Koban, Michael, and Kevin L. Swinson. Chronic REM-sleep deprivation of rats elevates metabolic rate and increases UCP1 gene expression in brown adipose tissue. Am J Physiol Endocrinol Metab 289: E68 –E74, 2005. First published February 22, 2005; doi:10.1152/ajpendo.00543.2004.—A cluster of unique pathologies progressively develops during chronic total- or rapid eye movement-sleep deprivation (REM-SD) of rats. Two prominent and readily observed symptoms are hyperphagia and decline in body weight. For body weight to be lost despite a severalfold increase in food consumption suggests that SD elevates metabolism as the subject enters a state of negative energy balance. To test the hypothesis that mediation of this hypermetabolism involves increased gene expression of uncoupling protein-1 (UCP1), which dissipates the thermodynamic energy of the mitochondrial proton-motive force as heat instead of ATP formation in brown adipose tissue (BAT), we 1) established the time course and magnitude of change in metabolism by measuring oxygen consumption, 2) estimated change in UCP1 gene expression in BAT by RT-PCR and Western blot, and 3) assayed serum leptin because of its role in regulating energy balance and food intake. REM-SD of male Sprague-Dawley rats was enforced for 20 days with the platform (flowerpot) method, wherein muscle atonia during REM sleep causes contact with surrounding water and awakens it. By day 20, rats more than doubled food consumption while losing ~11% of body weight; metabolism rose to 166% of baseline with substantial increases in UCP1 mRNA and immunoreactive UCP1 over controls; serum leptin decreased and remained suppressed. The decline in leptin is consistent with the hyperphagic response, and we conclude that one of the mediators of elevated metabolism during prolonged REM-SD is increased gene expression of UCP1 in BAT.

Rapid eye movement; oxygen consumption; uncoupling protein-1

WHEN TOTAL- OR RAPID EYE MOVEMENT- SLEEP DEPRIVATION (REM-SD) is enforced in rats for one to several weeks, pathologies develop that lead to significant morbidity. Collectively referred to as “sleep deprivation effects” (reviewed in Ref. 34), the cluster of syndromes was first described by Rechtschaffen and Bergmann (35) using the sophisticated disk-over-water (DOW) paradigm. The pathologies are reliably produced and include hyperphagia, weight loss, elevated energy expenditure, increased plasma catecholamines, hypothyroidism, reduction in core temperature, deterioration in physical appearance (34), reduced levels of anabolic hormones (10), and declines in integrity of the immune system (14). The etiology of most SD pathologies remains obscure, although several advances have recently been made (10, 12, 14). Adaptation to unmitigated sleep loss is not possible, because it is always fatal, whether the deprivation is total (9) or selective for REM sleep (26). REM-SD is achieved simply and effectively with the platform (i.e., flowerpot) method. The setup has a rat on a small platform (e.g., inverted flowerpot) surrounded by water. Enforcement takes advantage of muscle atonia during REM sleep, resulting in making contact with or falling into the water; the rat abruptly awakens and repeats the cycle. Electroencephalography of platform rats confirms that one-half to all of REM sleep and varying amounts of non-REM sleep are lost (20, 23, 27, 28), therefore validating its utility.

Comparisons between the two methods show that some syndromes are similar: hyperphagia and weight loss (5, 30, 31, 34, 44, 49) and decreased plasma thyroid hormones (3, 13, 31) and leptin (10, 21; this study). Importantly, because many serious pathologies manifest after more than 1 wk (34), whether or not other DOW syndromes come about with the platform method remain unanswered, because almost all of the studies involving the latter have been short term, typically with a time course of 96 h or less (1, 5, 7, 30, 31, 40–43, 49).

