Energy Expenditure is Affected by Rate of Accumulation of Sleep Deficit in Rats

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Study Objectives: Short sleep is a putative risk factor for obesity. However, prolonged total sleep deprivation (TSD) leads to negative energy balance and weight loss in rodents, whereas sleep-restricted humans tend to gain weight. We hypothesized that energy expenditure ($\dot{V}O_2$) is influenced by the rate of accumulation of sleep deficit in rats.

Design and Intervention: Six Sprague-Dawley rats underwent chronic sleep-restriction (CSR, 6-h sleep opportunity at ZT0-6 for 10 days) and stimulus-control protocols (CON, 12-h sleep opportunity for 10 days, matched number of stimuli) in a balanced cross-over design. Four additional rats underwent TSD (4 days). Sleep was manipulated using a motor-driven walking wheel.

Measurements and Results: Electroencephalography, electromyography, and body temperature were measured by telemetry, and $\dot{V}O_2$ by respirometry. Total sleep deficits of 55.1 ± 6.4 hours, 31.8 ± 6.8 hours, and 38.2 ± 2.3 hours accumulated over the CSR, CON, and TSD protocols, respectively. Responses to TSD confirmed previous reports of elevated $\dot{V}O_2$ and body temperature. These responses were attenuated in CSR, despite a greater cumulative sleep deficit. Rate of rise of $\dot{V}O_2$ was strongly correlated with rate of accumulation of sleep deficit, above a threshold deficit of 3.6 h·day$^{-1}$.

Conclusion: The change in $\dot{V}O_2$ is affected by rate of accumulation of sleep deficit and not the total sleep loss accrued. Negative energy balance, observed during TSD, is strongly attenuated when brief daily sleep opportunities are available to rats (CSR), despite greater accumulated sleep deficit.

Keywords: Body temperature, chronic sleep restriction, core sleep, energy balance, energy metabolism, oxygen uptake, sleep debt, total sleep deprivation

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represents a reduction below physiologic sleep requirement in rats. The results of the present study provide the first tentative estimate of core sleep time in rats.

Here we tested the hypothesis that responses in whole-body energy metabolism (measured indirectly as rate of oxygen uptake, $O_2$) and body temperature (Tb) vary with the rate of accumulation of sleep deficit by comparing these responses in rats under conditions of prolonged TSD and CSR.

METHODS

Subjects

All procedures were performed in accordance with the guidelines established by the Canadian Council on Animal Care and were approved by the animal care committee at the University of Toronto.

Ten male Sprague-Dawley rats were used: 6 in the CSR and control (CSR/CON) group, and 4 in the TSD group. Mean weight was 406 ± 114 g (mean ± SD, n = 10) at the time of surgery. For the CSR/CON group, each rat was studied in both conditions in a counterbalanced design with a minimum of 7 days recovery in the rat’s home cage between studies. Except during recordings, all rats were individually housed in standard plastic cages (home cage), with standard rat chow and water available ad libitum. The colony and experiment rooms were maintained on a 12-hour:12-hour light-dark cycle at 23°C ± 1°C.

Surgical Procedures

Animals were anesthetized using isoflurane (1%-2% in air/ O$_2$ mix) and instrumented with a multichannel radio transmitter (model TL10M3-F50-EET, Data Science International, St. Paul, MN). The transmitter enabled continuous recording of Tb (°C), electroencephalography (EEG), and electromyography (EMG). The transmitter, containing the temperature sensor, was implanted in the peritoneum and bipolar fronto-parietal EEG electrodes and bipolar EMG electrodes were tunneled subcutaneously to the head and neck, respectively. Right frontal (coordinates, expressed relative to bregma, AP +2 mm, ML 2 mm) and right parietal (AP -4 mm, ML 2 mm) EEG electrodes were affixed to the skull using miniature stainless-steel screws (size 080) and were sealed using acrylic dental cement (Plastics One, Roanoke, VA). The EMG was recorded using multistrand stainless-steel electrode wires embedded into the right dorsal cervical muscle. All signals were referenced to an electrode implanted over the left parietal cortex (AP -2 mm, ML 2 mm).

Rats were administered analgesic (buprenorphine, 0.015 mg·kg$^{-1}$·sc) during surgery and twice daily for 2 subsequent days. They were allowed a minimum of 7 days to recover before experiments began.

Experimental Protocol

After recovery from surgery, animals were acclimatized to a walking-wheel respirometer (described below) for several hours on each of 3 days before being housed in the apparatus for the remainder of the CSR, CON, or TSD protocol. After a full day of further habituation to the wheel (without rotation), recordings began with a resting baseline day, during which the wheel was immobilized and the rats were allowed ad libitum sleep (Figure 1). Body weight and the masses of food and water consumed were recorded daily.

