ± 0.026*</td>
<td>0.139 ± 0.022†</td>
</tr>
<tr>
<td>LD-10</td>
<td>45.94 ± 1.80</td>
<td>0.812 ± 0.035</td>
<td>0.993 ± 0.031</td>
<td>0.957 ± 0.115</td>
<td>0.199 ± 0.011</td>
</tr>
<tr>
<td>SD-10</td>
<td>34.14 ± 1.15*</td>
<td>0.853 ± 0.005†</td>
<td>0.281 ± 0.036†</td>
<td>0.296 ± 0.031*</td>
<td>0.089 ± 0.011†</td>
</tr>
</tbody>
</table>

Values are means ± SE in g. EWAT, EWAT, and RWAT, epididymal, inguinal, and retroperitoneal white adipose tissue; IBAT, interscapular brown adipose tissue; LD-5 and LD-10, long days for 5 and 10 wk; SD-5 and SD-10, short days for 5 and 10 wk. *P < 0.05 vs. LD-5 or LD-10; †P < 0.05 vs. LD-5, LD-10, or SD-10; ‡P < 0.05 vs. all other groups.

### Table 2. Body and adipose tissue masses in castrated and sham-operated hamsters 1 and 5 wk after surgery

<table>
<thead>
<tr>
<th></th>
<th>Body (g)</th>
<th>EWAT (g)</th>
<th>IWAT (g)</th>
<th>RWAT (g)</th>
<th>IBAT (g)</th>
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<tbody>
<tr>
<td>1 wk</td>
<td></td>
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</tr>
<tr>
<td>Sham</td>
<td>43.07 ± 0.82</td>
<td>0.943 ± 0.016</td>
<td>0.550 ± 0.074</td>
<td>0.181 ± 0.008</td>
<td>0.282 ± 0.027</td>
</tr>
<tr>
<td>Cast</td>
<td>37.69 ± 0.76*</td>
<td>0.784 ± 0.053*</td>
<td>0.529 ± 0.099</td>
<td>0.129 ± 0.023</td>
<td>0.197 ± 0.008*</td>
</tr>
<tr>
<td>5 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>45.59 ± 1.50</td>
<td>0.889 ± 0.037</td>
<td>0.626 ± 0.116</td>
<td>0.131 ± 0.024</td>
<td>0.370 ± 0.097</td>
</tr>
<tr>
<td>Cast</td>
<td>37.92 ± 2.11*</td>
<td>0.679 ± 0.130</td>
<td>0.617 ± 0.102</td>
<td>0.107 ± 0.014</td>
<td>0.245 ± 0.041</td>
</tr>
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</table>

Values are means ± SE in g. Sham, sham-operated; Cast, castrated. Significant difference between pairwise means: *P < 0.05 vs. Sham 1 wk or Sham 5 wk.
pression, whereas WAT β3-AR gene expression was increased and ectopic UCP-1 expression was induced in RWAT. SD exposure also triggered increased IBAT UCP-1 and PGC-1 mRNA, but IBAT leptin and β3-AR gene expression were unaffected. Although protein levels were not measured in this study, mRNA levels for the genes examined here (e.g., leptin, PGC-1, and UCP-1) have been shown to correlate well with protein levels (25). These SD-induced changes in mRNA levels were not due to SD-induced gonadal regression or decreased circulating testosterone, because castration alone in LD-housed hamsters had no effect on mRNA expression for any of the genes examined. Many of the SD-induced changes in gene expression in WAT were evident at 5 wk (i.e., increased β3-AR mRNA in EWAT and RWAT, decreased leptin mRNA in RWAT and IWAT, and presence of UCP-1 mRNA in RWAT). These alterations in gene expression appeared to occur independently of food intake, because food intake is not significantly decreased at this time (34), although food intake was not measured in the present study. Collectively, the present results show that there are photoperiod-specific changes in adipose gene expression and that these changes are neither secondary to photoperiod-induced changes in gonadal function nor likely due to changes in food intake.

Functional evidence is beginning to accumulate to suggest that the SNS innervation of WAT is the major

Fig. 1. A: representative autoradiograph from a ribonuclease protection assay of uncoupling protein-1 (UCP-1) mRNA from interscapular brown adipose tissue (IBAT) of Siberian hamsters housed in long days (LD) or short days (SD) for 5 or 10 wk. B: mRNA densitometry values for UCP-1 from IBAT of Siberian hamsters housed in LD or SD for 5 or 10 wk. *P < 0.05, LD vs. SD.

Fig. 2. mRNA densitometry values for peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1) from brown adipose tissue (BAT) of Siberian hamsters housed in LD or SD for 5 or 10 wk. See Fig. 1B legend for conventions. *P < 0.05, LD vs. SD.