Regardless of enforcement method, sleep-deprived rats exhibit hyperphagia with concomitant loss of body weight, two pathologies that cannot be explained by changes in digestive absorption efficiency (3) or development of diabetes (9). The only way to account for the ensuing state of negative energy balance is for metabolism to be elevated, and yet, the most commonly used and accepted procedure to measure metabolic rate, oxygen consumption, has never been employed for SD studies. To gain further understanding of some of these phenomena, we wanted to elucidate the relationship between REM-SD and increased metabolism. No inquiry has addressed this problem, but even a cursory review of mammalian metabolic physiology points to brown adipose tissue (BAT) as a logical place to begin. BAT is the primary site of regulatory nonshivering thermogenesis in rodents (15), and heat production is mediated by upregulation of uncoupling protein-1 (UCP1; reviewed comprehensively in Ref. 6). For this study, our objectives were to test the hypotheses that 1) chronic REM-SD of rats leads to progressively elevated metabolic rate with a time course corresponding to increased gene expression of UCP1 in BAT and 2) leptin, which acts centrally as a satiety signal to blunt appetite, would be depressed, permitting the development of hyperphagia.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (4–5 mo of age, Harlan) were housed individually in standard plastic cages with ad libitum chow (Teklad) and water; ambient temperature averaged 24°C, and photoperiod was a 12:12-h cycle with lights on at 0800. Experimental
procedures were approved by the Institutional Animal Care and Use Committee of Morgan State University, and they comply with National Institutes of Health guidelines.

The REM-SD paradigm. Two Plexiglas REM-SD tanks were each divided into five compartments of 30 × 30 × 40 cm. Compartments had a 10-cm-high column onto which platforms of different diameters can be interchangeably attached. Ten-centimeter platforms were used for REM-SD and 15-cm ones for tank controls; rats in home cages were additional controls. Smaller platforms (6–7 cm) are typically used for most REM-SD studies of ~96 h duration (1, 5, 7, 30, 31, 40–43, 49), but our observation has been that rats become completely exhausted beyond 96 h. To minimize confinement as a restraint-like stressor, 10-cm platforms were used to provide more space and mobility.

Inlet and outlet ports on opposite ends allow continuous water flow to carry away waste and debris and flood each chamber to 1 cm below the platform surface. Rats increasingly experience water immersions after ~2 wk, so water temperature was adjusted to ~30°C to lessen cooling effects. Food and water are easily accessible; rats can groom and rest by lying down. When rats on 10-cm platforms lapse into REM sleep, they lose muscle tone, make facial contact with or fall into the surrounding water, abruptly awaken, and the cycle is repeated. Thus the platform method is selective for abolishing REM sleep. The 15-cm platform is large enough so that, within a day or two, rats learn to position themselves to sleep with minimal or no water immersions. Tank control rats usually remain dry even after 20 days; however, there can still be loss of REM sleep (27, 28), although adaptation may eventually occur (28).

Experimental design. For 2 wk, rats were accustomed to routine handling and the novel environment of the REM-SD tanks by placement on 10-cm platforms; one day later, another five rats were added into the surrounding water, abruptly awaken, and the cycle is repeated.

The recovery period was unremarkable, and there were no mortalities for mammals (47), which we employ herein.

Semiquantitative RT-PCR of BAT UCP1 mRNA. A separate cohort of rats (n = 15) was REM-sleep deprived as described above, and after 5, 10, and 20 days, squads of five rats were killed by CO2 inhalation; controls were rats in home cages (n = 5). Excised interscapular BAT pads were freeze-clamped and stored at −75°C. Total RNA was extracted with TRI Reagent (Molecular Research Center), and sample integrity was confirmed by visual inspection of ethidium bromide-stained RNA after denaturing (formaldehyde) gel electrophoresis. Two micrograms of input RNA was reverse transcribed (Superscript First Strand Synthesis System, Invitrogen Life Technologies) and then PCR amplified with the primers illustrated in Table 1 (Integrated DNA Technologies).

GAPDH, 36B4 (acidic ribosomal phosphoprotein PO), and β-actin were included as “housekeeping” or reference markers, genes used for normalization because they are constitutively expressed and believed not to appreciably change with experimental conditions. After optimization, final reaction conditions were 40 cycles, 1 min each of 95°C denaturation, 58°C annealing (57°C for GAPDH), and 72°C elongation, with a final 10-min elongation step. Ampiclons (n = 5 per time point) were run on gels, and fluorescence intensity (FI) in arbitrary units of each ethidium bromide-stained band was digitally acquired and corrected for background with the Kodak EDAS 290 system using Kodak 1D Image Analysis Software, version 3.60 for Windows. Averaged data were normalized as the ratios of net FI of UCP1 to each reference gene.