A motor-controlled walking wheel (diameter 35 cm, width 16 cm, 3.5 rpm) enclosed within a Plexiglas respirometry chamber was used to prevent sleep during scheduled times of the day. Specifically, during the 10-day CSR protocol, the rats were allowed a 6-hour sleep opportunity each day beginning at the time of lights on (Zeitgeber time, ZT0-6), and they were kept awake at all other times (daily sleep-deprivation interval; ZT6-24). During the sleep opportunity, the wheel was immobilized. During the sleep deprivation interval, the wheel was activated intermittently (8 s on, 8 s off) to enforce wakefulness while allowing brief resting periods to minimize total daily exercise and facilitate the implementation of a control protocol. Computer recordings began at ZT6 so that the daily sleep opportunities are treated in Results as occurring at the end of each 24-hour day.

In the CON protocol, the same number and duration of wheel rotations was scheduled into a total of 12 hours per day by using a wheel-rotation cycle of 8 seconds on and 2.7 seconds off. The CON-stimulus schedule was arranged to accommodate the circadian rhythm of the animals such that they had 75% of their sleep opportunity distributed in the light period and 25%
in the dark period. CON stimuli were presented in 3 × 1-hour periods (each separated by 3-h sleep opportunities) during daytime and 3 × 3-hour periods (each separated by 1-h sleep opportunities) during the night. Following the 10 days of CSR or CON stimuli, 3 recovery days were recorded, when the wheel was immobilized, allowing ad libitum sleep (Figure 1).

In the TSD protocol, habituation and resting baseline days were followed by an active baseline day in which the wheel rotated for 1 hour in every 6-hour period. This allowed a comparison of data from rats walking on the wheel before and during TSD. The wheel rotated intermittently (8 s on, 8 s off) for the full 24 hours during the 4 TSD days. The animals were then allowed ad libitum sleep during 3 recovery days with the wheel immobilized (Figure 1). Data from recovery were compared with corresponding data from the resting baseline day. Comparison of wheel-walking periods during the active baseline day with inactive wakefulness during corresponding periods on the resting baseline day enabled evaluation of the metabolic cost of wheel walking.

Sleep Scoring
Sleep-wake states were scored by off-line automated analysis, as described in detail elsewhere. Briefly, a radio receiver (PhysioTel RPC-1, Data Sciences International) located under the wheel-respirometer detected EEG and EMG signals from the radiotransmitter, which were then decoded and amplified using an analog adapter (Model DL-10, Data Sciences International). Analog signals were then passed to a Pentium 4 PC, sampling at 400 Hz, using custom LabVIEW software (National Instruments Corp., Austin, TX) for signal conditioning and data acquisition and Maclab/8 hardware (ADInstruments, Inc., Grand Junction, CO) for visual display backup.

Behavioral states of wakefulness (WAKE), REM sleep, and non-REM sleep (NREM) were recorded in 5-second epochs throughout the study. Visual scoring was performed for at least 1 hour on each day of the study to validate the computer-based scoring system. Real-time video monitors were used for observation of behavior during visual validation of sleep-state recordings. Epochs were scored as WAKE when the EEG contained low voltage and high frequency, with high or variable EMG voltage, or when the animal was observed on video to be active. Epochs were scored as NREM if the EEG contained high-amplitude and low-frequency waves with low-to-moderate-amplitude EMG and as REM if the EEG was of low amplitude with prominent theta waves (6-9 Hz) together with very-low-amplitude EMG.

Mass-specific Metabolic Rate and Tb
Metabolic rate was estimated as the rate of oxygen consumption ($\dot{V}O_2$, mL·min$^{-1}$·kg$^{-1}$ standard temperature and pressure, dry [STPD]) measured by open-circuit respirometry, as described in detail elsewhere. Briefly, in each 5-second epoch, a calibrated O$_2$ analyzer (Servomex model 572, Novatech Inc., UK) measured the fractional concentration of O$_2$ in the dry incurrent (FiO$_2$) and excurrent (FeO$_2$) air. $\dot{V}O_2$ was calculated as:

$$\dot{V}i (FiO_2 – FeO_2) / 1 – (1 – RER) FeO_2$$

where $\dot{V}i$ is the chamber incurrent dry airflow rate (mL·min$^{-1}$ STPD) and RER (respiratory exchange ratio) is assumed to be 0.85. Animals were studied in pairs, each within its own wheel respirometer. The source of the air sampled by the O$_2$ analyzer was switched as follows: on a continuously repeating 34-minute cycle using timed solenoid-actuated valves: wheel-respirometer 1 (15 min), wheel-respirometer 2 (15 min), and 4.7 min). The first 3 minutes of data were ignored after each change of sampling source to purge the dead space in the system.