Fig. 3. mRNA densitometry values for β3-adrenergic receptor (β3-AR) from epididymal (EWAT), inguinal (IWAT), and retroperitoneal white adipose tissue (RWAT) and IBAT of Siberian hamsters housed in LD or SD for 5 or 10 wk. See Fig. 1B legend for conventions. *P < 0.05, LD vs. SD. †P < 0.05 vs. all other groups.

Fig. 4. mRNA densitometry values for leptin from EWAT, IWAT, RWAT, and IBAT of Siberian hamsters housed in LD or SD for 5 or 10 wk. See Fig. 1B legend for conventions. *P < 0.05, LD vs. SD.
Table 3. mRNA expression in EWAT, IWAT, RWAT, and IBAT in castrated or sham-operated hamsters 1 and 5 wk after surgery

<table>
<thead>
<tr>
<th></th>
<th>EWAT</th>
<th>IWAT</th>
<th>RWAT</th>
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<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Cast</td>
<td>Sham</td>
<td>Cast</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.274 ± 0.026</td>
<td>0.285 ± 0.056</td>
<td>0.489 ± 0.023</td>
<td>0.428 ± 0.041</td>
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<tr>
<td></td>
<td>0.320 ± 0.093</td>
<td>0.332 ± 0.059</td>
<td>0.498 ± 0.102</td>
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<tr>
<td>(\beta_3)-AR</td>
<td>0.028 ± 0.002</td>
<td>0.029 ± 0.003</td>
<td>0.038 ± 0.006</td>
<td>0.034 ± 0.002</td>
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<tr>
<td></td>
<td>0.033 ± 0.004</td>
<td>0.030 ± 0.005</td>
<td>0.039 ± 0.007</td>
<td>0.027 ± 0.004</td>
</tr>
<tr>
<td>PGC-1</td>
<td>0.013 ± 0.001</td>
<td>0.008 ± 0.002</td>
<td>0.009 ± 0.001</td>
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<tr>
<td>UCP-1</td>
<td>0.001 ± 0.008</td>
<td>0.001 ± 0.008</td>
<td>0.001 ± 0.001</td>
<td>0.008 ± 0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE in fmol/µg RNA. \(\beta_3\)-AR, \(\beta_3\)-adrenergic receptor; PGC-1, peroxisome proliferator-activated receptor-\(\gamma\) coactivator-1, UCP-1, uncoupling protein-1.

means by which the photoperiod alters body fat in Siberian hamsters, buttressing the neuroanatomic data showing the sympathetic innervation of this tissue (12, 38, 39). First, the more internally located WAT depots (i.e., EWAT and RWAT) are mobilized before the more externally located depots (i.e., IWAT) (4), suggesting a neural regulation of lipolysis with differential SNS innervation and/or drive to internal vs. external depots. Indeed, norepinephrine turnover (i.e., sympathetic drive) is greater in EWAT than in IWAT after 5 wk of SDs, corresponding with the more rapid reduction in EWAT mass at this time (38). Second, surgical or chemical denervation of IWAT largely, but not completely, blocks SD-induced increases in lipid mobilization by Siberian hamsters (12, 39). Interestingly, denervation combined with adrenal demedullation, the latter eliminating the other arm of the SNS, completes this blockade (12). Finally, the functional melatonin (MEL1a) receptor (30) that mediates the photoperiod-induced seasonal changes in body fat and other responses is colocalized on neurons that have been labeled as part of the sympathetic outflow from brain to WAT by a viral transsynaptic tract tracer (31). The following discussion of gene expression changes in WAT and BAT complements these functional and neuroanatomic studies and seems to support even more strongly the notion that the SNS is a primary mediator of SD-induced decreased body fat by Siberian hamsters.

We found that SDs significantly increased \(\beta_3\)-AR gene expression in WAT. Although it might be expected that the SD-induced increase in sympathetic drive on adipose tissues would downregulate \(\beta_3\)-ARs or promote postreceptor desensitization (16), the \(\beta_3\)-AR appears to be regulated primarily at the transcriptional level (8). This contrasts with the \(\beta_1\)- and \(\beta_2\)-ARs that are desensitized by phosphorylation after sustained stimulation (8). Therefore, the increased \(\beta_3\)-AR mRNA of SD-housed hamsters in the present study occurred despite the typical sustained increase in SNS stimulation observed in SDs (38) and demonstrates transcriptional upregulation of this gene by SD exposure. Furthermore, the possible subsequent increases in the \(\beta_3\)-ARs could enhance the ability of adipocytes to respond to sympathetic stimulation. Consistent with this notion, \(\beta_3\)-AR mRNA of the more internally located EWAT depot was upregulated after 5 wk of SDs, corresponding with the increased norepinephrine turnover (28) and reduced fat mass at this time (4). Collectively, these results suggest that the SD-induced decreases in WAT mass likely are due to increased SNS drive to peripheral adipose tissue depots, and they indicate that SDs may enhance the ability of adipocytes to respond to noradrenergic stimulation.