Western blotting of BAT UCP1 protein. BAT was homogenized [25 mM Tris-HCl, 1 mM EDTA, 1% Triton X-100, 0.5% Na-deoxycholate, pH 7.4, 50 µg protease inhibitor cocktail (Sigma, P8340), 1 mM PMSF], sonicated (5 W, 5 s), and centrifuged (1,000 g, 10 min). Lipid-free supernatant was collected and stored at −75°C. Protein content was determined in triplicate with the bicinchoninic acid assay (Pierce). BAT protein (20 µg) was electrophoresed in SDS-10% polyacrylamide minigels and electroblotted onto nitrocellulose (NitroBind, Osmonics). In addition, BAT and liver protein (20 µg) from cold-acclimated rats (9 days at 5°C) were run as positive and negative controls, respectively. Blocked (5% nonfat dry milk) nitrocellulose was probed with rabbit anti-UCP1 antisera (Calbiochem, cat. no. 36B4).

Table 1. Genes and primer sequences used in PCR amplification

<table>
<thead>
<tr>
<th>Product</th>
<th>Gene</th>
<th>Primer Sequence</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCP1</td>
<td>f</td>
<td>5'-ATACTGCGAGATGACGTTCC-3'</td>
<td>229</td>
</tr>
<tr>
<td>36B4</td>
<td>f</td>
<td>5'-GAGGTCACTGTGCCAGCTCA-3'</td>
<td>360</td>
</tr>
<tr>
<td>-Actin</td>
<td>f</td>
<td>5'-AGCAGCCATGTCAGACTCA-3'</td>
<td>182</td>
</tr>
</tbody>
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UCP1, uncoupling protein-1; 36B4, acidic ribosomal phosphoprotein PO; f, forward; r, reverse.
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662045; no cross-reactivity to UCP2 or UCP3) at 1:1,000 dilution (2.1 μg IgG/ml). Secondary antibody was horseradish peroxidase-conjugated mouse anti-rabbit IgG antibody (Jackson ImmunoResearch Laboratories, cat. no. 211-035-109) at 1:20,000 (20 ng/ml). All incubations were for 1 h at room temperature; immune complexes were detected by chemiluminescent (SuperSignal West Pico, Pierce) exposure to RX-B blue X-ray film (Daigger). Images were digitally acquired and analyzed as with RT-PCR described above.

Leptin RIA. Trunk blood was collected by cardiac puncture from rats killed by CO2 inhalation (as part of the BAT UCP1 study), and serum was harvested. Also included were archived serum samples of control and REM-sleep-deprived rats (n = 2 per time point) from a pilot study. All samples (total n = 7 per time point) were assayed in duplicate in a single run using the Linco rat leptin RIA kit; coefficient of variation between duplicates averaged 4.7%.

Data analyses. All data (means ± SE) are presented as percentages of baseline or cage controls, where those quantities were assigned a value of 100%. Parametric one-way ANOVA was used with Dunnett’s posttest, but if standard deviations were significant (Barlett test), nonparametric Kurskal-Wallis (KW) testing was done with Dunn’s posttest. Analyses were conducted using GraphPad InStat, version 3.06, and GraphPad Prism, version 4.2 (GraphPad Software).

RESULTS

REM-SD results in hyperphagia and loss of body weight. Patterns of change in food consumption and body weight are shown in Fig. 1 to emphasize two points. Because hyperphagia and weight loss are characteristics of chronic sleep deprivation in rats (5, 30, 31, 34, 44, 49), it was essential to demonstrate that they occurred as a simple confirmation of the utility of 10-cm platforms to enforce the paradigm. Second, if rats on the 15-cm platforms were appropriate controls, we would expect minimal changes in food consumption or body weight over the time course of the experiment.

Figure 1A shows that REM-sleep-deprived rats (n = 11) increased average food consumption to 220% of baseline by day 20 (39.5 ± 0.8 to 86.7 ± 19.9 g/day per kg0.67; KW = 105.95, P < 0.0001). Food consumption data were not corrected for washed-out crumbs, but the pattern and extent of hyperphagia agree with published results with the DOW (9, 26, 34) or platform (5, 31, 44, 49) methods. Within 1 day of recovery, food consumption returned to baseline levels. Body weights declined steadily [Fig. 1C; F(0.5,260) = 3.483, P < 0.0001] and rats lost an average of 11% of their initial mass by day 20. There was restoration of body weight during recovery so that after 4 days it was similar to baseline values. Comparable changes in food consumption and body weight were obtained for rats REM-sleep deprived for UCP1 gene expression studies (data not shown). For tank control rats (n = 5), there were no significant changes in food intake (Fig. 1B) or body weight (Fig. 1D) compared with baseline. We conclude that REM-SD was enforced with 10-cm platforms and that 15-cm platforms were suitable for tank controls.