Air in the wheel respirometer was fully mixed using 2 fans to ensure first-order washout kinetics (gas washout time constant, 9.7 min). This allowed a simple correction to be applied to FeO$_2$ to improve the temporal resolution of $\dot{V}O_2$ measurements:

$$\Delta Fe'O_2 = \Delta FeO_2 / Z,$$

where $\Delta FeO_2$ is the change in excurrent fractional O$_2$ concentration over time interval $\Delta t$ (which exceeded 1 min in this study) and:

$$Z = 1 – exp [-\dot{V}i \cdot \Delta t / V'_c].$$

where $V'_c$ is the effective chamber volume (see for details) in the wheel respirometers. $\dot{V}O_2$ and Tb were recorded for established WAKE, NREM, and REM bouts of 1-minute or longer duration. Except where noted, only $\dot{V}O_2$ and Tb data from ZT0-6 (i.e., the time of the daily sleep opportunity in the CSR protocol) are reported here. This was done to avoid the confounding influence of wheel-induced exercise on the effects of CSR. Hence, in CSR and CON protocols, comparisons were made against baseline using only those data collected during times when the wheel was immobilized. Since this confound was unavoidable in the TSD protocol, the data on TSD days were compared with those obtained during a stimulus interval on an additional active baseline day (see Figure 1). Data were analyzed in ZT0 to ZT6 in the TSD group to enable circadian time-matched comparison with CSR group data.

Statistical Analysis
For the CSR/CON study, all normally distributed homoscedastic data were entered into 1-way repeated measures analysis of variance (ANOVA), and the Holm-Sidak multiple comparisons posthoc test was used to compare against the appropriate control or baseline group. In cases in which data were not normally distributed, a Friedman repeated-measures ANOVA on ranks was used, followed by Tukey posthoc comparisons when appropriate, or a posthoc Dunnett test was used for multiple comparisons against baseline. In comparisons of CSR versus TSD, a 1-way ANOVA was used with the Holm-Sidak multiple-comparisons posthoc test. Unless indicated otherwise, values are expressed as means ± SEM. The level of statistical significance was set at P ≤ 0.05. All statistical tests were performed using Sigmastat software (version 3.5, Systat Software Inc., Point Richmond, CA).

RESULTS

Computer Scoring Algorithm
Overall concordance between visual scoring and the computer autoscore (total of 78,007 epochs visually analyzed in 10

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eliminated more than 90% of NREM sleep and virtually all REM sleep (P < 0.001). The other 2 CSR/CON rats made persistent attempts to sleep in the wheel, dozing during many of the short (8-s) intervals between wheel rotations. These 2 animals registered a significant number of NREM epochs, as scored by computer, but very few REM epochs. One rat obtained from 34% (CSR day 2) to 90% (CSR day 9) of its ZT6 to ZT24 base-line NREM time, and the other obtained 20% (CSR day 1) to 63% (CSR day 9). However, it was noted in both cases that this NREM sleep was highly fragmented, with the rats awakening within 10 second in almost every bout. In all other respects, there were no significant differences between the 2 subgroups of rats, so data were pooled for further analyses.

**Tb and Metabolic Rate**

**CSR condition**

**Wake:** Tb was significantly higher than baseline on CSR day 3 (P = 0.04, Figure 3) but was not significantly different from baseline at any other time, including the 3 recovery days (P = 0.670, Figure 3). Baseline $\dot{V}O_2$, recorded during spontaneous wakefulness during ZT0 to ZT6, was 16.9 ± 1.0 mL·min$^{-1}$·kg$^{-1}$. The $\dot{V}O_2$ of awake animals tended to increase progressively across CSR days, but this trend did not attain statistical significance on any CSR day (P = 0.548, Figure 4A).

**Sleep:** In the CSR study, the $\dot{V}O_2$ recorded during NREM and REM sleep in ZT0 to ZT6 of the resting baseline day was 14.5 ± 0.9 and 14.1 ± 0.9 mL·min$^{-1}$·kg$^{-1}$, respectively. These values were not different from each other, but both were significantly lower than the corresponding WAKE value (P = 0.005). Sleeping $\dot{V}O_2$ (during both NREM and REM sleep) did not differ significantly from baseline on any of the CSR and recovery days (Figure 4B). During recovery, REM $\dot{V}O_2$ was significantly lower than WAKE $\dot{V}O_2$ (P = 0.009), animals) was 87.4% ± 1.5% (87.4% ± 2.4% of wake epochs, 93.4% ± 0.9% of NREM epochs, and 80.4% ± 3.7% of REM epochs).