The results of the present study revealed another manifestation of the increased SNS drive on WAT, the recruitment of dormant brown adipocytes and/or the induction of transdifferentiation of white adipocytes in RWAT depots, as evidenced by the SD-induced increase in WAT UCP-1 gene expression in this tissue. As the present data suggest, brown adipocytes can be revealed within otherwise white adipocyte populations when the sympathetic drive on WAT is increased, such as occurs with cold exposure (15). Whether this represents stimulation of otherwise dormant brown adipocytes or transdifferentiation of white to brown adipocytes is under continuing debate. Regardless of the true origin of these cells, the presence of UCP-1 mRNA.

![Fig. 5. RT-PCR determination of UCP-1 mRNA from RWAT of Siberian hamsters housed in LD or SD for 5 or 10 wk.](http://ajpregu.physiology.org/)

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indicates the presence of brown adipocytes in RWAT. The SNS activation of these cells within RWAT provides another possible mechanism underlying the SD-induced decreased RWAT mass: an increased oxidation of WAT-derived lipid in situ via these apparent brown adipocytes.

In addition to the ectopic expression of UCP-1 mRNA in RWAT, SDs increased BAT UCP-1 mRNA. This is in accordance with the SD-induced increase in BAT UCP-1 of the common spiny mouse (*Acomys cahirinus*) (22). Because UCP-1 mRNA expression is regulated, in part, by PGC-1, the demonstration that SD-housed hamsters had increased BAT PGC-1 mRNA expression compared with LD-housed animals, together with previous research indicating that SDs trigger increases in the SNS drive to BAT (26), provides a likely mechanism for this increased UCP-1 expression. Therefore, the SD-induced increase in sympathetic drive on BAT appears to result in increases in the stimulation of PGC-1 that, in turn, stimulate UCP-1 gene expression and translation resulting in the possible oxidation of WAT-derived lipid fuels to increase BAT thermogenesis and thereby help contribute to the SD-induced decreases in body fat by Siberian hamsters.

Leptin mRNA expression and WAT pad masses were significantly reduced in SD- compared with LD-housed hamsters in the present study. This SD-induced decreased WAT pad mass is consistent with previous results in this species (4, 34), as is the decreased leptin gene expression (20) and associated decreases in circulating leptin concentrations (21). The mechanism underlying these decreases in leptin gene expression and translation by SDs is not understood but may be related to the ability of leptin to regulate WAT adipocyte function via the central nervous system modulation of the sympathetic activity to adipose tissues. For example, we have demonstrated that leptin downregulates its own gene expression in WAT through modulation of sympathetic tone and that this downregulation depends on the presence of the β3-AR subtype (11). Furthermore, leptin can induce UCP-1 expression in BAT and WAT through the SNS (11). These and other studies demonstrate that the level of circulating leptin communicates the amount of energy stored in adipose tissue to the brain, at least in mice, and suggest that leptin and its corresponding receptor could form a "lipostatic" feedback loop that acts to increase energy expenditure and decrease food intake. According to this model, reductions in circulating leptin in SDs should reduce SNS activation and consequently reduce energy expenditure while increasing food intake. Food intake by Siberian hamsters is not changed early in SD exposure, however, and instead is eventually decreased (34). Thus the role of leptin in the control of seasonal cycles of adiposity is not clearly defined.

Collectively, the present results and our previous studies of body fat alterations in Siberian hamsters discussed above suggest that their SD-induced decreased adiposity appears to be promoted by a coordinated suite of changes in WAT and BAT gene transcription ultimately facilitating lipid mobilization and utilization of lipid fuels.

**Perspectives**

Although our understanding of seasonal cycles of adiposity is deepening, it is far from completely understood, and many hypotheses related to this phenomenon require modification. For example, we proposed a relatively simple hypothesis where the SD-induced increases in WAT lipid mobilization exclusively involved activation of sympathetic outflow from brain to WAT via the SNS innervation of WAT (39). We recently found that the other arm of the SNS is significantly involved in this process. That is, adrenal demedullation (to eliminate circulating catecholamines, especially epinephrine) and sympathetic denervation of WAT are required to block completely the SD-induced decreases in body fat (12). Thus, together with SD-induced increases in activity of both of these arms of the SNS, we also postulate that the resulting response to these sympathetic effectors may be augmented by increased sensitivity to catecholamines on the basis of the SD-induced increased β3-AR gene expression in WAT found in the present study. We further suggest that the sympathetically mediated processes discussed above collectively promote increases in oxidation of these WAT-derived lipid fuels by BAT, a tissue that itself has augmented respiratory and oxidative capacity due to SD exposure, as indicated by our finding of increased UCP-1 and PGC-1 gene expression in this tissue in the present study. Finally, the sympathetically mediated recruitment of dormant and/or transdifferentiated brown adipocytes in specific depots (e.g., RWAT) may lead to increases in the oxidation of lipids in situ by these cells.

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