REM-SD increases metabolic rate. VO2 was measured to determine the effects of chronic REM-SD on resting metabolic rate. Figure 2 (n = 11; ○) shows that it steadily increased, peaking at 166% of baseline by day 20 (1,137 ± 15 to 1,884 ± 89 ml O2/h per kg0.67; KW = 95.38, P < 0.0001). When rats were in recovery, it quickly fell to baseline levels within 48 h. Metabolism of tank control rats (n = 5; ○) showed no changes compared with baseline. The relation between increased metabolic rate and decreased body weight of REM-sleep-deprived rats is presented in Fig. 3, showing a strong correlation (Pearson r = 0.949, P < 0.0001).

There were no trends or significant differences in average RQ of REM-sleep-deprived rats (0.886 ± 0.01; data not

![Fig. 1. Average changes as %baseline for food consumption (g/day per kg0.67; A and B) and body weight (g; C and D) of rapid eye movement-sleep-deprived (REM-SD) rats (n = 11; A and C) and tank controls (n = 5; B and D) over 20 days. The vertical dotted line at day 20 on the abscissa marks the end of the REM-SD or tank control experiment; days 21–28 are the recovery period, when rats were returned to home cages. *P < 0.05, **P < 0.01, ***P < 0.001 compared with baseline (day 0).](http://ajpendo.physiology.org/)

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shown). To check system performance toward the end of the experiment, two rats were fasted to deplete glycogen stores; metabolism is then sustained by fat oxidation with RQ values declining to $0.7$. As expected, RQ averaged 0.720 and 0.710 after 24 and 48 h of fasting, respectively.

**Gene expression of UCP1 in BAT is upregulated during REM-SD.** With a major goal being to determine the effects of REM-SD on UCP1 gene expression in BAT, we first performed RT-PCR. Abundance of mRNA is shown in Fig. 4A of pooled UCP1 and 36B4 amplicons ($n = 5$ per time point). Cage control rats constitutively had low amounts of UCP1 mRNA, but it tripled by day 5. After 10 and 20 days, UCP1 mRNA rose more than 7- and 11-fold, respectively [$F_{(3,16)} = 316.98, P < 0.0001$]. Levels of 36B4 mRNA remained consistent; however, mRNA for GAPDH declined modestly, and for β-actin it increased between days 10 and 20 (data not shown). To eliminate any question of the increases in UCP1 mRNA being the result of a nonspecific response, the average ratios of UCP1 to each reference gene were taken, and this normalization was used as a more stringent evaluation of the index of change. Averaged data ($n = 5$ per time point) are summarized in Fig. 4B for the UCP1-to-36B4 ratio. The principal finding is that normalized UCP1 mRNA levels markedly increased with time of REM-SD ($F_{(3,16)} = 137.07, P < 0.0001$). Highly significant changes were also found when UCP1 was normalized to GAPDH and β-actin (data not shown). Fig. 4B, inset, shows the relation between resting metabolic rate and normalized UCP1 mRNA. The obvious feature of this correlation is that as resting metabolic rate increased there was a concurrent increase in UCP1 mRNA abundance. Next, to confirm that changes in mRNA are reflected in protein levels, we performed Western blot analysis. As Fig. 5 illustrates, rats had very high levels of the 32-kDa UCP1 protein in BAT from day 5 up to day 20. To highlight the specificity of the response (and that of the antibody) additional controls were included. That is, because immunoreactive UCP1 is strongly detected in BAT following cold adaptation (see DISCUSSION), BAT protein from rats acclimated to 4°C for 9 days was run as a positive control; also, because UCP1 expression occurs exclusively in BAT, liver protein served as a negative control. The results were unequivocal: there was abundant immunoreactive UCP1 in BAT of cold-acclimated rats, but none was found in liver.
Leptin levels rapidly decrease during REM-SD. Serum leptin levels were assayed (n = 7 per time point), and, as shown in Fig. 6, REM-SD led to a rapid decline in circulating hormone to ~35% of controls by day 5, and this low level was maintained to the end of the experiment on day 20 (KW = 10.220.05 compared with controls (n = 7) during 20 days of REM-SD (also see Ref. 11). The implication is that metabolic regulation remains intact, and some of its mechanistic features may be gleaned by examining hormonal profiles. It is well established that increased norepinephrine (NE) and thyroid hormones, during cold adaptation, for example, are responsible for up-regulating UCP1 (37; reviewed in Ref. 6). Chronically sleep-deprived rats present differently, however, in that circulating catecholamines are high (3, 11, 32) but they are profoundly hypothyroxicemic (3, 13, 31). It is noteworthy that a compensatory increase in plasma epinephrine occurs when NE release is blocked by guanethidine (32), suggesting that maintaining high plasma catecholamines is vital. Perhaps more importantly, hypothyroxia is offset by a substantial increase in activity of the type II deiodinase in BAT (2), and presumably, this adaptive response maintains appropriate levels of triiodothyronine to ensure persistent, elevated thermogenesis. Further understanding of the regulatory processes again comes from