**Sleep Deficit**

Sleep deficit was calculated by subtraction of the total daily NREM and REM sleep time during baseline from the respective times measured during the CSR, CON, and TSD protocols. A significant sleep deficit was found to accumulate in all 3 experimental protocols; CSR, CON, and TSD (Figure 2A; TSD, 38.2 ± 2.3 h; CSR, 55.1 ± 6.4 h; CON, 31.8 ± 6.8 h, P < 0.001). The rate of accumulation of sleep deficit differed significantly among groups (Figure 2B; TSD, 9.6 ± 2.5 h·d$^{-1}$; CSR, 5.5 ± 0.7 h·d$^{-1}$; CON, 3.2 ± 0.6 h·d$^{-1}$, P < 0.001). Hence, the CON group was treated here as a more moderate CSR group. A detailed description of sleep and sleep homeostasis in these animals is to be reported elsewhere.

During the daily wheel-walking intervals, the stimulus was highly effective in 4 of the 6 rats used in CSR and CON protocols and all 4 rats used in the TSD protocol. Wheel rotation eliminated more than 90% of NREM sleep and virtually all REM sleep (P < 0.001). The other 2 CSR/CON rats made persistent attempts to sleep in the wheel, dozing during many of the short (8-s) intervals between wheel rotations. These 2 animals registered a significant number of NREM epochs, as scored by computer, but very few REM epochs. One rat obtained from 34% (CSR day 2) to 90% (CSR day 9) of its ZT6 to ZT24 base-line NREM time, and the other obtained 20% (CSR day 1) to 63% (CSR day 9). However, it was noted in both cases that this NREM sleep was highly fragmented, with the rats awakening within 10 second in almost every bout. In all other respects, there were no significant differences between the 2 subgroups of rats, so data were pooled for further analyses.

**Figure 2**—Sleep deficit, expressed as mean (+ SEM) change from resting baseline sleep time (sum of NREM and REM sleep). A. Total sleep deficit accumulated (h) during 4 days of total sleep deprivation (TSD, n = 4; black), 10 days of chronic sleep restriction (CSR, n = 6; white), and 10 days of stimulus control (CON, n = 6; grey). B. Mean (+ SEM) rate of accumulation of sleep deficit (h·day$^{-1}$). Asterisks denote a statistically significant difference (*P < 0.001; **P < 0.0001).

**Figure 3**—Mean (± SEM) body temperature of awake rats during ZT0-6 in resting baseline (BL), during sleep interventions (chronic sleep restriction, CSR, n = 6; stimulus control, CON, n = 6; total sleep deprivation, TSD, n = 4) and during recovery (REC). White circle indicates resting baseline value for TSD, dark circle at time 0 indicates active BL value for TSD (see text for details). *A statistically significant difference (P < 0.05) from corresponding BL value.
whereas NREM $\dot{V}O_2$ was statistically similar to both WAKE and REM $\dot{V}O_2$ (Figure 4B).

**CON condition**

**Wake:** Tb was higher than baseline on CON days 2 and 3 ($P = 0.009$) but was not significantly different from baseline at any other time, including the 3 recovery days ($P = 0.340$, Figure 3). Baseline $\dot{V}O_2$ was $17.5 \pm 1.3$ mL·min$^{-1}$·kg$^{-1}$ before CON, which was statistically similar to the corresponding value in the same rats before CSR. The $\dot{V}O_2$ did not differ significantly from baseline throughout the CON study in awake rats ($P = 0.340$; Figure 4A).

**Sleep:** The $\dot{V}O_2$ during baseline in the CON study was $13.9 \pm 0.7$ and $14.0 \pm 0.9$ mL·min$^{-1}$·kg$^{-1}$ in NREM and REM sleep, respectively. This was not statistically significantly different from the corresponding resting baseline values in the CSR and TSD studies ($P = 0.088$). Sleeping $\dot{V}O_2$ (during both NREM and REM sleep) did not differ significantly from baseline on any of the CON or recovery days.

**TSD condition**

**Wake:** Tb increased by 0.8°C to 1.0°C above active baseline on days 2 to 4 of TSD ($P < 0.001$), remained above baseline on the recovery day 1 ($P = 0.011$), and returned to resting baseline level on recovery days 2 and 3 (Figure 3). Tb was statistically similar on active baseline and resting baseline days. $\dot{V}O_2$ was significantly elevated by wheel walking; the $\dot{V}O_2$ was raised to $24.0 \pm 1.7$ mL·min$^{-1}$·kg$^{-1}$ STPD on the active baseline day, compared with $18.3 \pm 1.4$ mL·min$^{-1}$·kg$^{-1}$ STPD on the resting baseline day (i.e., a 31% increase; $P < 0.001$). Moreover, the awake $\dot{V}O_2$ increased gradually over the 4 TSD days and was statistically significantly higher than the active baseline value on TSD days 2 to 4 ($P = 0.008$, Figure 4A). Recovery was rapid, with the awake $\dot{V}O_2$ returning to resting baseline levels within recovery day 1 ($P = 0.113$).

**Sleep:** A 2-way repeated-measures ANOVA determined that there were no differences in metabolic rate among WAKE, NREM sleep, and REM sleep ($P = 0.086$), nor between resting baseline and recovery days 1 to 3 ($P = 0.082$). However, these marginal $P$ values are inconclusive, given the low sample size ($n = 4$) and low statistical power of this test in both factors State and Day (power = 0.371 and 0.380, respectively). Sleep was too sporadic and bouts too short to permit measurement and analysis of sleep metabolic rate during TSD days 1 to 4.

**Food Consumption, Water Intake, and Body Weight**

**CSR condition**

Daily food consumption remained at baseline levels (32.2 ± 2.7 g·day$^{-1}$) throughout the CSR and REC periods, except CSR day 2, when it was significantly reduced (by 18%, $P = 0.001$, Figure 5A). Water intake fell by approximately 21% below the resting baseline level on CSR day 1 ($P = 0.004$) and remained near that level during the remainder of CSR but returned to baseline levels (48.7 ± 4.0 g·day$^{-1}$) during recovery (Figure 5B). Body weight was significantly below baseline (by 3.6% ± 0.3%) on CSR days 2 to 10 ($P < 0.001$) but returned to baseline on recovery days 1 to 3 (Figure 5C).

**CON condition**

Daily food consumption remained at baseline levels (35.8 ± 2.1 g·day$^{-1}$) throughout the CON and recovery periods (Figure 5A). Similarly, water intake was equivalent to that of the resting baseline level on all days of the CON study (Figure 5B). Body weight decreased significantly on CON day 1 and remained suppressed (by 4.6% ± 0.2%) throughout the CON protocol ($P < 0.001$). Body weight returned to resting levels on recovery days 1 to 3 (Figure 5C).

**TSD condition**

Food consumption decreased (by 17.5%) on TSD day 1 ($P = 0.025$) but gradually returned to baseline level over the remainder of the experiment and remained at that level during recovery days 1 to 3 (Figure 5A). Water intake increased...
progressively during TSD to 39% higher than baseline on TSD day 4 (P = 0.041), returning to baseline levels on recovery days 1 to 3 (Figure 5B). Body weight decreased progressively to 83% of baseline values by TSD day 4, but the statistical significance of this was equivocal (P = 0.070, power = 0.408). Body weight returned to baseline values during recovery days 1 to 3 (Figure 5C).

**Sleep Deficit and Metabolic Rate**

Metabolic rate was significantly elevated in awake rats when sleep deficit exceeded 20 hours in the TSD group (P = 0.008) but not in the CSR or CON conditions (Figure 6).

We applied a simple additive 2-step model\(^{12,15}\) to gain further insight into the relationships between metabolic rate and rate of accumulation of sleep deficit. The model is expressed as:

\[
\Delta V_O^2 = \alpha + \beta \cdot \Delta S,
\]

where \(\Delta V_O^2\) is the change in metabolic rate from baseline, \(\alpha\) is the first-day effect (change in metabolic rate on day 1), \(\beta\) is the daily rate of change of metabolic rate over the subsequent days, and \(\Delta S\) is the daily rate of accumulation of sleep deficit. The data were derived for each rat individually and are illustrated in Figure 7. Figure 7A shows that the change in metabolic rate occurring on the first day (\(\alpha\)) was not correlated with the sleep deficit accumulated on day 1.

In contrast, Figure 7B demonstrates a highly significant correlation between rate of change of metabolic rate following day 1 (\(\beta\)) and daily rate of accumulation of sleep deficit. There was no correlation between \(\alpha\) and \(\beta\) (P = 0.122), indicating that, for each individual rat, the progressive response (\(\beta\)) was not related to the magnitude of the initial response (\(\alpha\)). The regression line in Figure 7B was drawn through the individual animal data and explains 48% of the variance; a regression (not shown) through the group average data was found to explain 99% of the variance. The slope of the regression line (\(\beta\)) indicates that, following day 1, for each hour of daily accumulated sleep deficit, metabolic rate increased by 0.17 mL \(O_2\)·min\(^{-1}\)·kg\(^{-1}\) (i.e., approximately 1% of baseline \(V_O^2\)). \(\beta\) was negative in the CON study, representing a gradual return to baseline following the initial elevation of \(V_O^2\) on day 1 (see Figures 4A and 7B), whereas \(\beta\) was positive in the CSR study, indicating a trend for a progressive increase in metabolic rate over days (Figures 4B and 7B). Paired t tests between \(\beta\) values for CSR and CON indicated a significant difference between conditions (P = 0.003).