DISCUSSION

In this first study of the platform method to enforce REM-SD of rats for as long as 20 days, there are two significant findings. One is that, by measuring $V_{\text{O}2}$, we establish that REM-SD significantly elevates resting metabolic rate. The second is that results from RT-PCR and Western blotting offer evidence of increased gene expression of UCP1 in BAT as a mechanism mediating the hypermetabolic state. Previous investigations of DOW rats involving calculations of energy expenditure (9, 26) and using doubly-labeled water (3) as indexes of metabolism showed that these parameters increased about twofold, which agrees with present results.

An issue arises in that the platform method activates the hypothalamic-pituitary-adrenal (HPA) axis (1, 30, 40–44, 49), with nonspecific stress as a potentially confounding factor. Interestingly, despite many sleep deprivation pathologies being severe, an equivalent HPA response does not occur with the DOW method (34). A confounding stress factor cannot be ignored, but it is intriguing that, HPA stimulation or not, the methods have similar outcomes: rats are hyperphagic and lose body weight (5, 30, 31, 34, 40, 44, 49), they have increased energy expenditure (3, 9, 26) or elevated resting $V_{\text{O}2}$ (this study), and they are hypothyroxicemic (3, 13, 31) with low leptin (11, 31; this study).

Our results are consistent with current understanding of thermogenesis. UCP1 is a unique 32-kDa inner mitochondrial membrane protein that allows proton leakage and short-circuits cellular respiration. As a consequence, the thermodynamic energy of the proton-motive force is dissipated as heat rather than being conserved by ATP formation (6). Accordingly, conditions that mandate augmented thermogenesis will increase UCP1 gene expression in BAT, as we demonstrate here for REM-SD. In control rats, constitutive levels of UCP1 mRNA were low, which is expected for normothermic, non-stressed rodents (6). As time of REM-SD lengthened, however, mRNA abundance rose with corresponding increases in metabolic rate, but an unexpected outcome was the disproportionately enormous change in UCP1 protein compared with the approximately threefold rise in its mRNA at day 5. Early on, even as gene transcription increases, synthesis of the protein occurs much more briskly, and these events probably cause metabolism and thermogenesis to rapidly ramp upward.

Other stress modalities can affect UCP1 expression, and two pertinent examples are given. When small mammals are challenged by cold exposure, UCP1 is vigorously induced (6). Recalling that REM-SD enforcement is by water contact, acute cold stress may have occurred despite warm water flowing through the tanks. This scenario is unlikely, however, because by day 10, when both metabolic rate and UCP1 gene expression were already significantly elevated, rats remained dry. In fact, they did not experience frequent immersions until the last quarter of the experiment. The second example is immobilization, a potent psychological stressor. When applied acutely, UCP1 mRNA does not change (36) but repeated and chronic episodes improve cold tolerance (25) with increased UCP1 mRNA and protein (18). It seems, then, that chronic REM-SD causes rats to respond as if they were adapting to cold but without a temperature effect being present. In addition, the similarities of REM-SD to these examples denote that it probably has both physical and psychological attributes as a stressor.

Hypermetabolism during REM-SD is not a process running out of control. If it were, $V_{\text{O}2}$ could not have declined so rapidly to baseline levels (<48 h) when rats were in recovery (also see Ref. 11). The implication is that metabolic regulation remains intact, and some of its mechanistic features may be gleaned by examining hormonal profiles. It is well established that increased norepinephrine (NE) and thyroid hormones, during cold adaptation, for example, are responsible for up-regulating UCP1 (37; reviewed in Ref. 6). Chronically sleep-deprived rats present differently, however, in that circulating catecholamines are high (3, 11, 32) but they are profoundly hypothyroxicemic (3, 13, 31). It is noteworthy that a compensatory increase in plasma epinephrine occurs when NE release is blocked by guanethidine (32), suggesting that maintaining high plasma catecholamines is vital. Perhaps more importantly, hypothyroxia is offset by a substantial increase in activity of the type II deiodinase in BAT (2), and presumably, this adaptive response maintains appropriate levels of triiodothyronine to ensure persistent, elevated thermogenesis. Further understanding of the regulatory processes again comes from