**DISCUSSION**

The present study found that the effects of sleep loss on energy metabolism and Tb are influenced by the rate of accumulation of sleep deficit in rats. During prolonged TSD, sleep deficit accumulated at a rapid rate and was associated with a progressive hypermetabolism, confirming the results of previous studies.\(^7,8,9,19,35\) However, accumulation of a similar (CON) or greater (CSR) total sleep deficit at slower rates did not elicit a significant elevation of metabolic rate, suggesting that daily sleep opportunities can ameliorate the metabolic effects of sleep deficit.

Using the DOW technique, Rechtschaffen and colleagues found that TSD is lethal after approximately 3 weeks.\(^8,19\) Those seminal studies showed that unrelenting TSD elicits physiologic responses, which include increased energy expenditure (hypermetabolism), weight loss, hyperphagia, increased Tb, adrenosympathetic activation, tachycardia, and compromised immune system function (lesions in skin and gut and reduced immunoreactivity to infection). A modified technique recently found similar results,\(^20\) and long-term (20 days) TSD induced using the platform technique, which causes a strong suppression of REM and partial suppression of NREM sleep,\(^21,22\) was
also shown to induce similar physiologic responses in rats. Moreover, in a recent study in which the DOW technique was used to induce chronic severe fragmentation of sleep with only moderate sleep loss (as estimated from an analysis of a subset of the animals), a similar set of responses occurred.

Both the DOW and platform techniques use water to maintain arousal, and rats become wet under those conditions. It is possible that heat loss is augmented in wet animals due to evaporative cooling, leading in turn to a compensatory increase in metabolic thermogenesis. The dry walking-wheel technique was utilized in the present study to avoid this confound. We found that TSD led to hypermetabolism in dry rats, thus supporting the original conclusion of Bergmann et al that the sleep deprivation–induced hypermetabolism did not result from water exposure.

The present data also argue against the hypothesis that repetitive arousal, a potential confound in the DOW technique, is a major contributing factor in the development of TSD-induced hypermetabolism. Arousal from sleep has been shown to activate an autonomic stress response and could conceivably have contributed to the observed sleep-deprivation effects, including increased energy expenditure, in the TSD rats. Such a mechanism has received indirect support from studies of healthy human subjects, who were found to develop an elevated metabolic rate during an experimental sleep-fragmentation protocol, and from patients with obstructive sleep apnea, who exhibited a significant elevation of metabolic rate that was reversed following stabilization of sleep by continuous positive airway pressure treatment (although this relationship disappears when corrected for lean body mass). The walking-wheel apparatus was deployed in a continuous schedule (i.e., not contingent on the behavioral state of the animals) specifically to address this problem. Although all TSD rats did briefly fall asleep at times during the 4-day protocol, the method was found to prevent sleep onset for the majority of the time, and the rats in this study certainly experienced far fewer arousals than did those in the DOW apparatus, which was designed to awaken the animals after every spontaneous sleep onset. Despite this, hypermetabolism occurred in the walking-wheel apparatus to an extent that was similar to that in the first quarter (approximately 5 days) of the DOW studies. Moreover, we found that 2 rats in the CSR group obtained a significant total quantity of NREM sleep in the wheel, but this sleep was highly fragmented, and the responses in metabolic rate, Tb, and body weight of these rats did not differ notably from that of the other rats, again suggesting that the repeated arousals had little impact on responses and that brief sleep bouts (microsleeps) may confer little functional benefit.

Finally, it is unlikely that the exercise levels per se were unduly stressful. In this study, the rats walked a total distance of 2.25 km per day, which is not more than that of published reports of over 3-km distances that are run spontaneously by Sprague-Dawley rats with voluntary access to running wheels. Control data in the TSD protocol indicated that wheel walking elevated metabolic rate by only approximately 31% above resting values, which represents a low intensity of exercise. Voluntary exercise in humans and animals has only small and inconsistent effects on sleep architecture, even after much higher levels of exercise intensity than those induced by the walking wheel in this study, and has not been shown to strongly suppress sleep.

Hyperphagia has been observed in rats subjected to prolonged TSD using the DOW and platform methods. However, it is a response that develops relatively slowly, reaching significance only after approximately a week or more of sleep deprivation in the rat. A recent study reports a more rapid onset of hyperphagia in rats deprived of sleep using a modified DOW approach. In the present study, food consumption decreased on the first day of walking-wheel TSD (Figure 5A), an initial response also reported for rats subjected to the DOW and platform techniques. Thus, the present results are consistent with most previous data for 4-day TSD. In contrast, there was no indication of a progressive increase in food consumption over the 10 days of the CSR and CON protocols, in which sleep deficit matched or exceeded that of the TSD condition (Figure 5A). This implies that the hyperphagic response may be attenuated by short daily sleep episodes. However, because of the development of a sleep deficit in the CON protocol, it is not possible to exclude the possibility that the wheel stimulus itself may have influenced food intake. In long-term TSD, hyperphagia has been linked to decreases in circulating leptin levels, orexin activation with a subsequent increase in the expression of neuropeptide Y mRNA in the hypothalamic arcuate nucleus in rats, and suppression of the nocturnal rise in leptin levels in sleep-deprived human subjects. It would be of interest to know which, if any, of these mechanisms is involved in the apparent blunting of the response during CSR. Such a mechanism may be of clinical relevance given the reported correlations among short sleep, appetite, and obesity in humans (see 4 and 11 for critical reviews of this controversial question).

Body mass was found to decrease (Figure 5C) in rats during all 3 protocols (TSD, CSR, and CON). In TSD, the reduction in mass was progressive over the 4 days, with rats losing an average of 4% of their baseline weight per day. In contrast, the rats lost weight for only the first 2 days of the CSR and CON protocols, remaining steady at the lower level (approximately 95% of baseline) for the remaining 8 days. In all 3 protocols, restoration of body mass was rapid during recovery. The progressive fall in body weight during TSD was not accompanied by sustained reductions in food and water consumption. Assuming that efficiency of ingestion and assimilation were unchanged, this suggests that the TSD rats were in a state of negative energy balance. In contrast, during CSR and CON, the animals appeared to maintain neutral energy balance following the first 2 days of sleep restriction, suggesting that the effect of acute profound sleep debt (TSD) on energy balance is attenuated by daily sleep opportunities under conditions of chronic sleep deficit.

The data shown in Figure 4 have been replotted in Figure 6 to emphasize the point that the effects of sleep deprivation on energy expenditure are attenuated when daily sleep opportunities are allowed. For the purposes of illustration, the metabolic-rate data for TSD have been corrected for the effect of activity by subtraction of the baseline elevation of O₂ attributable to wheel walking (active baseline – resting baseline difference shown in Figure 4A) from all TSD data (this assumes that the mechanical efficiency of wheel walking remains unchanged by sleep debt). It is clear that hypermetabolism was
not a simple function of sleep deficit, since energy metabolism was significantly elevated above baseline in TSD when sleep loss exceeded 20 hours, whereas, in CSR and CON, energy metabolism increased only slightly, even at much higher accumulated levels of sleep deficit. Instead, our analysis (Figure 7) suggests that, after day 1, the change in $\dot{V}O_2$ is related to the rate of accumulation of sleep deficit and not to the absolute deficit accrued.

It is clear from Figure 7 that there was substantial variability in both the first day ($\alpha$) and subsequent progressive ($\beta$) responses between rats, supporting previous reports of considerable intersubject variability in sleep-wake patterns in rodents$^{38}$ and neurobehavioral responses to sleep debt in humans.$^{39}$ Figure 7A shows that the change in metabolic rate occurring on the first day was not correlated with the sleep deficit accumulated on day 1. Alpha was not statistically different between groups and, hence, was not related to absolute sleep deficit, rate of accumulation of sleep deficit, or extension of wakefulness. This suggests that the “first-day effect” may be a nonspecific consequence of experimental conditions, such as anxiety induced by the novelty or behavioral constraints imposed on the animals by activation of wheel rotation.

In contrast, after day 1, the rate of change of metabolic rate was strongly correlated with daily rate of accumulation of sleep deficit (Figure 7B). Further studies of longer-term CSR are needed to determine whether the asymptotic $\dot{V}O_2$ is proportional to the initial rates of change described here. Figure 7 identifies a threshold rate of accumulation of sleep deficit below which there is no effect on metabolic rate. In Figure 7B, the regression line crosses 0 at 3.6 hours of sleep deficit per day, implying that rats may be able to tolerate a daily loss of up to one third of their ad libitum sleep without any effect on energy expenditure.

The question of how much sleep is needed has not been definitively answered for human subjects and is likely to vary considerably between individuals.$^{40}$ Nevertheless, convergent data from studies of the correlations between sleep time and various clinical outcomes, such as mortality,$^{41}$ obesity,$^{11}$ diabetes,$^{42}$ and hypertension,$^{43}$ indicate that approximately 7 to 8 hours per day may be an optimal average value within large populations. Van Dongen and colleagues$^{12,15}$ addressed this question by titrating daily sleep over 14 consecutive days (i.e., a CSR protocol) and examining aspects of neurobehavioral function in healthy adult men. Overall, their data indicated that performance was impaired if daily sleep was less than 8.2 hours. Thus, in healthy humans, core sleep estimated in terms of neurobehavioral function appears to be similar to normal sleep duration,$^{12}$ whereas the present data suggest that the core sleep of rats, estimated in terms of whole body metabolic rate, may be approximately 6 to 8 hours or 60% to 75% of ad lib-
It is possible that rats may spontaneously over sleep in the unnatural conditions of the laboratory cage (implying that the apparent sleep deficit in CON rats may have been caused by oversleeping in baseline rather than under-sleeping during the CON protocol), as was shown recently for captive sloths. It is also likely that sleep loss may affect some physiologic and behavior mechanisms more readily than others, and a definitive determination of minimum sleep need must await identification of the appropriate (i.e., most sensitive) sleep-dependent marker.