![Fig. 5. Western blot showing UCP1 protein in BAT of REM-SD rats. Lane 1 (C), cage control; lane 2 (d5), day 5 of REM-SD; lane 3 (d20), day 20 of REM-SD; lane 4 (L), liver (negative control); lane 6 (CA), BAT of cold-acclimated rat (positive control); arrow, 32 kDa; 2-min film exposure (UCP1 protein was detectable in cage controls after a 10-min film exposure).](http://ajpendo.physiology.org/)

![Fig. 6. Serum leptin (ng/ml) levels were assayed by RIA and are shown as % changes compared with controls (n = 7) during 20 days of REM-SD (n = 7 per time point). *P < 0.05 compared with controls.](http://ajpendo.physiology.org/)
cold-adapted mammals, where changes in sympathetic activity promptly stimulate heat production during acute cold exposure or cause its inhibition with warmth (48). Hence, elevated plasma catecholamines found in sleep deprivation (3, 11, 32) resemble those of cold-adapted rats (6) and hint that the rapidly triggered metabolic “on” and “off” switch may be sympathecically regulated. Substantiating this view is that, following relief from sleep deprivation, VO₂ (this study) and energy expenditure (11) expeditiously returned to baseline levels and that most anabolic hormones are suppressed (10).

We expected that RQ would trend toward 0.7, an indicator of fat oxidation, because body weight declines with depletion of fat depots (9, 26, 30) and there is rapid mobilization of tissue glycogen (24a). An operational problem with the calorimetry system was ruled out because fasted rats had values of ~0.7. With average RQ being ~0.89, rats were oxidizing a mixture of fuels, but mostly carbohydrate. To explain our results, a closer examination of the hyperphagic response may be instructive. Clearly, hyperphagia was insufficient to sustain increased metabolism, but, as Suchecki et al. (40) propose, it may be that REM-SD brings about a need to augment gluconeogenesis to satisfy growing demands for fuel. Their argument is appealing because, if fat were mobilized, not for oxidation, but rather for gluconeogenesis with attendant carbohydrate oxidation, RQ values would probably be much above 0.8. Another consideration is the caloric content of the chow fed to our rats. About 58% of its metabolizable energy is carbohydrate. Hence, increased carbohydrate ingestion, entirely as a function of hyperphagia, may account for the high RQ values.

As shown in the present study and elsewhere (10, 21), leptin levels declined within a few days of sleep deprivation. Suppression of leptin might be viewed as an appropriate response to obviate its anorectic effect, but the changes occurred before there were any significant alterations in food consumption, and even though leptin secretion is correlated to adiposity (16), the pace of fat depletion cannot account for the low levels found early in the regimen. Instead, it is more likely that increased sympathetic activity to white adipose tissue (19), which down-regulates the leptin gene (33), allowed increased food intake. Finally, it is important to note that the sleep deprivation effects discussed so far are not unique to rodent models. In studies of human sleep deprivation or sleep disorders (e.g., apnea, insomnia), the pathophysiological consequences have many similarities to those of rats. For instance, there is heightened sympathetic tone and altered carbohydrate metabolism (38); food intake increases, coincident with elevated ghrelin (an orexinogen) and decreased leptin in the circulation (39); immune functions decline (8); and VO₂ is elevated (4). Sleep loss can also exacerbate morbidity in critically ill patients (17), contribute to health problems of persons of low socioeconomic status (45), and may even lead to premature mortality (29).

In summary and conclusion, our results show that 20 days of REM-SD brings about progressive increases in resting metabolic rate and hyperphagia while body weight is lost. We provide evidence that one of the mechanisms mediating hypermetabolism is a pronounced increase in thermogenic capacity, as illustrated by robust gene expression of UCP1 in BAT. Finally, we show that leptin decreases markedly, consistent with elevated sympathetic activity and the need to maintain hyperphagia. Apparently, the increased metabolic rate is a necessary (although curious) outcome of REM-SD that must be strongly defended, but its physiological advantage, if any, awaits clarification.

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