There has been only 1 study reporting calorimetric measurement of energy expenditure in patients with insomnia. This report indicated that metabolic rate was elevated in insomniacs, on average, by approximately 6%. Interestingly, a group of subjects presenting with sleep-state misperception (people who complained of insomnia but had normal sleep upon polysomnographic evaluation) also had a similar 6% elevation of metabolic rate relative to matched control subjects. Daily energy expenditure was elevated by 16% in patients with obstructive sleep apnea, who had normal total amounts of sleep (8-9 h·d⁻¹) but with arousals approximately once every minute of sleep. Ryan et al found a similar elevation of daily energy expenditure in patients with obstructive sleep apnea, but this was eliminated when the data were corrected for lean body mass. Patients with narcolepsy, who present with hypersomnolence and disrupted sleep patterns, did not exhibit hypermetabolism, and, indeed, a group of nonobese narcoleptic patients had a significantly lower (13%) energy expenditure than did age- and weight-matched control subjects. A similar relative hypometabolism was reported in a transgenic mouse model of narcolepsy, which exhibits disrupted sleep patterns but normal daily sleep amounts.

The small rise in metabolic rate in humans with insomnia is considerably less than the pronounced hypermetabolism observed in rats subjected to acute TSD or chronic severe sleep fragmentation but is similar to rats under conditions of CSR. The present study found that metabolic rate in rats is more closely correlated with the daily rate of accumulation of sleep deficit than with the absolute sleep deficit. However, this was true only when the rate of accumulation of sleep deficit exceeded a threshold of approximately 3.6 hours per day (Figure 7B). In contrast, human neurobehavioral performance has been found to be more closely linked with cumulative excess wakefulness than with cumulative sleep deficit. Cumulative excess wakefulness has been calculated as the sum of wake time exceeding the habitual wake times of the human subjects. It is possible that the threshold for rate of accumulation of sleep deficit described here for rats and the threshold used for calculation of excess wakefulness in humans are different analytic descriptions of a common underlying process. Both analyses provide insight into the fundamental question as to how much sleep is needed, but further research is needed to resolve this issue.

In summary, the present study in rats has confirmed that 4 days of TSD results in weight loss, hypermetabolism, and increased Tb. In contrast, 10 days of CSR evoked responses that were qualitatively similar to those of TSD but were quantitatively diminished. In CSR, weight loss was slight and did not worsen after the second day, the elevation of Tb was attenuated and transient, returning to baseline after the third day; hypermetabolism was apparent only during wakefulness and was reduced to a statistically insignificant trend in CSR and completely absent in CON. The data show that, across the 3 protocols, the responses in metabolic rate were not directly proportional to absolute sleep deficit but, instead, were related to rate of accumulation of sleep deficit, above a threshold of 3.6 hours per day.

Short sleep duration in humans may increase the risk for weight gain and obesity, implying that sleep debt may be associated with a positive energy balance in affected individuals. Large epidemiologic studies suggest, however, that the risk of weight gain from short sleep duration may be trivial because hundreds of hours of accumulated sleep deficit lead to weight increases of less than 1 kilogram per year (reviewed in 1). The positive (or neutral) energy balance associated with short sleep in humans contrasts with the profoundly negative energy balance described in rats during prolonged TSD. It has been suggested that this may represent a difference between species, experimental protocols, or both. However, the present study is able to reconcile these divergent observations by suggesting the hypothesis that the effect of insomnia on energy balance may depend on the rate of accumulation of a sleep debt, rather than on the total accumulated debt. A rodent model of CSR may, therefore, prove to be useful for understanding the mechanisms underlying the modulation of energy metabolism by sleep loss, which, in turn, may contribute to our understanding of the causal links, if any, between short sleep and obesity.

ABBREVIATIONS

- BL, baseline
- CSR, chronic sleep restriction
- CON, sleep restriction stimulus control
- DOW, disc over water
- EEG, electroencephalogram
- EMG, electromyogram
- REC, poststimulus recovery
- Tb, body temperature
- TSD, total sleep deprivation
- VO₂, rate of oxygen consumption
- ZT, Zeitgeber time

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DISCLOSURE STATEMENT

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